COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

The effect of Hydroethanolic extract of *Curcuma longa* Linn (*Curcuma domestica* Valeton) Rhizome on Colon, Liver and hematological parameters on a model of 1,2-Dimethyl hydrazine induced colorectal cancer in male Wistar albino rats

By

Gelila Woredebrhan (B.Sc)

November 2019

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

Investigator - Gelila Woredebrhan

Main advisor- Dr. Gnanasekaran Natesan (Assistant professor)

Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University

Dr. Maria Degef T/mariam (Assistant professor)

Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University

Dr. Daniel Seifu (Associate professor)

Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University

Dr. Yonas Bekuretsion (Associate professor)

Department of Pathology, School of Medicine, College of Health Sciences, Addis Ababa University

Dr. Wondemagegnehu Tigeneh (Associate professor)

Oncology center, School of Medicine, College of Health Sciences, Addis Ababa University

A thesis submitted to the school of Graduate studies of Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Science in Medical Biochemistry

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

This is to certify that the thesis prepared by Gelila Woredebrhan, entitled "The effect of Hydroethanolic extract of *Curcuma longa* Linn (*Curcuma domestica* Valeton) Rhizome on Colon, Liver and hematological parameters on a model of 1,2-Dimethyl hydrazine induced colorectal cancer in male Wistar albino rats" is submitted in partial fulfillment of the requirement for the degree of Master of science in Medical Biochemistry compiles with the regulation of the university and meets the accepted standards with respect to the originality and quality.

Signed by the examining committee:

External examiner

Dr. Mathewos Assefa **Signature ……** **Date ……**

Main advisor

Dr. Gnanasekaran Natesan **Signature ……** **Date ……**

Co- advisors:

Dr. Maria Degef T/mariam **Signature ……** **Date ……**

Dr. Daniel Seifu **Signature ……** **Date ……**

Dr. Yonas Bekuretsion **Signature ……** **Date ……**

Dr. Wendemagegnehu Tigneh **Signature ……** **Date ……**

Distribution Agreement

This thesis is as partial fulfillment of the requirements for the degree of Master of Science from Addis Ababa University, I hereby grant to Addis Ababa University and its agents the non-exclusive license to archive, make accessible, and display on the World Wide Web. I understand that I may select some access restriction as part of the online submission of this thesis or dissertation. I retain all ownership right to the copy right of the thesis. I also retain the right to use in future works (such as articles or books) all or parts of this thesis.

Gelila Woredebrhan …… Date ……..

ACKNOWLEDGMENTS

Most importantly, I am extremely grateful to God and our Mother Virgin Marry for being with me through all the good and bad times during my study. I might want to recognize Addis Ababa University, College of Health Sciences, and School of Medicine and Department of Medical Biochemistry for its money related and strategic help. My thankfulness additionally goes to my home organization Dilla University for sponsoring me. I admiringly recognize the commitment of my advisors (Dr. Yonas, Dr.Wondemagegnehu, and Dr. Daniel) without you this research would have not been imaginable. Every one of you gave me important direction and backing with high worry from the point of title selection to the finish up of the paper. I am grateful having my advisors, Dr. Gnanasekaran Natesan and Dr. Maria Degef T/mariam, for their persistent assistance in forming this study. I truly value their basic suggestions and energetic bits of advice to deal with tackle issues. Their agreeable dialogs and direction made this study imaginable. My gratitude goes to Dr. Yonas, Mr. Moti Sori, Dr. Bereket and Dr. Demese for good cooperation from the Department of Pathology for their kind help with the histopathology slide preparation and examination. I would like to show appreciation for the farmer from which the plant collected; the national herbarium, AAU for their support during plant authentication; EPHI staffs for their support during analysis of blood and serum; department of Biochemistry/staffs for their professional support and friendship approach; Department of Physiology and School of Pharmacy, CHS, AAU; Mr. Tesfaye Getachew, Mr. Molla, and Mrs. Hageritu who support me with procedure involving in experimental animals. My special thanks go to Mr. Winner K. Dewelle (Ph.D. scholar) for his valuable and constructive suggestions during the development of this research. His willingness to give his time so generously has been very much appreciated. He has taught me more than I could ever give him credit for here. Finally, I want to thank my life partner (Tizalegn Tesfaye), my family, and my friends for providing me support and continuous encouragement throughout my years of study. This accomplishment would not have been possible without them.

Table of contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AOM	Azoxymethane
APC	Adenomatous polyposis coli
AST	Aspartate aminotransferase
CMC	Carboxymethyl cellulose
COX	Cyclooxygenase
CRC	Colorectal cancer
CSF	Colony stimulating factor
DMH	1,2 Dimethyl hydrazine
DPPH	2,2 Diphenyl-1-picrylhydrazyl
EPO	Erythropoietin
Hct	Hematocrit
HEECLR	Hydroethanolic extract of *Curcuma longa* rhizome
Hgb	Hemoglobin
HM	Herbal medicine
IARC	International Agency for Research on Cancer
ID	Iron deficiency
IL	Interleukin
LDH	Lactate dehydrogenase
LHR	Lymphocyte to Hemoglobin ratio
LMR	Lymphocyte to Monocyte ratio
LPL	Lipoprotein lipase
MAM	Methyl azoxy methanol
MAPK	Mitogen activated protein kinase
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean corpuscular volume
MHR	Monocyte to Hemoglobin ratio
MPV	Mean platelet volume

NAD+	Nicotinamide adenine dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NF-KB	Nuclear factor –B
NHR	Neutrophil to hemoglobin ratio
NLR	Neutrophil to lymphocyte ratio
OECD	Organization for Economic Co-operation and Development
PAK	P-21-activated kinase
PC	Platelet count
PCT	Platelet crit
PDW	Platelet distribution width
PKC	Protein kinase C
PLR	Platelet to lymphocyte ratio
RDW-CV	Red cell distribution width- coefficient of variation
RDW-SD	Red cell distribution width- standard deviation
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STAT	Signal transducer and activator of transcription
TAFLD	Toxicant associated fatty liver disease
TAG	Triacyl glycerol
TAM	Tumor associated macrophage
TCF	Ternary complex factor
TIL	Tumor infiltrating lymphocyte
TLC	Thin layer chromatography
TNF-α	Tumor necrosis factor- alpha
TNM	Tumor, Node, Metastasis
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

ABSTRACT

Background: Cancer incidence, mortality, and burden are growing at an alarming rate around the globe. Colorectal cancer is ranked as the fourth most lethal cancer in the world and in Ethiopia it accounts 7%. Certain herbal medicines protect the body from cancer by improving cellular defense mechanisms (anti-inflammatory, detoxification, and antioxidant).*Curcuma longa* is one of herbal medicine that has the potential to fight cancer.

Objectives: The aim of this study was to assess the effect of Hydroethanolic extract of *Curcuma longa* Linn. rhizome on colon, liver and hematological parameters on a model of 1,2-dimethylhydrazine induced colorectal cancer in male Wistar albino rats.

Methods: 80 male Wistar albino rats divided into three groups. The group I intraperitoneal administration of normal saline 2mL/kg for 15 weeks, group II intraperitoneal injection of 20 mg/kg of 1,2- Dimethyl hydrazine for 15consecutive weeks, and group VI intraperitoneal injection of 20 mg/kg of 1,2- Dimethyl hydrazine and simultaneously oral administration of 500 mg/kg of extract for 15 weeks. After 15 weeks group II rats were divided into four groups and the groups were renamed (group II, III, IV, and V).Three different doses (250mg/kg, 500mg/kg, and 1000mg/kg daily) of hydroethanolic extract of *Curcuma longa* rhizome were administered for 8 weeks for groups III, IV, and V respectively. At the end of the experiment, the rats were anesthetized and blood was collected by cardiac puncture for whole blood and biochemical analysis. Rats were sacrificed, liver and colon were isolated for histopathological investigation.

Results: The results indicated that the elevated level of total White blood count, neutrophil and monocyte and decreased level of lymphocyte, hemoglobin, hematocrit and platelet count seen in cancer rats. The affected parameters were effectively restored by hydroethanolic extract of *Curcuma longa*. The Extract has been found to have immunomodulatory, anti-metastasis, hepatoprotective, and chemo-preventive effects.

Conclusion: *Curcuma longa* grown in Ethiopia has anti-oxidant, immune-modulatory, detoxifications boosting, chemo-preventive, and anti-metastasis activities due to the presence of adequate secondary metabolites.

Keywords: Colorectal cancer, 1,2- Dimethyl hydrazine, *Curcuma longa (Curcuma domestica),* Wistar albino rats

1. INTRODUCTION

1.1. Background

Cancer is the consequence of a series of molecular changes that interrupt the normal function of cells. They are abnormal cells in which the cell division regulation process is disturbed (Lowhan., 2003). Cancer is a leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018 (WHO, 2018). Colorectal cancer (CRC) is the third most common cancer in males and the second in females worldwide (www.wcrf.org). It is the most common malignant cancer in the gastrointestinal tract (Kolligs, 2016).

Cancer is a complicated disease and it's the result of environmental toxins that serve as the driving force for the initiation of tumor development and progression. Environmental toxin accumulation is one prominent risk factor for cell damage which turns to cancer formation (Parsa, 2012). The liver neutralizes a large range of toxic chemicals, each these produced internally and those coming from the environment. The liver performs various roles in detoxification which is the mechanism of eliminating and cleansing toxins from the body. The detoxification program is important to stop and reverse cellular damage, prevent excessive inflammation, and essential for the prevention of cancer. High degrees of exposure to carcinogenic agents coupled with the sluggish function of detoxification enzymes will increase toxin accumulation in the body and bring chronic inflammation (Pezzone and Murray, 1999). Chronic inflammation or chronic activation of the immune system is linked with the development of tumors (Byrne and Dalgleish, 2001; Robinson *et al.*, 2016). Chronic inflammation is able to promote the malignant transformation of normal epithelial cells in the gastrointestinal tract to cancer. Host inflammation responses in carcinogenesis are through multiple mechanisms, such as the formation of reactive oxygen and nitrogen species from mononuclear phagocytes and leukocytes, immune response, and pro-inflammatory cytokines (Ida *etal.*, 2015). Reactive oxygen species (ROS) can induce the formation of oxidative Deoxyribonucleic acid (DNA) lesion products, including 8-oxo-7,8-dihydro-2ˈ-deoxyguanosine (8-oxodG), which is considered to be mutagenic (Kawanishi *et al.*, 2016).

Hematological parameters and markers changes are the result of the systemic inflammatory response and have been correlated with prognosis in several malignancies including colorectal

cancer. Any kind of severe disease or abnormality has a direct impact on blood parameters so it is necessary to study the changes in hematological parameters. A complete blood count (CBC) is a blood test that gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. The white blood cell count (total and differentials) predicts disease severity and mortality risk (Shrivastava *et al.*, 2017). In addition, changes in lipid profiles have long been associated with cancer because lipids play a key role in the maintenance of cell integrity and malignant proliferation (Mehrotra *et al*., 2009).

Scientists all over the world are focused on the herbal medicines to boost immune cells of the body against cancer without harming normal cells of the body (Sakarkar and Deshmukh, 2011). The terms "herbal medicine" (HM), "traditional herbal medicine" (THM), or "phytomedicine" refer to the use of plant material, such as whole plant, roots, bark, leaves, flowers, berries, or seeds, for medicinal purposes (WHO, 2005). It has a long tradition of use with the discovery of archaeological evidence of the use of herbal medicine by humans, dating as far back as the Neanderthal period. Certain HM protects the body from cancer by augmenting the detoxification process. Some biological response modifiers derived from herbs are recognized to hamper cancer progression cut back the lethal side effects of chemotherapy and radiotherapy (Tuasha *et al*., 2018).

Curcuma longa Linn., plant is a perennial herb belonging to the ginger family, Zingiberaceae. The Latin name is derived from the Persian word, "*kirkum*", which means saffron, in reference to the rhizomes vibrant yellow-orange color. It is also called 'Ird' in Amharic (Bhowmik *et al.*, 2008; Seifu and Tassew, 2014) and *Curcuma domestica* Valeton is an alternative name for *C.longa* Linn (WHO, 1997). It used in various foods for flavor as well as a coloring agent for foods and it has a wide spectrum of pharmacological activities(Nasri *et al*., 2014). The plant reached in Ethiopia during 800 AD probably from India. In 1972, two types of *C.longa* had been introduced from India and China and planted at Jimma, Metu, Bebeka, Tepi, Wenago, Hawassa, Magi and Bako. *C.longa* can be grown up to an altitude of 2,000m in areas with excessive rainfall. At present, because of the suitability of Southern humid regions, *C.longa* is widely grown there (Peethambaran *et al*., 2016*)*.

Curcumin (Diferuloylmethane), a polyphenol extracted from the plant *C. longa* Linn.*(*Lee *et al., 2013)*, which gives the yellow color to *C.longa*, was first isolated almost two centuries ago, and

its structure as diferuloylmethane was determined in 1910. Since the time of Ayurveda 1900 BC, numerous therapeutic activities have been assigned to *C.longa* for a wide variety of diseases (Aggarwal *et al.*, 2007).It has been determined that the chemical structure of this polyphenol substance shows anti-oxidant, anti-inflammatory, anti-angiogenic, anti-mutagenic, and anti-platelet aggregation properties (Kocaadam and Sanlier, 2015), hepatoprotective effect (Khater *et al.*, 2013)and hypolipidemic effect (Zingg *et al.*, 2013). It may exert anticancer effects in a variety of biological pathways involved in mutagenesis, apoptosis, tumorigenesis, cell cycle regulation, and metastasis (Perrone*et al.*, 2015). Laboratory studies have demonstrated that it inhibits colon tumorigenesis and tumor size in animals (Tayyem *et al.*, 2006).

1.2. Statement of the problem

Cancer is a fundamental public health challenge (Brown *et al.*, 2001). It causes about 1 in every 6 deaths worldwide, more than AIDS, tuberculosis, and malaria combined. It is the second-leading cause of death following cardiovascular diseases. The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018. One in 5 men and one in 6 women worldwide develop cancer during their lifetime, and one in 8 men and one in 11 women die from the disease. By 2040, the global burden is expected to grow to 27.5 million new cancer cases and 16.3 million cancer deaths (GLOBOCAN, 2018). The international burden of cancer is accompanied through the economic load, including direct costs, such as medication and rehabilitation costs, and indirect costs, associated with productiveness losses due to incapacity and premature death (Pearcea *et al.*, 2018). The statistics in cancer incidence and mortality show cancer burden is developing at an alarming rate around the globe.

Major cancer types in 2018 were cancers of the lung, breast, and colorectal in terms of incidence and are ranked within the top five in terms of mortality. These three cancer types are responsible for one-third of the cancer incidence and mortality burden worldwide. CRC is one of the most prevalent and fatal cancers worldwide. It is the highest contributor to cancer-related death with an incidence rate of approximately 1.4 million and a mortality rate of about 700,000 people per year globally (IARC, 2018). According GLOBOCAN 2018 report, the incidence of CRC in Ethiopia is 7%. It accounts for 10.4% in males and 5.4% in females.

According to the 2014 IARC report, the global fighting against cancer can't be won through treatment alone rather it needs urgent prevention strategies to prevent most cancers crisis. Clinical trials using most cancers drugs failed again and again because of costly therapeutic procedures discontinued after the loss of patients. Furthermore, the main problem in the scientific world for the unsuccessfulness of cancer therapy is due to a shortage of systematic research on roles of inflammation, oxidative stress, detoxification failure and impaired mitochondrial function on multistep carcinogenesis procedure (Maeda and Khatami, 2018).

Chemotherapy is the application of chemicals or drugs to kill cancer cells, and its effects are systemic. However, a large number of drawbacks are its limitations (Huang *et al.*, 2017). Such drugs would induce an ‘immune tsunami’ or ‘cytokine storm’ throughout the body. The overall toxicities of such drugs on the metabolic and detoxification processes could progressively lead to

severe damages to the function of normal organs such as the kidneys, liver, and heart, and it could further involve in coagulopathy and peripheral neuronal toxicity (Maeda and Khatami, 2018).

The age of cancer patients is still decreasing, and the entire scientific society is eager for new ways to fight against cancer (Kisková and Kassayová, 2019). Thus, it is interesting to seek alternative treatments for cancer that have minimal side effects or less toxic and not so expensive and more potent anticancer drugs as compared to the drugs available in the market (Roy *et al.*, 2017). Herbal medicines are generally low in cost, plentiful, and show very little toxicity or side effects in clinical practice. Much of the current research in cancer therapeutics is aimed at developing drugs or vaccines to target key molecules that can inhibit tumor cell growth, metastasis, and proliferation. The cancer-preventive and/ or protective activities of HM target cellular defenses (anti-inflammatory, detoxification, and antioxidant) (Huang *et al.*, 2017). *C.longa* is one of HM that has the potential to fight cancer. It has no significant side effects and is cheap and easily available. It's used for cancer patients as chemo-preventive and chemotherapeutics agents.

So, the aim of this study was to assess the effect of *Curcuma longa (curcuma domestica)* extract on colon, liver and hematological parameters on the model of 1,2- dimethylhydrazine induced colorectal cancer male Wistar albino rats.

1.3. Significance of the study

Cancer is one of the main causes of mortality in Ethiopia. Oxidative stress, inflammation, and detoxification failure are the fundamental driver for malignancy events. The current chemotherapy focused on the outcome of such fundamental causes. Treatment for cancer is costly and has a lot of side effects. Individuals don't know about how malignant growth happens and how to treat the disease effectively with easily accessible items. So, the disease treatment approach should concentrate on the reduction of oxidative stress, treatment of inflammation and liver protection for enhancement of detoxification process and boosting the immune system. Various studies were conducted on the anti-cancer property of *C.longa* but not well studied about the immuno-boosting mechanism and also no investigations in Ethiopia that can elucidate therapeutics and chemo-preventive effects of *C.longa* rhizome which grown in Ethiopia. This plant is effectively accessible and can be utilized in routine life. So, this investigation will incite the clinical significance of C.*longa* for malignant growth and it will move clinicians to prescribe *C.longa* for their patient combined with chemotherapy. The study also explains the anti-oxidant, anti-inflammatory and hepatoprotective effects of *C.longa* which are important keys for the treatment and prevention of cancer. Generally, results obtained from this study may empower the utilization of C.*longa* as chemo-preventive, hepatoprotective and chemotherapeutic treatment. Apart from that, this study will add knowledge for the scientific world about hematological parameters which give benchmark data about how inflammation contributes to malignant growth and the result obtained from this study may be used as baseline data for further study. Cancer patients can likewise profit by this finding.

2. LITERATURE REVIEW

2.1. Colorectal cancer

2.1.1. Colorectal cancer epidemiology

Colorectal cancer (CRC) is ranked as the fourth most lethal and commonly diagnosed cancer in the world (Gothai *et al.*, 2018). There is great variability in the worldwide incidence and mortality rates. Industrialized nations appear to have the greatest risk while most developing nations have lower rates. Unfortunately, this incidence is on the increase. North America, Western Europe, Australia and New Zealand have high rates for colorectal neoplasms (www.hopkinsmedicine.org). According to the 2018 GLOBOCAN report, the worldwide cases of CRC in males, females and both sex was 10.9%, 9.5%, and 10.2 % respectively. While the Worldwide death report of CRC was 9.2% in both sex, 9.5% in females and 9% in males. The new cases of CRC in Africa account 6.9 % in male, 5.1 % in female and in both sex it account 5.9 %. In east Africa it accounts 6.1 % in male and 4.5 % in female. In Ethiopia, it account 7%, 10.4% in males and 5.4% in females.

Figure 1: Geographic distribution of sporadic colon cancer neoplasm (https://www.hopkinsmedicine.org/gastroenterology_hepatology/)

2.1.2. Staging of colorectal cancer

Staging is used mainly to determine appropriate treatment strategies. Staging consists of assessing the status of the tumor with regards to various factors, such as depth of tumor invasion into the colorectal wall, fat and fascia involvement, status of circumferential resection margin, invasion into surrounding structures, the involvement of local lymph nodes, and distant metastasis (Bruening *et al.*, 2014). Using the TNM staging, the progression of the original primary tumor is denoted by the letter T (tumor); N (node) indicates whether the tumor has spread to lymph nodes; M (metastasis) represents whether the tumor has metastasized to distant organs in the body, most commonly the liver or lungs. T, N, and M are followed by numbers giving further information on the stage of the disease (https://www.roche.com/med-colorectal-cancer).

Table 1: TNM classification of CRC.

The stages of colorectal cancer (TNM)	
Stage	**Classification**
Stage I	The tumor is localized to the lining of the colon. T1 -T2, N0, M0
Stage II	The tumor grows into the outer lining of the colon or surrounding tissue. T3-T4, N0, M0
Stage III	Cancer has metastasized to the lymph nodes. Any T, N1-N2, M0
Stage IV	Cancer has metastasized to distant organs in the body. Any T, Any N, M1

2.1.3. Inflammation and colorectal cancer

CRC especially sporadic form is most strongly related to lifestyle factors. The epidemic of obesity and physical inactivity has a great impact on disease patterns. Likewise, an altered metabolism has consequences at the cellular and molecular level with implications for cancer initiation and growth (Hagland *et al.*, 2013). Recent data have expanded the concept that inflammation is a critical component of tumor progression (Coussens and Werb, 2002).

Chronic inflammation is a critical component of CRC initiation and progression. This is supported by finding of strong associations between inflammatory bowel disease (IBD) and CRC, and by findings supporting the positive effects of chronic Non-steroidal anti-inflammatory drugs use in CRC (Colussi *et al*., 2013).

Cancer and inflammation are linked by two pathways: the extrinsic and intrinsic pathways (Mantovani *et al.*, 2008). In the extrinsic pathway, tumor initiation and development are triggered by inflammation or infection. In the intrinsic pathway, somatic alterations and genetic mutations activate signaling pathways (β-catenin/WNT, TGFβ signaling, P53, Myc, etc) that lead to an inflammatory response (Mantovani *et al.*, 2008). Both pathways can meet regardless of the origin, mediators, and effectors of inflammation (such as inflammatory cells), cytokines and growth factors create an optimal environment for tumor-cell proliferation, survival, and metastasis (Balkwill *et al.*, 2005).In support of the role of chronic inflammation in CRCs, researchers studied the role of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α, the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3) protein, Interleukin (IL)-6 and the C-reactive protein (Colussi *et al.*, 2013).

Chronically elevated levels of TNF-α promote tumor growth, proliferation, and metastasis. IL-6 is a cytokine involved in the regulation of the acute phase of inflammation and, in its own transduction pathway, stimulates the transcription of STAT3(Colussi *et al.*, 2013).Cancer cells produce cytokines and chemokines that attract a diverse immune-cell infiltrate composed of macrophages, neutrophils, and lymphocytes. These infiltrating immune cells can produce cytotoxic mediators, such as ROS, matrix metalloproteinases and cytokines (TNF-α), ILs and interferons(Coussens and Werb, 2002).

Persistent activation of the immune system and failure of the inflammatory response results in chronic inflammation. The chronic inflammatory microenvironment fosters genomic lesions and promotes tumor growth. One effector mechanism includes the production of free radicals by the host such as reactive oxygen intermediates (ROI),hydroxyl radicals, superoxide, reactive nitrogen intermediates (RNI), nitric oxide and peroxynitrite (Hussain *et al.*, 2003). Notably, ROI and RNI increase the risk of DNA mutations via oxidative stress and nitration of DNA bases. Finally, the failure of cell death and repair programs in chronically inflamed tissues leads to continuous DNA replication and cellular proliferation (Gun *et al.*, 2019).

2.1.4. Molecular mechanism of colorectal cancer

The pathogenesis of CRC varies according to genetic or epigenetic changes. Such genetic and epigenetic alterations are directly responsible for a specific event within the sequence that leads to CRC, by contributing to the "initiation" of neoplastic transformation of healthy epithelium and/or determining the "progression" towards more malignant stages of the illness (Colussi *et al.*, 2013). Among these specific events around 75% of CRCs have mutations in the adenomatous polyposis coli (APC) tumor suppressor gene (Christie *et al.*, 2013). Mutation in the APC activates the Wnt signaling pathway by increasing β-catenin levels. β-catenin is translocated to the nucleus and enhances the transcription of various oncogenes with T-cell factor transcription factors (Tariq and Ghias, 2016). High β-catenin levels are noted in gastrointestinal tumors (Bakker *et al.*, 2013). The effects of APC restoration in mice are demonstrated on tumor regression by the conversion of cancer cells back to normal. Mutations in other genes of this pathway, particularly in β-catenin, may also lead to CRC. These mutations are found in 48% of CRCs without APC mutation (Tariq and Ghias, 2016). β-catenin activates a set of 162 Wnt pathway target genes in a colon cancer cell line (Watanabe *et al.*, 2014). Mutations of K-ras lead to a permanently active state that permits the cell to evade apoptosis and acquire a growth advantage for the cancer cell. P_{53} loss of function is frequently present in the later stages of colorectal tumorigenesis. BRAF mutations are often associated with a poorer CRC prognosis (Colussi *et al.*, 2013).

2.1.5. Liver and Colorectal cancer

Hepatocytes are the most abundant cell type in the liver, accounting for 90% mass of the liver. Their primary role is toxin neutralization (Crispe, 2016). The liver is the first line of defense against potentially harmful xenobiotics, and it is, therefore, the target organ that is most commonly affected by commercially-produced chemicals and environmental pollutants (Al-Eryani *et al.*, 2015). These result in the generation of reactive moieties that can cause tissue injury and modification of hepatic metabolism (Cichocki *et al.*, 2017).

Toxicant associated fatty liver disease is a recently identified form of non-alcoholic fatty liver disease (NAFLD) associated with exposure to industrial chemicals and environmental pollutants.

Numerous studies have been conducted to test the association between industrial chemicals/environmental pollutants and fatty liver disease both in vivo and in vitro (Al-Eryani *et al.*, 2015). Several mechanisms intoxicant associated fatty liver disease occurred including hepatocyte necrosis, pro-inflammatory cytokine elevation, decreased antioxidants, and mitochondrial dysfunction (Wahlang *et al.*, 2013).The association between NAFLD and colorectal adenoma has been investigated in multiples studies (Muhidin *et al.*, 2012; Shen *et al.*, 2014).

2.1.6. Alteration of hematological and biochemical parameters during colorectal cancer

2.1.6.1. Alteration in white blood cell and its differentials

The peripheral blood count is a basic and highly informative hematological tool at the clinician's disposal in screening, diagnosis, and monitoring of disease progression and therapeutic response. Peripheral blood interpretation is important for a successful clinical practice (Adewoyin and Nwogoh, 2014). Inflammation plays a critical role in cancer onset and development. Several systemic inflammatory factors can be used to predict the prognosis for CRC and alter WBC count (Wu *et al.*, 2017).

2.1.6.1.1. Alteration in neutrophil count

The inflammatory response is accompanied by an increase in circulating neutrophil levels and a decrease in circulating lymphocyte levels. A high concentration of neutrophils is known to promote tumor progression and it can suppress the antitumor effect of lymphocytes. An imbalance of neutrophils and lymphocytes in peripheral blood can be associated with tumor development (Demir *et al.*, 2015).Neutrophils are an important component of peripheral blood, and have the ability to promote the development of tumors, due to their effect on tumor-related angiogenesis in addition to this they are a primary source of circulating angiogenesis-regulating chemokines, growth factors, and proteases. In many patients with advanced cancer, elevated counts of neutrophils in the blood are found. How tumors induce neutrophilia is uncertain, but the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) is a possible mechanism in several types of cancer (Wu *et al.*, 2019). In addition, other cytokines such as granulocyte colony-stimulating factor (G-CSF), IL-1, and IL-6 produced by tumors seem to contribute to elevated neutrophil numbers in the blood. This neutrophilia is associated with poor prognosis in several types of cancers including colorectal cancer. It represents evidence for the

concept of cancer-related inflammation-inducing tumor progression (Uribe-Querol and Rosales, 2015). On Kaplan–Meier analysis of the whole cohort, age, Tumor, Nodal, and Metastasis stage, venous invasion, margin involvement, peritoneal involvement, and tumor perforation, and white cell and neutrophil count was associated with survival of cancer (Watt *etal.*, 2015).

2.1.6.1.2. Alteration in lymphocyte count

Lymphocytes participate in cytotoxic cell death and inhibition of tumor cell proliferation and migration. Lymphopenia usually indicates disease severity and can make cancer cells escape from the immune of tumor-infiltrating lymphocytes (TILs).TILs are formed by lymphocytes migrating into the tumor microenvironment. It has been proved that decreased levels of TILs predict worse survival in patients with CRC (Wu *et al.*, 2017).

2.1.6.1.3. Alteration in monocyte count

Monocyte promotes tumor progression and metastasis (Evani *et al.*, 2013). Several pro-inflammatory cytokines, secreted from monocytes, are associated with poor prognosis in cancer patients, such as TNF-α and IL-1 (Landskron *et al.*, 2014).Tumor-associated macrophages, derived from circulating monocytes, have a role in suppressing adaptive immunity and promoting angiogenesis, invasion, and migration. The peripheral monocyte count was associated with the density of tumor-associated macrophages (TAMs) in the cancer microenvironment (Cassetta and Pollard, 2018).

2.1.6.2. Alteration in platelet count and its indices

In malignancy, tumor cells can activate platelets by direct contact, or via the release of mediators such as ADP, thrombin, thromboxane-A2 or tumor-associated proteinases (Lou *et al.*, 2015). Many of the major structural components of platelets and platelet receptors that contribute to hemostasis have also been found to relate to malignancy progression. In addition to coagulation-related proteins, platelets also store proteins within the alpha granule that can regulate angiogenesis and metastasis (Wojtukiewicz *et al.*, 2017). Several studies have suggested an increase in platelet activation in the blood of patients with cancer (Plantureux *et al.*, 2018).

2.1.6.3. Alteration in RBC and its indices

Anemia in cancer patients is due to decreased the production of RBCs resulting from nutritional deficiencies; insufficient production of RBCs because of the presence of chronic disease; due to reduction of erythropoietin (EPO); bone marrow infiltration by the tumor or bone marrow suppression resulting from anti-cancer treatment (Schrijvers, 2011). High levels of pro-inflammatory cytokines and increased oxidative stress may contribute to the development of cancer-related anemia. The tumor which related to inflammation has a positive correlation with red blood cell distribution width (RDW) (Li *et al.*, 2018). Tumor growth can lead to malnutrition, which causes changes in erythropoiesis. It is well known that patients with CRC have a tendency for hemorrhage, which reduces iron storages. These lead to the variation of red blood cell size and increase RDW (Song *et al.*, 2018).

2.1.6.4. Alteration of Neutrophil-to-Lymphocyte ratio, Platelet-to-Lymphocyte ratio, and Lymphocyte-to-Monocyte ratio

Several studies have demonstrated that many systemic inflammatory factors can be used to predict the prognosis for CRC patients, such as platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and lymphocyte-to-monocyte ratio (LMR) (Wu *et al.*, 2017). NLR is a predictive parameter for tumor staging and a prognostic marker in CRC. NLR may be considered as the balance between pro-tumor inflammatory status and anti-tumor immune status. Its prognostic role has been evaluated in several studies including patients affected either by locally or advanced stage of CRC (Ozgehan *et al.*, 2014).

Ozdemir and his colleague's retrospective study a series of 281 patients, that includes stage I to IV CRC, local recurrences and presence of distant metastases suggested that high NLR value and was predictive of shorter survival rate (Ozdemir *et al.*, 2014). Pre-treatment PLR and LMR could be also used as prognostic predictors in CRC patients. Elevated PLR was associated with poor prognosis (Chen *et al.*, 2018).

2.1.6.5. Alteration of Triglyceride

Lipid metabolism disorders play an important role in carcinogenesis and development as well since they cause abnormal expression of various genes, proteins, and deregulation of cytokines and signaling pathways (Long *et al.*,2018). Hyperlipidemia can lead to enhanced neutrophil infiltration, which increases CRC metastasis. There are three primary aspects that relate hyperlipidemia with CRC metastasis: promotes the initial metastatic properties of CRC; stimulates CRC cells to leave the vasculature, and facilitates the development of CRC metastasis (Shen *et al.*, 2018). In a metastatic microenvironment, high levels of plasma triglyceride contribute to ROS-induced oxidative stress. ROS degrade molecules such as PKC and protein tyrosine phosphatases and regulate downstream molecules of mitogen-activated protein kinase (MAPK) and p21 activated kinase (PAK), which are both involved in cancer migration. Serum triacylglycerol concentrations are involved in the pathogenesis of colon cancer (Shen *et al.,* 2018).

Higher serum triglycerides level was significantly associated with an increased prevalence of both non-advanced and advanced colorectal adenomas (Li *et al.*, 2011; Yang *et al.,* 2013).

2.2. 1,2 -Dimethyl hydrazine(DMH)

Animal models have been viewed as critical tools necessary to study potential preventative and therapeutic strategies of colorectal carcinogenesis. For research purposes, the two major animal models of colorectal carcinogenesis are colorectal tumors induced by chemical or environmental agents in rodents, which represent sporadic CRC, and genetically modified mice, which represent the hereditary familial adenomatous polyposis and hereditary non-polyposis colorectal cancer syndromes (Johnson and Fleet, 2013). The two most commonly used chemical substances in animal models for induction of sporadic CRC are azoxymethane (AOM) which is a direct inducer, and 1,2 dimethyl hydrazine (DMH), which is an indirect inducer of carcinogenesis. The mechanisms of development of CRC by DMH and AOM is the same with naturally occurring CRC in humans (De-souza and Costa-Casagrande, 2018).

The DMH/AOM model of colon carcinogenesis is a valid, well-appreciated, and widely used model of experimental colon carcinogenesis. It shares many similarities to human sporadic Colon Cancer(Perše and Cerar, 2010).

DMH and AOM are pro-carcinogens, which require metabolic activation by cytochrome P450 enzymes. They undergo a series of reactions through intermediates AOM and methylazoxymethanol (MAM) to the ultimate carcinogenic metabolite, highly reactive methyldiazonium ion. DMH undergoes N-oxidation to form AOM, which, upon hydroxylation, yields MAM which is unstable, with a half-life of approximately 12 hr. It subsequently decomposes to yield formaldehyde and a highly reactive methyldiazonium ion, MAM can be excreted into the bile and transported to the Colon or enter directly into epithelial cells of the colon from blood circulation (Perše and Cerar, 2005).It is responsible for methylation of the DNA bases, including epithelial cells in the proliferative compartment of the crypts, which result in a great loss of colonic cells by apoptosis, an increase in proliferation, and an apparent increase in mutations of colonic epithelial cells (Hamiza *et al.*, 2014). Some studies have also demonstrated that rat colon epithelial cells are capable of metabolizing DMH into the carcinogenic metabolite without previous metabolism by other tissues or colon bacteria (Abdelaziz *et al.*, 2016). DMH is highly toxic and carcinogenic and affects a number of body organ including the liver. It is metabolized in the liver and produces highly reactive electrophiles i.e., carbonium ions and alkyl free radicals which severely damage the liver causing necrosis and fatty infiltration, methylate nucleobases and disrupt the polysomal assembly (Sharma and Sharma, 2011).

2.2.1. DMH and Colorectal cancer

MAM alkylates the DNA bases, resulting in the formation of DNA adducts, including O6-methylguanine (O^6-mG) and N^7-methylguanine (N7-mG). Persistence of O6-mG can lead to mutation in oncogenes and initiation of tumorigenesis (Megaraj *et al.*, 2014).DMH treatment generates free radicals in colonic tissue. Thus, DMH treatment decreases the level of antioxidant enzymes(Hamiza *et al.*, 2012).The metabolite of DMH causes DNA mutations from G:C to A:T in genes (K-ras, Apc, β-catenin). Following DMH treatment, the epithelial cells undergo pathogenesis from minor lesion aberrant crypt foci to adenomas and malignant adenocarcinomas (Rosenberg *et al.*, 2009).

In the progression of DMH carcinogenesis, the reactive metabolite causes the methylation of DNA in colonic epithelial cells and β-catenin gene mutation (Tanaka, 2009). Mutation in β-catenin or APC prevents the phosphorylation and consequently β-catenin proteosomal

degradation in cytoplasm leading to increased level of cytoplasmic β-catenin. This is resulted in accumulation of β-catenin/TCF/LEF complexes in the nucleolus and activation of downstream target oncogenes such as c-myc, c-jun and cyclin D1 leaded to colon cancer (Shang*et al.*, 2017).

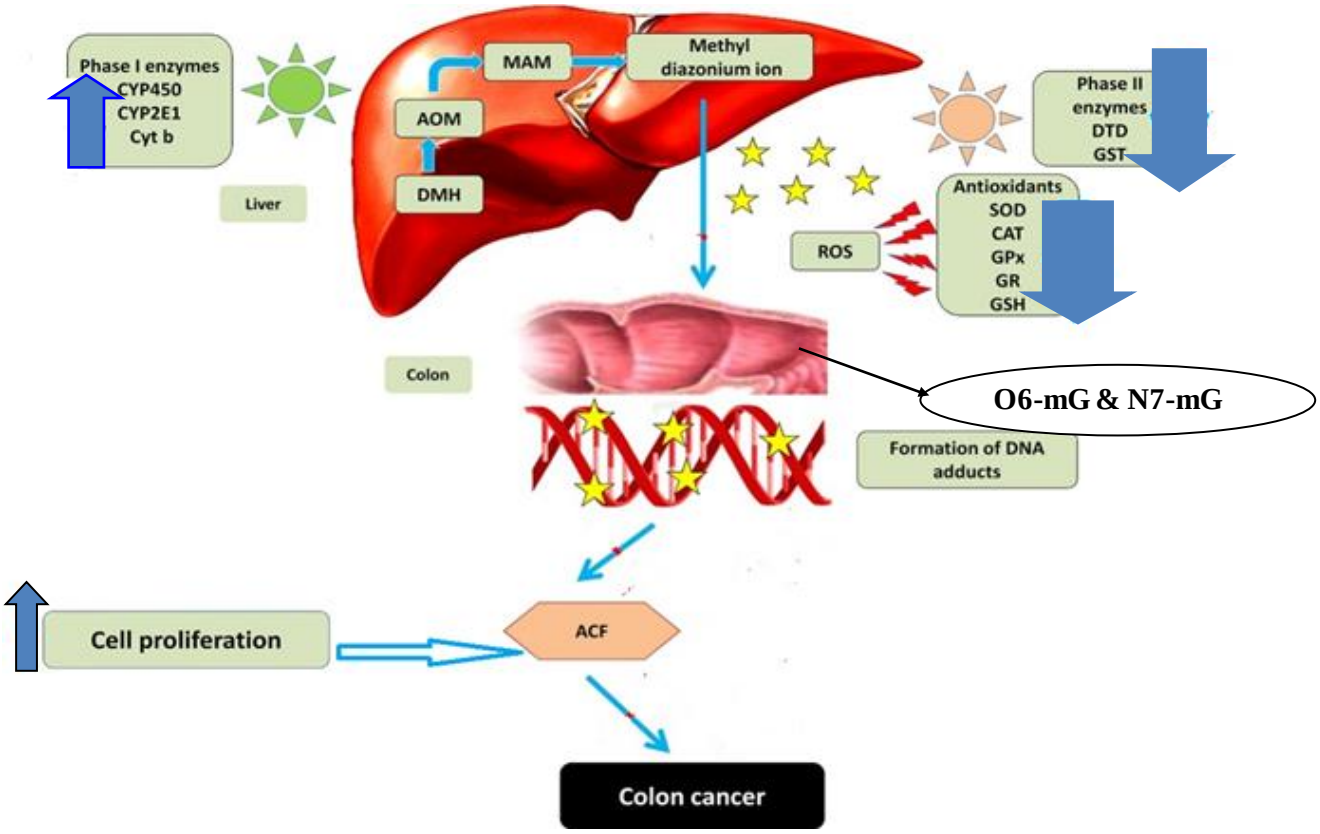


Figure 2: DMH reactive metabolites in the colon, adapted from (Thangaraj *et al.*, 2018). ACF: Aberrant crypt foci; AOM: Azoxymethane; CAT: Catalase; DTD: DT-Diaphorase; GST: Glutathione S-transferase; MAM: Methyl azoxy methanol; SOD: Super oxide dismutase; ⬆ arrow showing increasing; ⬇ arrow showing decreasing.

2.3. *Curcuma longa* Linn. (*Curcuma domestica* Val.)

Curcuma longa (C. longa) contains many secondary metabolites like phenols, alkaloids, tannins, steroids, saponins, flavonoids, and many others. Phytochemicals are the non-nutritive chemical constituents of plants that help to protect the plant for defense and prevent them from being infected by diseases. They can also protect humans against a variety of diseases (Oghenejobo *et al.*, 2017).

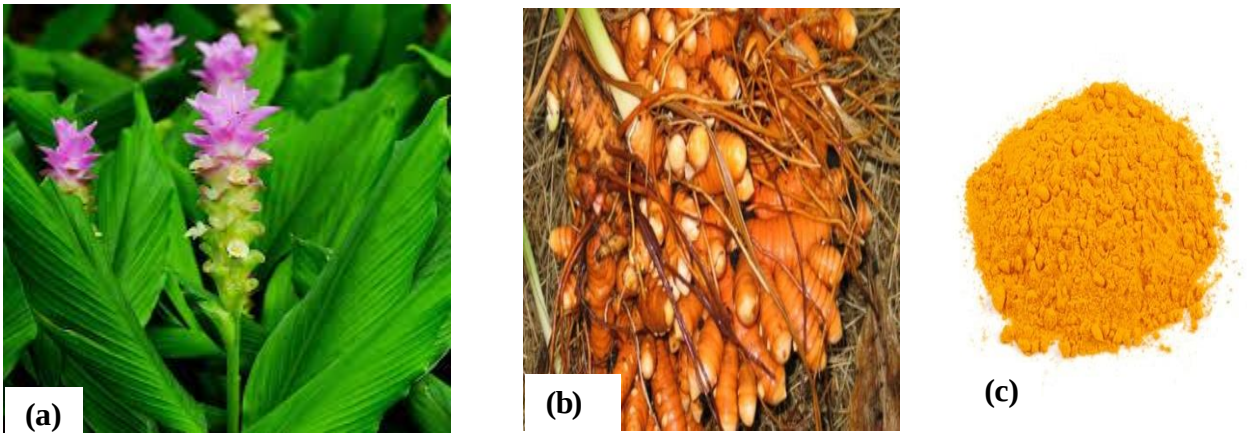


Figure 3: **(a)** *C.longa* in its natural habitat (https://www.easytogrowbulbs.com/); **(b)** *C. longa* rhizome (https://www.pinterest.com/); **(c)** Powder of *C.longa (https://www.alibaba.com/)*

2.3.1. Biochemical composition of *Curcuma longa Linn (C.longa)*

C.longa contains a wide variety of phytochemicals, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerones, and turmeronols(Elhassaneen *et al.*, 2017). Its main yellow compounds are composed of curcumin, demethoxycurcumin, and bis-demethoxycurcumin, and most of the yellow color of *C.longa* can be explained by the presence of these curcuminoids (Iijima and Joh, 2014). Curcuminoids comprise 3–6% of *C.longa* powder (Aggarwal*et al.*, 2007). The remaining two curcuminoid accounts, Demethoxycurcumin about 15–20 % and bisdemethoxycurcumin about 3%. Curcumin is the most studied and makes up 70–75% curcuminoids. Curcumin is insoluble in water but is easily soluble in ethanol and acetone (Hegde *et al.*, 2012). *C.longa* has a peculiar smell due to the aromatic volatile oil components. This oil component contains 25% turmerone, 11.5% curdine and 8.55% ar-turmerone (Oghenejobo *et al.*, 2017).

C.longa also contains nutritive components. It contains 5.1 % fat, 6.3%protein, 69.4% carbohydrate, 3.5% minerals and 3.5% moisture (Kocaadam and Sanlier, 2015).

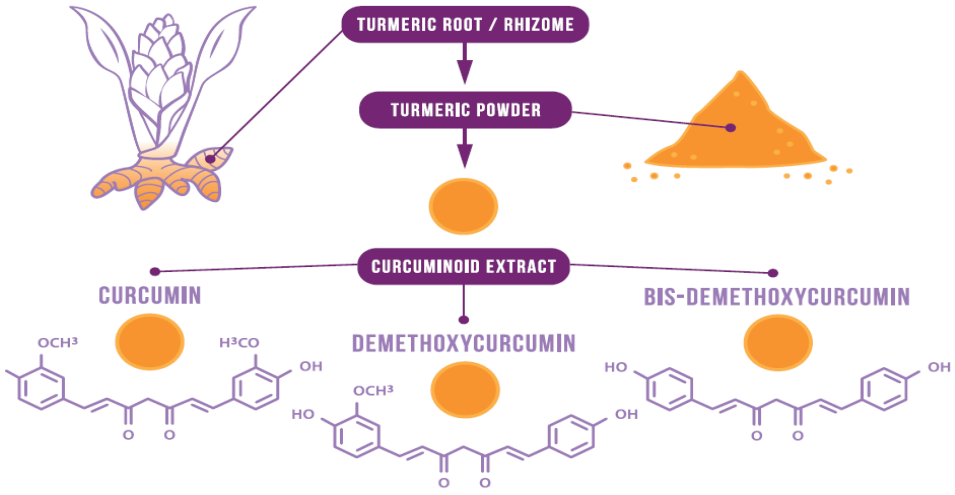


Figure 4: Structure of curcuminoids, adapted from (Janeen *et al.*, 2016)

2.3.2. Medicinal use of *C.longa*

C.longa has a long tradition of use in the Chinese and Ayurvedic systems of medicine in India, particularly as an anti-inflammatory agent, antioxidant, hepatoprotective, and anti-carcinogenic (Hewlings and Kalman, 2017).

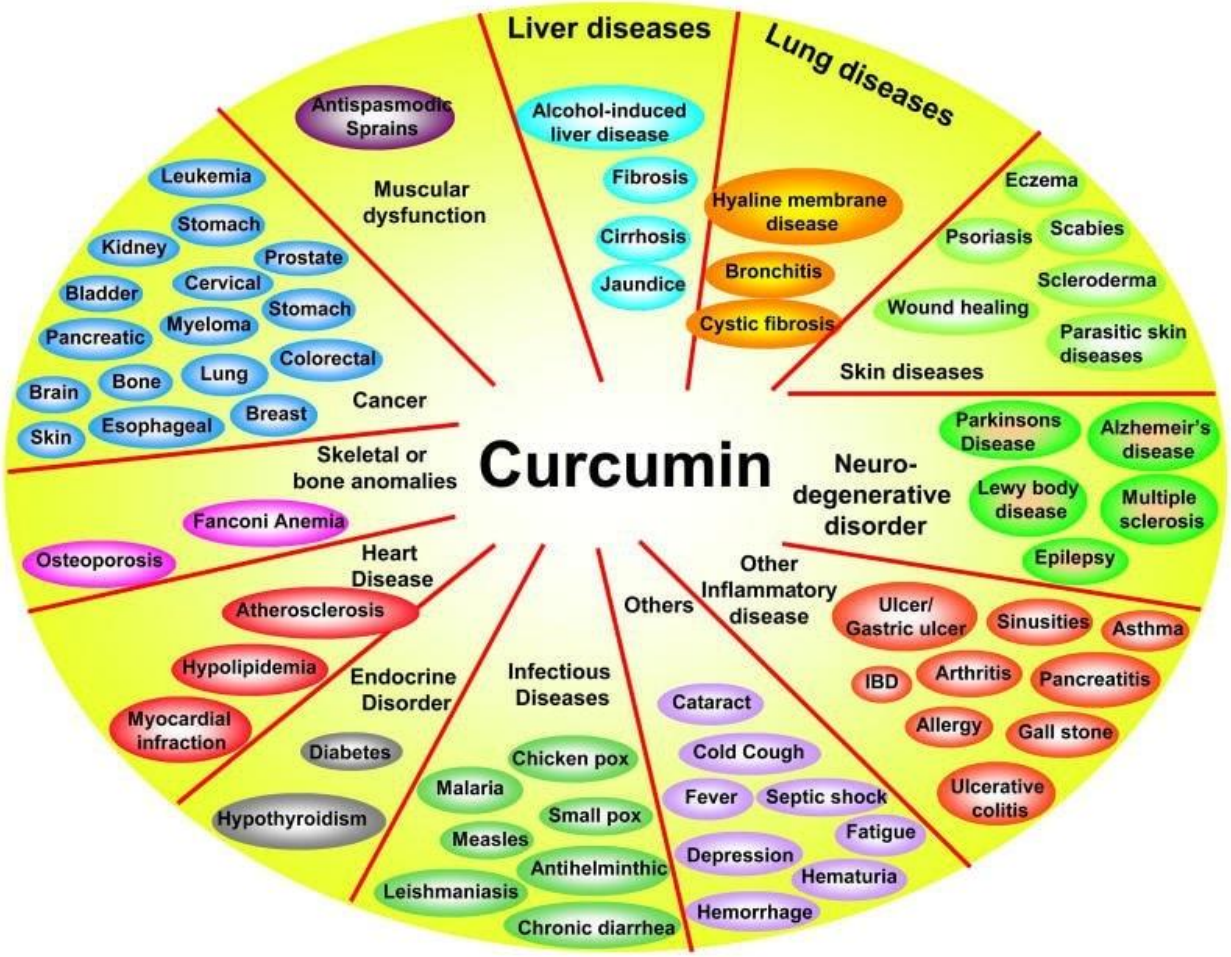


Figure 5: Medicinal use of *C.longa* ***(https://www.zetpilnutrition.com/trying-to-capitalize-on-the-power-of-curcumin/)***

2.3.2.1. Antioxidant effect of *C.longa*

The antioxidant activity of curcumin depends upon the presence of both the central methylene hydrogens and the phenolic hydrogen. It has a conjugated structure and shows a typical radical trapping ability as chain-breaking antioxidant properties. Generally, it has a dual effect in oxygen radical reactions, thus it can act as a scavenger of hydroxyl radicals or it catalyzes the formation of hydroxyl radicals (Pandey *et al.*, 2011).Curcumin and other curcumunoid have the ability to induce glutathione-*S*-transferase, inhibit free radical generation and act as free radical scavengers, inhibiting lipid peroxidation (Jagetia and Rajanikant, 2015). It is known to protect biological membranes against peroxidative damage. Peroxidation of lipids is known to be a free-radical-mediated chain reaction, leading to the damage of the cell membranes, and the inhibition

of peroxidation by curcumin is mainly attributed to the scavenging of the reactive free radicals involved in the peroxidation (Khater *et al.,* 2013). It also chelates with iron and disarms the oxidative properties. It inhibits the free radical damage to biomolecules both in vitro and in vivo conditions by prevention and intervention processes which makes it very unique natural antioxidant (Pandey *et al.,* 2011). In human hepatocyte L02 cell line study showed curcumin was able to avoid the ROS formation by increasing superoxide dismutase (SOD) activity and reduced glutathione levels after treatment with the antimicrobial feed additive quinocetone as a generator of free radicals (Pulido-Moran *et al.,* 2016). *C.longa* may stabilize the cell membrane and restore various blood variables (Sharma and Sharma, 2011).

2.3.2.2. Anti-inflammatory activity of *C.longa*

Curcumin was found to anti-inflammatory response. The natural anti-inflammatory activity of curcumin is competing with steroidal drugs and nonsteroidal drugs (Bagad *et al.,* 2013). Its anti-inflammatory property appears to be mediated through the inhibition of the induction of cyclo-oxygenase (COX-2), Lipoxygenase (LOX), Inducible nitric oxide synthase (iNOS). It also inhibits the production of cytokines such as interferon-gamma and TNF-α; activation of transcription factors like NF-κB, and AP-1 (Zhou *et al.,* 2011). The volatile oils and curcumin of *C.longa* exhibit potent anti-inflammatory effects. Oral administration of curcumin for acute and chronic inflammation was found to be as effective as cortisone or phenylbutazone and also reduced inflammatory swelling through inhibition of neutrophil aggregation associated with inflammation (Kumar and Sakhya, 2013).

.

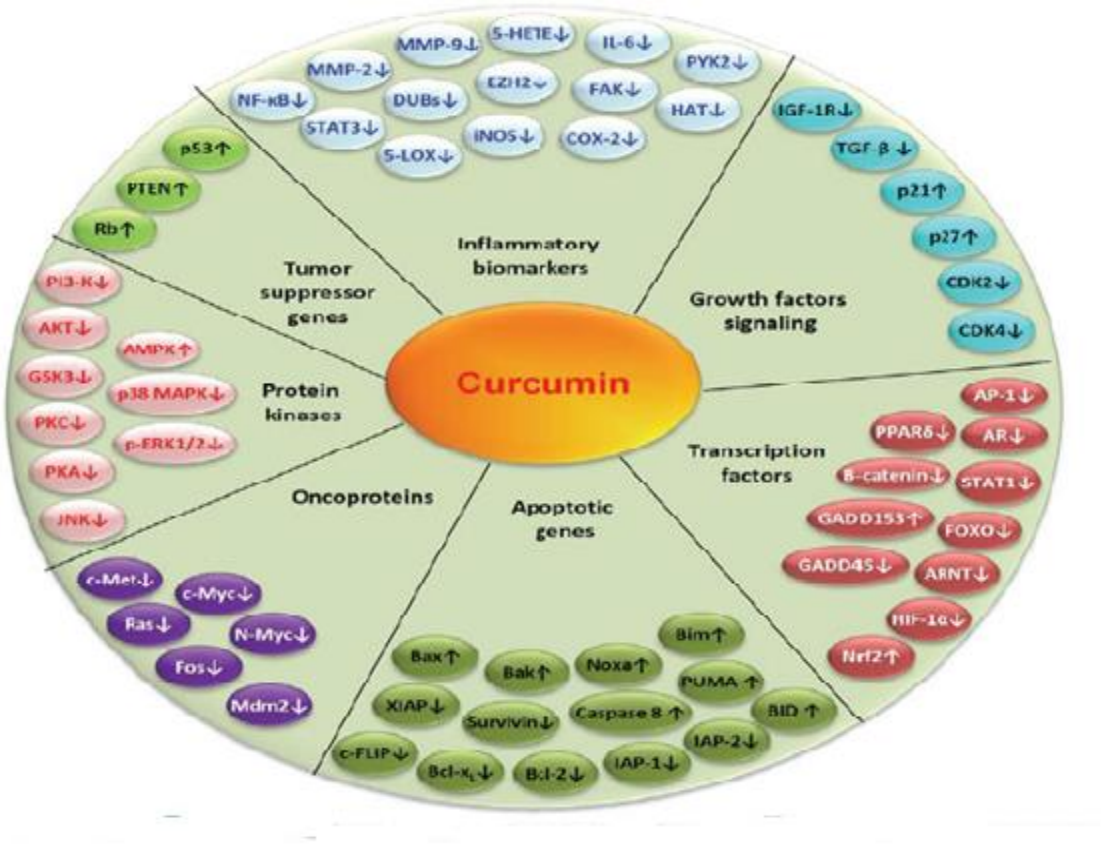


Figure 6: Molecular targets of curcumin, adapted from (Aggarwal*et al*., 2007)

2.3.2.3. Hepatoprotective effect of *C.longa*

Curcumin is known to protect the liver against toxic chemicals. There is evidence that curcumin enhances liver detoxification by increasing the activity of glutathione-S-transferase, an enzyme that conjugates glutathione with a wide variety of toxins to facilitate their removal from the body (Saifi *et al.*, 2015).Studies have shown its hepatoprotective effects in protecting animal livers from a variety of hepatotoxic insults, including carbon tetrachloride (CCL_4) (Deshpande *et al.*, 1998), galactosamine (Kiso *et al.*, 1983), acetaminophen (paracetamol) (Donatus *et al.*, 1990), and *Aspergillus* aflatoxin (Soni *et al.*, 1992). Its hepatoprotective effect is mainly a result of *C.longa's* antioxidant properties. In rats with CCL_4-induced acute and subacute liver injury, curcumin administration significantly decreased certain liver enzyme values, resulting in decreased liver injury in the test animals compared to controls (Park *et al.*, 2000). *C.longa* extract inhibited fungal aflatoxin production by 90 percent when given to ducklings infected with *Aspergillus parasiticus* at concentrations of 5-10 mg/mL.

C.longa and curcumin were also found to reverse biliary hyperplasia, fatty changes, and necrosis induced by aflatoxin production. Curcumin alone had no effect on aflatoxin production (Soni *et al.*, 1992).

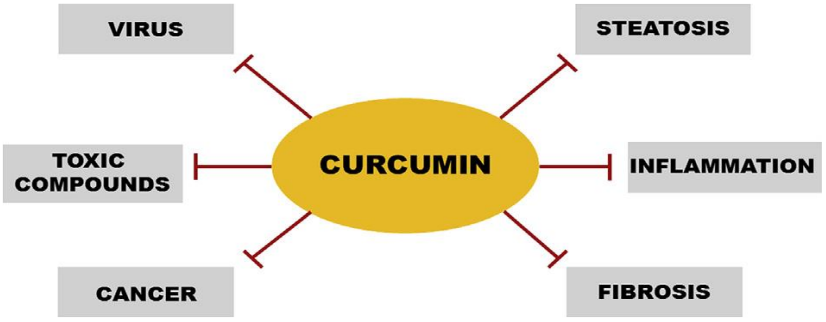


Figure 7: Some health benefits of curcumin on liver damage, adapted from (Reyes-Gordillo *et al.*, 2017). ⊣ arrow showing inhibition.

2.3.2.5. Hypolipidemic effect of *C.longa*

C.longa plays a crucial role in the reduction of lipid levels (Santoshkumar *et al.*, 2016). Earlier studies have found that *C.longa* is known to possess hypolipidemic activity (Ali Hussain, 2002). The hypolipidemic effect probably results from increased elimination of bile in the liver and also increased hepatic cholesterol 7α-hydroxylase activity (Babu and Srinivasan, 1995; Santoshkumar *et al.*, 2016). *C.longa* extract has significantly raised HDL levels both in healthy and diabetic rats and significantly reduced serum TC, TG, LDL levels (Santoshkumar *et al.*, 2016). For *C.longa*, early studies suggested that the main active component, curcumin, is responsible for the reduction of cholesterol in experimental rats (Hussein *et al.*, 2014).

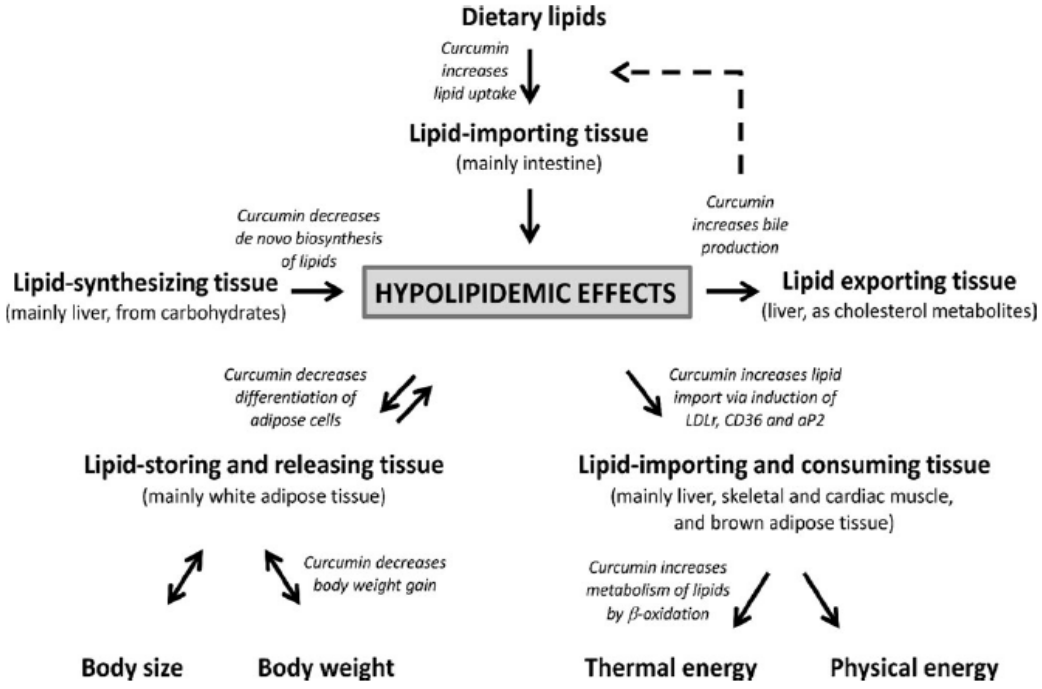


Figure 8: Molecular and cellular mechanisms of hypolipidemic effects of curcumin, adapted from *(Zingg et al., 2013)*

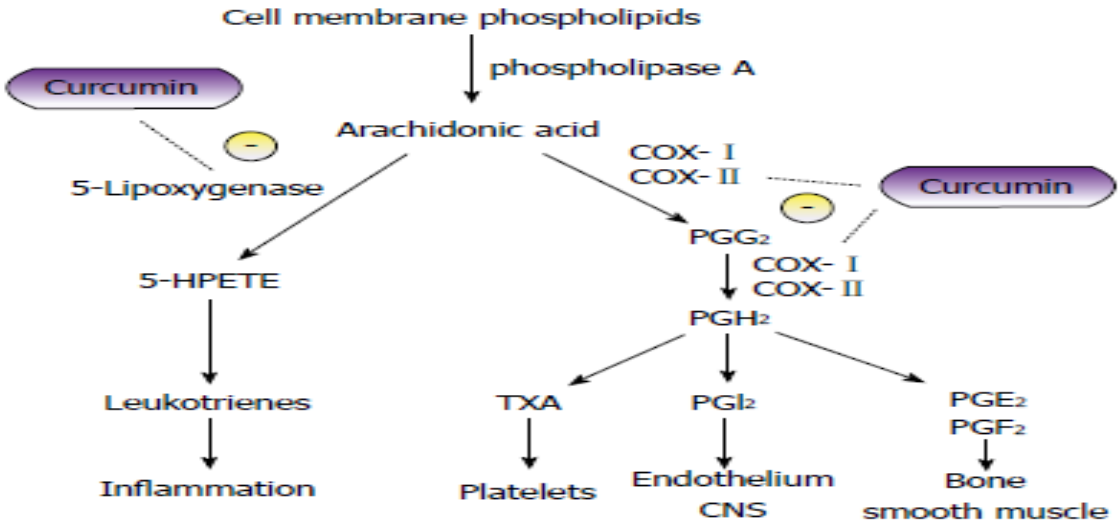


Figure 9: Curcumin and its potential inhibitory effects on the metabolic pathway of arachidonic acid, adapted from (Park and Conteas, 2010). COX: Cyclooxygenase; HPETE: hydroperoxyl eicosatetraenoic acid; PG: Prostaglandin; TXA: Thromboxane A; ⊖ indicating inhibition

2.3.2.6. *C.longa* and colorectal cancer

Several studies have demonstrated curcumin has the ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth (Nagpal and Sood, 2013). *C.longa* and curcumin are also capable of suppressing the activity of several common mutagens and carcinogens in a variety of cell types in both in vitro and in vivo studies(Lee *et al.*, 2013). The anti-carcinogenic properties of *C. longa* are due to increase glutathione levels, which induce hepatic detoxification of mutagens and carcinogens. Curcumin also induces apoptosis of cancer cells and it inhibits angiogenesis (Nasri *et al.*, 2014).

CRCs overexpress EGFR in > 90% of clinical patients, the literature indicates that EGFR is involved inactivating the JAK-STAT signaling pathway for the survival of Cancer stem cell (CSC). In fact, EGFR was reported to be responsible for maintaining the survival of cancer stem-like cells (CSLCs) in EGFR-positive cancer. Therefore, EGFR downstream proteins, such as STAT3play a crucial role in activating Wnt signaling in colon cancer (Cheng *et al.*, 2018). The oncogenic significance of activated STAT3 molecules is due to their effects on various parameters, such as apoptosis, cell proliferation, angiogenesis, and immune system evasion (Chakravarti *et al.*, 2006).Curcumin inhibits STAT3, which as it has already been mentioned plays key roles in cancer development and progression (Vallianou*et al.*, 2015). Experiments performed on human colon carcinoma cell (Lovo cell) growth has proved that Curcumin inhibits Lovo cells growth and induces apoptotic cell death in S and G2/M phases of the cell cycle(Chen *et al.*, 1999).Fifteen patients with advanced colorectal cancer were treated for up to 4 months with Curcuma extract at doses of 0.44 and 2.2 g/day, each containing 36–180 mg of curcumin. This dose did not induce any observed toxicity but resulted in a 59% decrease in lymphocytic glutathione S-transferase (GST) activity. A study was done by Sharma and his colleagues in the colorectal patients who treated with curcumin for two months confirmed the decline of cancer biomarkers after the treatment of curcumin (Sharma *et al.*, 2001). Curcumin doses between 0.45 and 3.6 g daily for up to 4 months in 15 patients with advanced colorectal cancer resulted in the dose-dependent inhibition of PGE2 production, a finding illustrating the efficacy of curcumin in the cure of colorectal cancer (Tomeh *et al.*, 2019).Curcumin prevents metastasis by decreasing platelet and leukocyte adhesion (Vachharajani *et al.*, 2010).

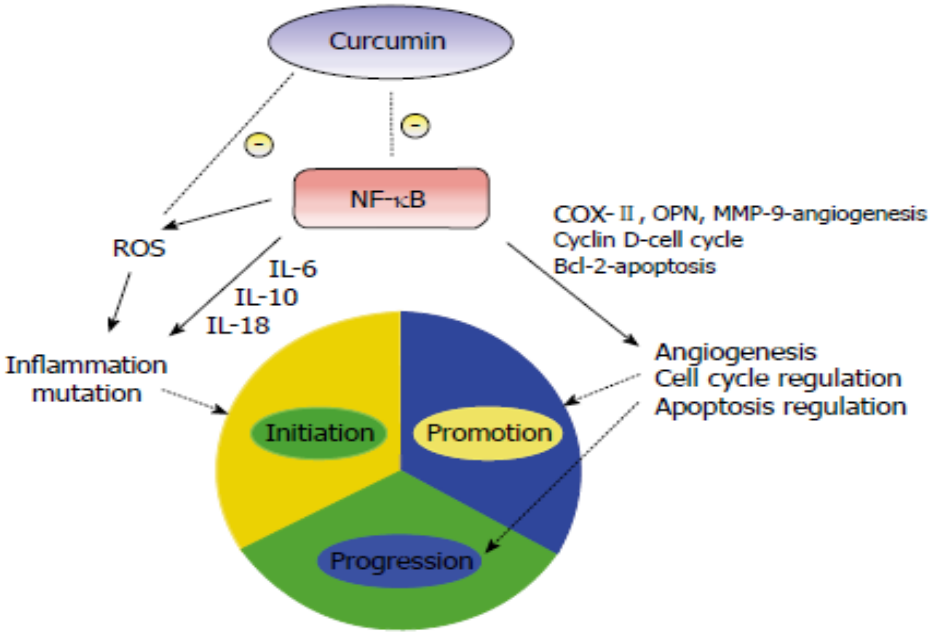


Figure 10: Curcumin and its effects on the three stages of carcinogenesis, adapted from (Park and Conteas, 2010). COX: Cyclooxygenase; IL: Interleukin; MMP: Matrix metalloproteinase; NF-κB: Nuclear factor-B; OPN: Osteopontin; ROS: Reactive oxygen species.

3. OBJECTIVE OF THE STUDY

3.1. General objective

- ✓ To assess the effect of Hydroethanolic extract of *Curcuma longa* rhizome on colon, liver and Hematological parameters on a model of 1,2- Dimethyl hydrazine induced colorectal cancer male Wistar albino rats

3.2. Specific objectives

- ✓ To find out phytochemical component and assessment of in vitro antioxidant activities of HEECLR
- ✓ To assess RBC, WBC and platelet indices altered by DMH induced colorectal cancer and alleviation potential of HEECLR
- ✓ To find out the alleviation potential of HEECLR on liver enzymes and histopathological changes of liver in colorectal cancer
- ✓ To examine histopathological variations of colon cancer and restoration potential of HEECLR
- ✓ To assess the chemo-preventive effect of HEECLR
- ✓ To find out the correlation between triglyceride and hematological and liver enzymes parameters

4. MATERIALS AND METHODS

4.1. Study area and period

The study was conducted at Department of Biochemistry, School of Medicine (SOM), College of Health Sciences (CHS), and Addis Ababa University (AAU) from September 2018 to May 2019.

4.2. Study design

Randomized experimental study

4.3. Study animals

Eighty male Wistar albino rats weighing 150-180 grams were obtained from the Department of Pharmacology, School of Pharmacy and the rats were placed in the animal house of the Department of Biochemistry, SOM, CHS, AAU. Acclimatized with rat pellet diet (obtained from Kality Animal Nutrition Production Ltd., Addis Ababa, Ethiopia) and water provided *ad libitum* for two weeks. The room was well- ventilated and maintained at room temperature and natural 12/12hr light and dark period. The animals were kept 8 rats in polypropylene cages (size of 59 cm x37 cm and depth of 20 cm) with a wire mesh top and a hygienic bed of husk (regularly changed every week). Cages were labeled and specific code was given (on the tail of rats) for all rats.

4.4. Chemicals and Solvents

1,2-Dimethylhydrazine Dihydrochloride ($C_2H_8N_2.2HCl$) (TCI, India.),Sodium hydroxide (NaOH) (Unichem Ltd., Lithuania.), Sodium chloride (NaCl) (MedSol pharmaceuticals, Ethiopia.), Ethanol (C_2H_5OH) (Research-Lab fine chem. Industries, India),Whatman filter paper no.2 (What man International Ltd., England.), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Sigma Aldrich, India.), Methanol (CH_30H) (Research-Lab fine chem. Industries, India.), Sodium Carboxy methyl cellulose (Unichem Ltd., Lithuania.), Ascorbic acid (BDH chemical Ltd., UAE), Hydrochloric acid (HCl) (Unichem. Ltd., Lithuania.), Iodine (Pharmachemic Trading Agency Co. Ltd, Malta), Potassium iodide (Pharmachemic Trading Agency Co. Ltd, Malta), Chloroform (Research-Lab fine chem.Industries, India), Sulfuric acid (Unichem Ltd.,Lithuania), Ferric chloride (Research-Lab fine chem. Industries, India), Formaline (Research-Lab fine chem. Industries, India).

4.5. Plant material

C.longa rhizome was collected from the farm area of Mizan Tepi. Tepi is located in southwestern Ethiopia in Sheka zone of Southern Nations and Nationalities' Peoples Regional State. It is located 611 Km away from Addis Ababa at Latitude of 7°10' 54.5" N and Longitude 35° 25.04' 28.2" E and Altitude of 1200 meters above sea level. The site receives mean annual rainfall of 1688 mm with maximum and minimum temperatures of 29.5°C and 15.3°C, respectively (Gebreazgaabher and Negash, 2015). The plant was kept in a plastic bag and transported to Addis Ababa within a day of collection. The Sample was pressed, identified and authenticated at National Herbarium, Department of Plant Biology and Biodiversity, AAU using Ethiopian flora volumes. It was identified as "*Curcuma domestica* Valeton'' as the family of "Zingiberaceae''with voucher numbered G-001 and deposited at National Herbarium, Department of Plant Biology and Biodiversity, AAU.

4.6. Extraction of plant material

The rhizomes of *C. longa* were washed and crushed using mortar and pestle. Then dried under shaded area for two weeks and powdered, and stored in a tight glass container for further use. 200 g of *C.longa* powder was macerated in 70% ethanol (1:8 ratio) and the solution was shaken for 24 hours under shaker with a speed of 150 rpm.Then the solution was filtered with nylon cloth followed by filter paper (Whatman no.1). Then the solvent was allowed to evaporate using a rotary evaporator at 45^0C and the remaining water was removed by lyophilization. The final product was stored at room temperature until use (Shakeri *et al.*, 2017).The above procedure was repeated until enough amount of extract obtained from 5000 grams. The plant extraction process was done in the Department of Biochemistry laboratory, SOM, CHS, AAU.

Rhizomes

Washed with tap water

Dehydration of extract

Evaporate solvent under rotatory evaporator

Grounded and leave under the shaded area

Filtration

Maceration under 70% ethanol

Powder form

Figure 11: Summary for the extraction process

4.7. Qualitative phytochemical analysis

The possible presence of phytochemicals in crude 70% ethanoic extract of rhizomes of *C. longa* was screened qualitatively.

4.7.1. Test for Saponins (Frothing Test)

5mL of HEECLR was mixed with 20mL of distilled water and then vigorously shaken in a test-tube and left to stand. Foaming (frothing) was observed which lasted for 15 minutes. This shows the presence of saponins (Mujeeb *et al.*, 2014).

4.7.2. Test for Tannins

5 mL of the extract was added to 2.0 ml of 1% HCl. Deposition of a red precipitate shows the presence of tannin (Usman *et al.*, 2009).

4.7.3. Test for Flavonoids

1 mL HEECLR treated with dilute ammonia (10%). The appearance of yellow color after adding ammonia solution and disappearance of yellow color after adding concentrated sulfuric acid was taken as positive result (Vimalkumar *et al.*, 2014).

4.7.4. Test for Alkaloids (Wagner's test)

2 mg HEECLR was acidified with 1.5 % 0f hydrochloric acid and few drops of Wagner s reagent (iodine in potassium iodide solution) were added and formation of brown/reddish brown precipitate indicates the presence of alkaloids (Anyasor *et al.*, 2014).

4.7.5. Test for Phenols (Ferric chloride test)

The extract (1 ml) was dissolved in 2.0 ml of chloroform in a test tube, and then 1 ml of conc. H_2SO_4 was added. Formation of reddish brown colour at the inter - phase confirms the presence of steroid (Usman *et al.*, 2009).

4.7.6. Test for steroids (Keller- Killiani test)

To 0.5 mL of the test solution, 2 mL of 3.5% $FeCl_3$, a small amount of glacial acetic acid and 2 mL of concentrated H_2SO_4 was added. The appearance of the reddish-brown ring at the interface is a positive indication for the presence of steroids (Sheel *et al.*, 2014).

4.8. DPPH Assay for testing the Antioxidant Activity of HEECLR

4.8.1. Qualitative assay for anti-oxidant property

Qualitative assay for the anti-oxidant property of HEECLR was based on the procedure of Soler-Rivas *et al*. with modifications. Different concentrations of the extract, 250 mg/100mL, 500 mg/100mL, and 1000 mg/100mL were taken in separate test tubes. 50 mL of 0.1 mM DPPH solution was prepared in a petri dish for easy immersion. 3µLof the sample from different concentrations was carefully loaded into 3 cmx4 cm size thin layer chromatography (TLC)plate and allowed to dry. The dry TLC plate was placed upside down for 10 seconds in a 0.1 mM DPPH solution and was dried by air. TLC layers gave a purple background with yellow spots at the location of those drops which showed radical scavenging activity. The intensity of the yellow color is subjected to the amount and nature of radical scavengers present in the sample (Soler-Rivas *et al*., 2000).

4.8.2. Quantitative assay for Anti-oxidant property

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical with a maximum absorbance at 517 nm. It can be readily scavenged by an antioxidant and gets converted into 1,1-diphenyl-2-picrylhydrazine. Different concentrations of the extracts were taken in separate test tubes. The volume was adjusted to 100 µl with methanol. Five mL of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand for 20 min at room temperature. The control was prepared as above without extract, and methanol was used for the volume correction. Changes in the absorbance of the samples were measured at 517 nm using a spectrophotometer (Jenway, Bibby Scientific Ltd, UK.). Radical scavenging activity was expressed as the inhibition concentration (IC_{50}), i.e., the concentration of extract necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) under the specified experimental condition(Loganayaki *et al*., 2013).

$$\text{DPPH Activity } \% = \frac{A\ control - A\ sample}{A\ control} * 100$$

Where; A is absorbance.

4.9. Acute oral toxicity study

Acute toxicity study was done according to the Organization for Economic Co-operation Development (OECD) guideline for testing of chemicals. Female rats were placed in two groups (each containing five rats). The acute oral toxicity of hydroethanolic extract of *C.longa* rhizome (HEECLR) was carried out in two different doses (2,000 mg/kg and 5,000 mg/kg). The extract was dissolved in 0.5 mL 2% carboxymethyl cellulose (CMC) and administered orally with oral gavage. The rats were observed for abnormalities including changes in the skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity, and behavior pattern. Such observations include tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The rats were examined for the first 24 hours followed by 14 days.

4.10. Preparation of 1,2-Dimethylhydrazine dihydrochloride

1,2-Dimethylhydrazine dihydrochloride (DMH) was dissolved in normal saline solution (0.9%NaCl); the pH was adjusted to 7.0 -7.5 with 1 M NaOH. It was prepared every week freshly. Before and after usage DMH powder was stored at 4^0c (Bekusova *et al.*, 2017). The maximum DMH that could be administered to the rats was 20mg/kg. The maximum saline volume was 2 mL/kg.

4.11. Experimental design

All rats except normal control group were given intraperitoneal injections of DMH to induce cancer in a dose of 20 mg/kg body weight once a week for 15 consecutive weeks(Youssef*et al.*, 2018). Animals in the normal control group were given an intraperitoneal injection of normal saline in a dose of 2mL/kg. After the cancer induction period, the HEECLR treatment period was carried out for eight weeks. The extract was orally administered at three different doses (250, 500 and 1000) mg/kg b.w daily. The extract was dissolved in 0.5mLof 2% Carboxymethylcellulose (CMC) (Ung *et al.*, 2010). The normal control group was given 0.5 mL of 2% CMC with oral gavage. DMH along with HEECLR (Co-treatment) group was given20mg DMH/kg body weight and 500mg/kg HEECLR at the same time for 15 weeks.0.5 mL of 2% CMC administration was given for the remaining 8 weeks.

The experimental animals were grouped as follows:

Group-I: Control group (Normal saline 2 mL/kg for 15 weeks + 0.5 mL 2 % CMC for 8 weeks)

Group-II: Cancer group (DMH 20 mg/kg for 15 weeks + 0.5 mL 2 % CMC for 8 weeks)

Group-III: DMH 20 mg/kg for 15 weeks + HEECLR 250 mg /kg for 8 weeks

Group-IV: DMH 20 mg/kg for 15 weeks + HEECLR 500 mg /kg for 8 weeks

Group-V: DMH 20 mg/kg for 15 weeks + HEECLR 1000 mg /kg for 8 weeks

Group-VI: Co-treatment group (20 mg/kg DMH with 500 mg/kg HEECLR for 15 weeks+ 0.5mL of 2% CMC administration for 8 weeks)

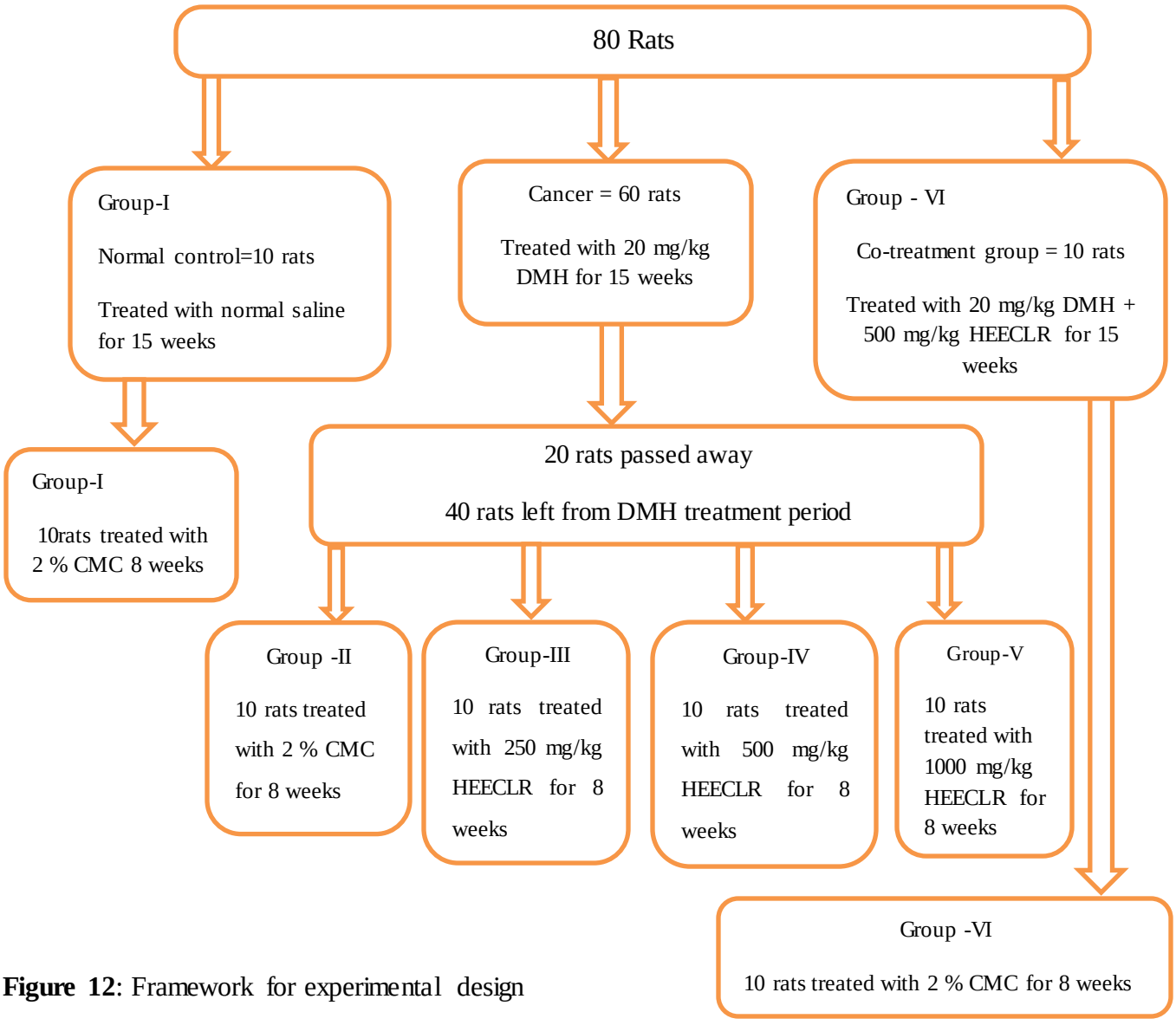


Figure 12: Framework for experimental design

4.12. Sample collection

23 weeks after the start of the experiment, the rats were fasted overnight and anesthetized using diethyl ether and 3mL of blood was drawn with a syringe by cardiac puncture. For complete blood count,1mL of blood was transferred to ethylene diamine tetraacetic acid (EDTA) coated tube and the remaining 2mL was transferred to the serum separator tube (SST) for liver enzyme and triglyceride analysis. The sample in EDTA coated tube was thoroughly mixed and kept in the test tube holder in an upright position and placed in an ice-box. The samples collected in SST were left in an upright position for 30 min to allow for the formation of clot and then subjected to centrifugation (5 min, 3000rpm); the serum is then transferred to a labeled Nunc tube and stored at -20^{o}c until analysis., after blood sample collection the rats were sacrificed by cervical dislocation and dissection was made and colon and liver were collected.

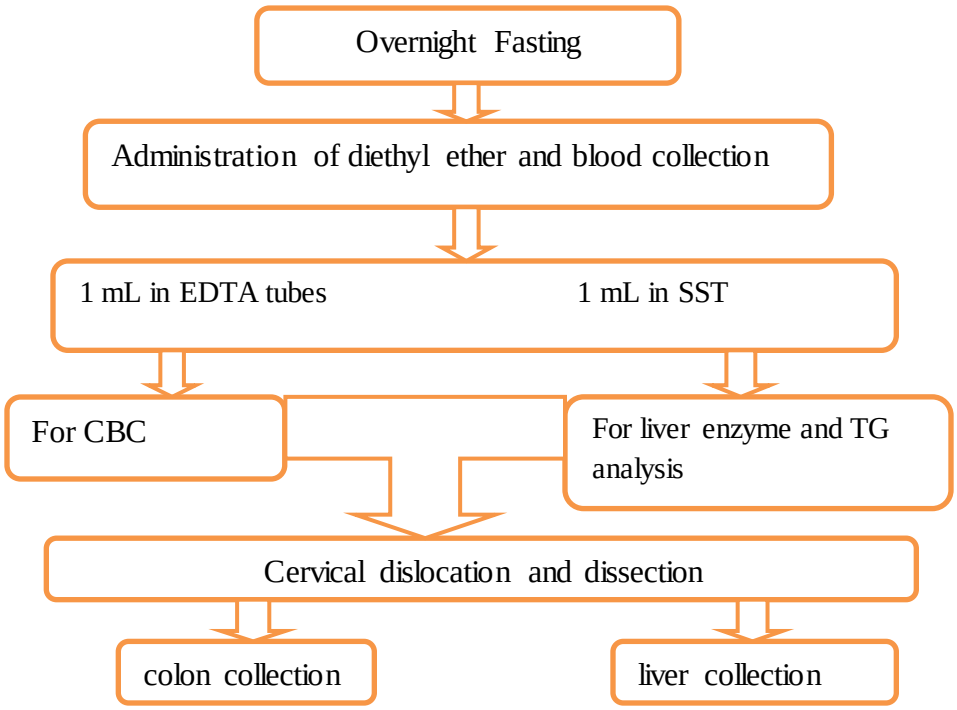


Figure 1: Sample collection framework. EDTA: Ethylene diamine tetraacetic acid; SST: Serum separator tube; TG: Triglyceride

4.13. Sample analysis

4.13.1. Complete blood count

Complete blood count was performed by an automated hematology analyzer (Sysmex U.S.A), in hematology and immunohematology reference laboratory at Ethiopian Public Health Institute (EPHI).

Calculation of NLR, MLR, LPR, NHR, LHR, MHR

- Neutrophil to lymphocyte ratio **(NLR)**: calculated by dividing neutrophil count by the absolute lymphocyte count

  NLR =Neutrophil count /Lymphocyte count
- Monocyte to lymphocyte ratio **(MLR)**: calculated by dividing monocyte count by the absolute lymphocyte count

  MLR = Monocyte count / Lymphocyte count
- Lymphocyte to platelet ratio **(LPR)**: calculated by dividing the lymphocyte count by platelet count

  LPR = Lymphocyte count / Platelet count
- Neutrophil to hemoglobin ratio **(NHR)**: calculated by dividing the neutrophil count by hemoglobin

  NHR = Neutrophil count / Hemoglobin
- Lymphocyte to hemoglobin ratio **(LHR)**: calculated by dividing lymphocyte count by hemoglobin

  LHR = Lymphocyte count / Hemoglobin
- Monocyte to hemoglobin ratio **(MHR)**: calculated by dividing monocyte count by hemoglobin

  MHR = Absolute monocyte count / Hemoglobin

4.13.2. Analysis of serum triacylglycerol and liver enzymes

Biochemical parameters measured were triglyceride, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP).All biochemical analyses were carried out by COBAS 6000(Roche Diagnostics GmbH, Mannheim, Germany) clinical chemistry analyzer in National Reference Laboratory for Clinical Chemistry, at EPHI.

4.12.2.1. Test principle for ALP

At alkaline pH of 10.3, ALP present in the sample catalyzes the hydrolysis of the colorless organic phosphate ester, p-Nitro phenyl phosphate, to the yellow-colored product p-Nitro phenol. The intensity of the yellow color is directly proportional to the ALP activity present in the sample. The absorbance was measured at 410 nm. ALP activity was calculated as:

Conversion factor: U/L x 0.0167 = µkat/L

p -nitrophenyl phosphate + H_2O $\xrightarrow{\text{ALP}}$ phosphate + para -nitrophenol

4.12.2.2. Test principle for ALT

ALT present in the sample catalyzes the reaction between L- alanine, and 2-oxoglutarateforming L-glutamate and pyruvate. NADH reduces the pyruvate formed in a reaction catalyzed by lactate dehydrogenase (LDH) to form L- Lactate and NAD+. The ALT activity was determined by assaying the rate of NADH oxidation, which is proportional to the reduction in absorbance at 340 nm over time. The rate of the NADH oxidation is directly proportional to the catalytic activity of ALT.

Conversion factor: U/L x 0.0167 = µkat/L

L-Alanine + 2-oxoglutarate $\xrightarrow{\text{ALT}}$ L-Glutamate +pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ L-Lactate + NAD^+

4.12.2.3. Test principle for AST

AST present in the sample catalyzes the transfer of an amino group from L- aspartate to 2-oxoglutarate to form oxaloacetate and L- glutamate. In the presence of malate dehydrogenase (MDH), the oxaloacetate is reduced by NADH, to form malate and NAD+. The rate of NADH oxidation is directly proportional to the catalytic activity of AST. It was determined by measuring the decrease in absorbance at 340 nm over time.

Conversion factor: U/L x 0.0167 = µkat/L

L-Aspartate + 2-oxoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ L-malate + NAD^+

4.12.2.4. Test principle for triacylglycerol

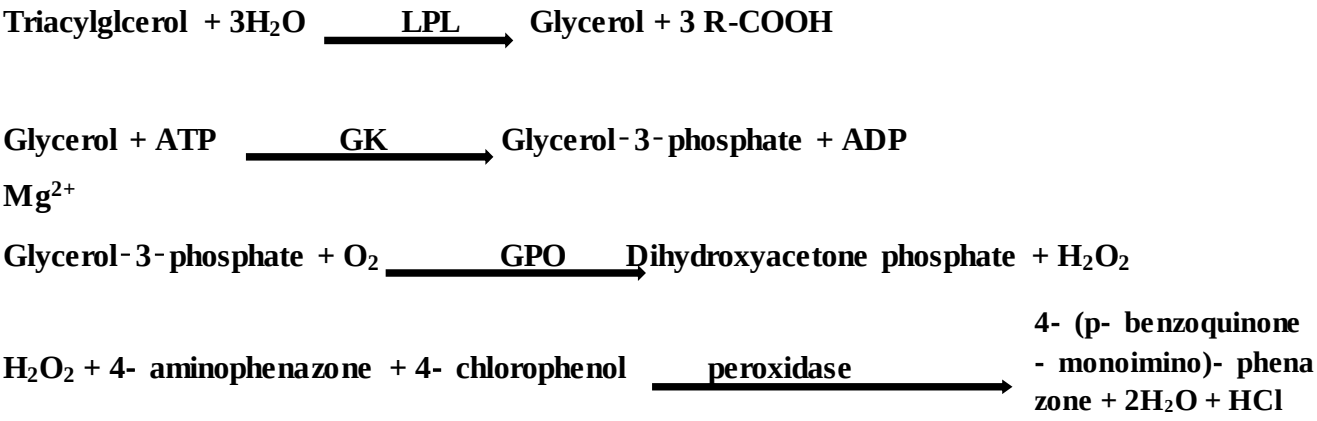
Triacylglycerol present in the sample is hydrolyzed by lipoprotein lipase (LPL) to glycerol and free fatty acids. In the presence of ATP, the glycerol is phosphorylated by glycerol kinase to form glycerol-3-phosphate. In the presence of oxygen, glycerol-3-phosphate is oxidized by glycerol phosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red-colored compound. The intensity of the red color is directly proportional to the concentration of triglyceride present in the sample. The absorbance was measured at 700/705nm.

Roche/Hitachi Cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors:

mmol/L x 88.5 = mg/dL **mg/dL x 0.0113 = mmol/L**

mmol/L x 0.885 = g/L **mg/dL x 0.01 = g/L**



4.13.3. Histopathological examination of colon and liver

The colon was excised, flushed with saline, cut open longitudinally along the main axis, and then again washed with saline. The inner surface was examined for visible macroscopic lesions. Both colon and liver Samples collected under labeled cassettes. Slide preparation and reading were done in the Department of Pathology, SOM, CHS, AAU. Slide picture was taken by a binocular microscope fitted with a camera (Leica ICC50 HD, UK).

4.13.3.1. Method of processing

1. Fixation: - The tissue was preserved/fixed in 10% formalin solution

2. Dehydration: - The preserved tissue was washed in running tap water for 4-6 minutes. Then, they passed through upgraded alcohol as follows: - 70% alcohol – 1hour, 85 % alcohol – 1hour, 96% alcohol – 1hour, Absolute alcohol I– 1hour, Absolute alcohol II – 1hour.

3. Clearing: - Clearing of tissue was done in xylene-1hour in xylene -I, then after in xylene II for 1 hour.

4. Infiltration: - Tissue was infiltrated with paraffin wax I, for 1and 1/2 hrs., paraffin wax II for 2 and 1/2 hrs. and paraffin wax III for overnight.

5. Embedding: - The cleared tissue was put in molten wax (melting point 56-58degree Celsius) for 12 hours in a cryostat. The paraffin blocks of tissue were made with the help of embedding cassettes.

6. Sectioning: - The serial paraffin sections of 5-micron thickness was cut by rotator microtome and floated in a water bath having temperature 45-50 degree Celsius. The section was made spread on the slide smeared with the adhesive solution (a mixture of equal 55 amount of glycerol and egg albumin). The slide was dried on a hot plate with having a temperature of 50 degrees Celsius.

7. Deparaffinization of sections: - The slide was put in xylene II, changes each for 5-10 min in order to remove the extracellular and intracellular wax.

8. Rehydration: - The slide was put in descending grades of alcohol i.e. absolute, 90 %, 70 % and 50% alcohol for 2 min for each. The slide was then washed in running tap water for 2 minutes and then taken for routine H & E staining.

Method of Staining

The slide was stained with Hematoxylin for 10 minutes. Then it washed in running tap water until the section becomes blue. Again, it stained in 1% eosin for 7- 10 min and washed in running tap water for 5 minutes. The slide was dehydrated through 70 % and 95% Alcohol for 3 minutes, then absolute alcohol I and absolute alcohol II for 1 and 1/2 hour. Finally, it cleaned by Xylene I and XyleneII for 5 minutes.

9. Mounted – By Dibutyl phthalate in xylene (DPX)

4.14. Ethical considerations

The study was carried out after obtaining ethical approval with reference number: Ref. No. SOM/BCHM/153/2010, protocol number: M.Sc.08/18 and meeting number DRERC 08/18 from the Department research ethics and review committee, SOM, CHS, AAU. All the animals' procedures were performed in accordance with the standard guidelines for care and use of laboratory animals.

4.15. Statistical analysis

All statistical analyses were performed using SPSS (version 21 for Windows, SPSS, IBM, and Chicago, IL, USA).The results were expressed as mean ± standard deviation (M ±SD) for each group. Statistical differences between the cancer group (G-II) with the remaining five groups (G-I, III, IV, V, VI) and also G-VI compared with G-I were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Statistical significance was accepted when $P<0.001$, $P<0.01$ and $P<0.05$.The relationship between variables was examined with Pearson's correlation test at $P<0.01$.

5. RESULT

5.1. The percentage yield of Hydroethanolic extract of *C.longa* rhizome (HEECLR)

The 5000gram of *C.longa* powder yields1200 gram of crude extract. The percentage yield of extract was given as:

$$\boldsymbol{HEECLR\ yield}\ \% = \frac{\boldsymbol{HEECLR\ (g)}}{\boldsymbol{Turmeric\ powder\ (g)}} * \mathbf{100} = \frac{\mathbf{1200\ g}}{\mathbf{5000\ g}} * \mathbf{100 = 24\%}$$

Therefore, the percentage yield of HEECLR was 24%.

5.2. Qualitative phytochemicals screening test of HEECLR

The phytochemical screening of HEECLR extract showed the presence of the following secondary metabolites as indicated in (Table 2).

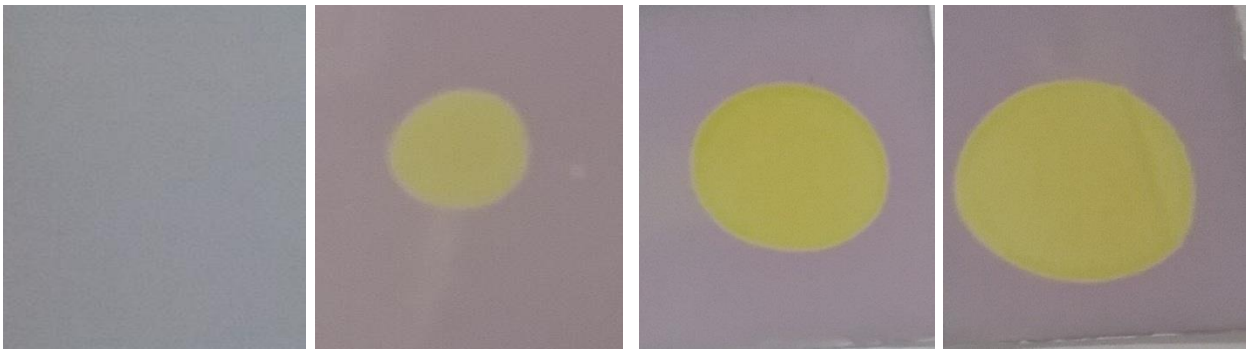
Table 2: Qualitative phytochemicals screening test of HEECLR

Phytochemical	**HEECLR**
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Phenols	+
Steroids	+

5.3 DPPH Assay for testing Antioxidant Activity of HEECLR

5.3.1. Qualitative assay for detection of the presence of anti-oxidant activity of HEECLR by TLC plate

Figure 13- shows qualitative assay for detection of the presence of anti-oxidant activity of HEECLR by TLC. The result indicated that antioxidant activity (zone of inhibition) increases when the concentration of extract increases. This showed that the zone of inhibition is directly proportional to the concentration of extract in the strong oxidizing agent of the DPPH methanol solution.



Control **250mg/100mL** **500mg/100mL** **1000mg/100mL**

Figure 2: Qualitative assay for detection of the presence of anti-oxidant activity of HEECLR by TLC plate

5.3.2. Quantitative assay for the anti-oxidant property of HEECLR by using UV-Visible spectrometer

Figure14 indicates a quantitative assay for the anti-oxidant property of HEECLR by using a UV-Visible spectrometer. The IC_{50} of HEECLR was found to be166.4µg/mL and the Ascorbic acid was 97.06µg/mL. The result showed that the HEECLR has anti-oxidant activity.

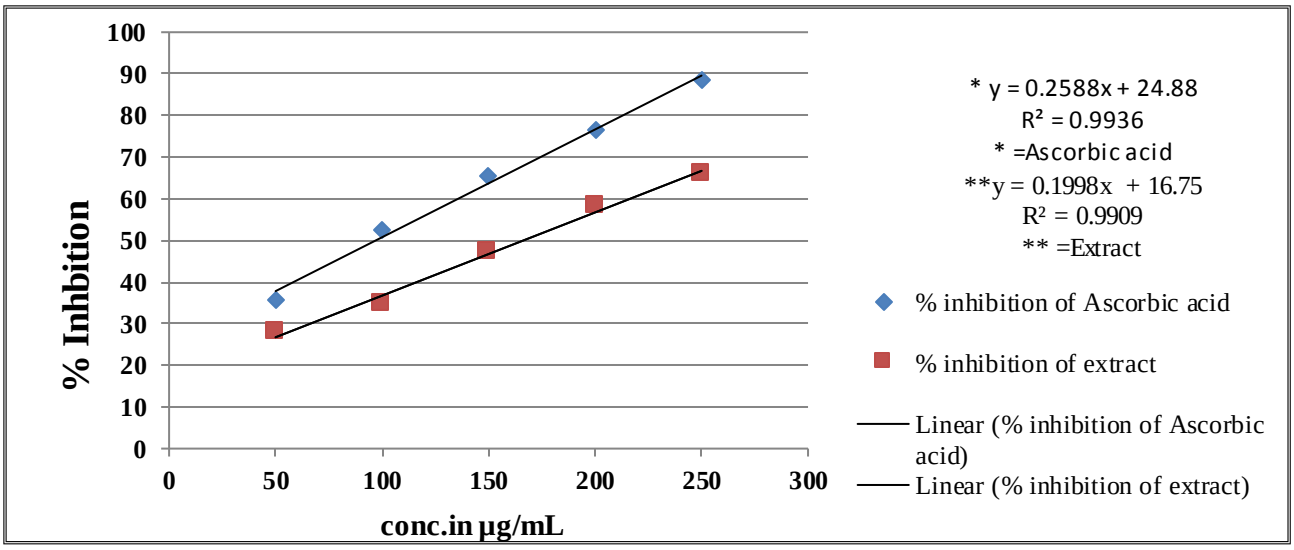


Figure 15*:* Percentage inhibition of ascorbic acid and extract at different concentration

5.4. Acute oral toxicity study

The animals in both doses (2000 and 5000) mg/kg did not display the change in behavior (tremors, salivation, and coma), skin effects like hair loss, breathing, and impairment in food intake and water consumption, postural abnormalities. No toxic symptoms or mortality was observed in rats. The result suggests that the extract has a medial lethal dose (LD_{50}) of greater than 5000mg/kg.

5.5. Death of rats per group

Data on Table 3 shows the death of rats during 8 weeks treatment period of the extract. It was seen in all the groups except the control group (G-I). Hence, we selected uniformly six rats for each group for sample analysis.

Table 3: Mortality of rats per groups

Group (G)No.	Number of rats per groups	Death of rats per group
G-I	10 rats	-
G-II	10 rats	4 rats
G-III	10 rats	3 rats
G-IV	10 rats	1 rat
G-V	10 rats	2 rats
G-VI	10 rats	2 rats

5.6. Effect of HEECLR on Hematological profile

5.6.1. Effect of HEECLR on white blood cells and its differentials

The total number of white blood cell, neutrophil, and monocyte number were found to be increased significantly ($p<0.01$) in cancer group (Group II), whereas HEECLR treatment bring down the elevated WBC, neutrophil and monocyte number in dose-dependent manner (250 mg/kg, 500 mg/kg, and 1000mg/kg). The lymphocyte number was observed to be decreased significantly ($p<0.01$) in the cancer group (Group II). It was significantly ($p<0.01$)elevated in doses (250, 500 & 1000)mg/kg of extract treated groups (Group III-V respectively).No significant difference was seen on WBC, neutrophil and monocyte number in HEECLR along with DMH administer group (Co-treatment group-VI) compared with the normal control group (G-I). There is a significant ($p<0.01$) difference in lymphocyte count between G-VI and G-II. Summarized results for effects of HEECLR on the total number of white blood cells, neutrophil, lymphocyte, and monocyte are shown in Table 4 expressed as M ±SD.

Table 4: Effect of HEECLR on the total number of white blood cells, neutrophil, lymphocyte, and monocyte.

Group (G) No.	Results (M ±SD)			
	WBC (10^3/µL)	**Neutrophil (10^3/µL)**	**Lymphocyte (10^3/µL)**	**Monocyte (10^3/µL)**
G-I	$7.67 \pm 0.16^{**b}$	$4.68 \pm 0.2^{**b}$	$2.59 \pm 0.09^{**b}$	$0.24 \pm 0.027^{**b}$
G-II	$16.26 \pm 0.37^{**a}$	$14.29 \pm 0.38^{**a}$	$1.36 \pm 0.038^{**a}$	$0.45 \pm 0.033^{**a}$
G-III	$12.55 \pm 1.13^{**b}$	$9.8 \pm 0.95^{**b}$	$2.21 \pm 0.21^{**b}$	$0.38 \pm 0.025^{**b}$
G-IV	$9.16 \pm 0.59^{**b}$	$6.51 \pm 0.71^{**b}$	$2.22 \pm 0.14^{**b}$	$0.27 \pm 0.021^{**b}$
G-V	$9.86 \pm 0.26^{**b}$	$7.06 \pm 0.35^{**b}$	$2.26 \pm 0.18^{**b}$	$0.38 \pm 0.02^{**b}$
G-VI	8.64 ± 0.67^{c}	6.02 ± 0.62^{d}	$2.17 \pm 0.097^{**b}$	0.3 ± 0.05^{c}

a**-mean difference is significant compared with Group- I at P. value < 0.01

b**-mean difference is significant compared with Group- II at P. value < 0.01

c -no significant mean difference compared with Group- I at P. value < 0.01

d-no significant mean difference compared with Group- I at P. value < 0.001

5.6.2. Effect of HEECLR on Red blood cell and its indices

No statistically significant difference was noticed in the levels of MCV, MCH & MCHC between the groups. In the cancer group (Group II) RBC, hemoglobin, and hematocrit significantly ($p<0.001$) decreased while RDW-SD and RDW-CV increased significantly ($p<0.001$). The dose of 500mg and 1000mg/kg HEECLR (Group IV and V)restore RBC, hemoglobin, and hematocrit significantly ($p<0.001$).However, we found the only numerical difference but no statically significant difference was observed in RBC, Hgb, Hct, RDW-SD, and RDW-CV in 250mg/kg dose of HEECLR treated group (Group-III).No Significant ($p<0.05$) difference was seen in RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW-SD, and RDW-CV between the co-treatment group (Group-VI) and Group-I.

Table 5: Effect of HEECLR on RBC, Hgb, Hct, MCV, MCH, MCHC, RDW-SD and RDW-CV

	Results (M ±SD)					
	Group (G) No.					
	G-I	**G-II**	**G-III**	**G-IV**	**G-V**	**G-VI**
RBC (10^6/µL)	9.32±0.09^{*b}	7.31±0.58^{*a}	7.32±0.54	9.03±0.43^{*b}	9.18±0.15^{*b}	8.89±0.49^{d}
Hgb (g/dL)	17.35±0.39^{*b}	13.74±0.89^{*a}	14.12±1.75	16.61±0.74^{*b}	17.08±0.58^{*b}	16.73±0.49^{d}
Hct (%)	50.02±2.34^{*b}	41.76±1.84^{*a}	41.91±5.38	49.99±2.52^{*b}	49.83±2.54^{*b}	49.01±1.54^{d}
MCV (fl)	53.69±2.91	58.71±2.55	57.88±5.38	55.38±2.69	54.25±2.77	55.19±2.20^{d}
MCH (pg)	18.48±0.55	19.45±0.89	18.61±0.78	18.47±0.64	18.49±0.70	18.83±0.61^{d}
MCHC (g/dL)	34.32±1.12	33.22±0.48	33.26±0.91	33.52±1.00	33.89±0.76	33.76±0.64^{d}
RDW-CV (%)	20.93±0.71^{*b}	26.31±2.41^{*a}	19.58±1.79	20.46±1.15^{*b}	19.95±1.04^{*b}	22.03±2.50^{d}
RDW-SD (fL)	32.25±1.32^{*b}	44.18±4.3^{*a}	35.91±4.26	32.23±1.48^{*b}	31.31±1.27^{*b}	33.38±1.83^{d}

a*- mean difference is significant compared with Group- I at P. value < 0.001

b*- mean difference is significant compared with Group- II at P. value < 0.001

d - no significant mean difference compared with Group- I at P. value < 0.05

5.6.3. Effect of HEECLR on platelet indices

In the cancer group (Group II), the platelet and Plateletcrit (PCT) levels were significantly reduced ($p<0.001$). Administration of extract in a dose of 500 mg/kg (Group-IV) and 1000mg/kg (Group-V) raise platelet and PCT significantly ($p<0.001$). There is no statistical ($P<0.05$) difference was appreciated in platelet and PCT when Co-treatment group (Group VI) compared with G-I. However, no statistically significant ($p<0.001$) change was seen in platelet and PCT in low dose extract administered group (Group-III). There was no statistically significant difference in levels of platelet distribution width (PDW) and mean platelet volume (MPV) between all groups.

Table 6: Effect of HEECLR on platelet and platelet indices

Group (G) No.	Results(M±SD)			
	PLT $10^3/\mu L$	**PCT (%)**	**PDW (fL)**	**MPV (fL)**
G-I	729.3± 21.27^{*b}	0.56±0.04^{*b}	8.81±0.24	7.93±0.21
G-II	408.33±25.42^{*a}	0.35±0.03^{*a}	9.13±0.71	7.90±0.30
G-III	506.5±82.76	0.46±0.05	7.81±0.79	7.43±0.42
G-VI	641.6±45.42^{*b}	0.6±0.035^{*b}	8.26±0.33	7.70±0.28
G-V	730.8±57.61^{*b}	0.58±0.05^{*b}	8.5±0.32	7.45±0.20
G-VI	612±75.52^{d}	0.49±0.06^{d}	7.69±0.44^{d}	7.61±0.25^{d}

a*- mean difference is significant compared with Group- I at P. value < 0.001

b*- mean difference is significant compared with Group- II at P. value < 0.001

d - no significant mean difference compared with Group- I at P. value < 0.05

5.6.4. Effect of HEECLR on NLR, LMR, LHR, NHR, MHR, PLR

In the cancer group (Group II)NLR, NHR and MHR were elevated significantly ($p<0.001$).Where different doses of extract (250, 500 & 1000)mg/kg (Group III-V respectively) administered groups significantly ($p<0.001$) restore NLR, LMR, LHR, NHR and MHR.A significant difference of NLR, LMR, LHR, NHR, MHR, PLR also observed in Group VI. No statistically significant change seen in PLR between all groups**.**

Table 7: Effect of HEECLR on NLR, LMR, LHR, NHR, MHR, PLR

Ratio (R)	Results (M ±SD)					
	Group (G) No.					
	G-I	G-II	G-III	G-IV	G-V	G-VI
NLR	1.8±0.138^{*b}	10.52±0.138^{*a}	4.43±0.27^{*b}	2.95±0.47^{*b}	3.14±0.38^{*b}	2.78±0.29^{*b}
LMR	10.67±1.51^{*b}	3.02±0.29^{*a}	5.74±0.70^{*b}	8.07±0.79^{*b}	5.97±0.84^{*b}	7.43±1.41^{*b}
LHR	0.15±0.007^{*b}	0.009±0.007^{*a}	0.16±0.11^{*b}	0.134±0.012^{*b}	0.132±0.012^{*b}	0.13±0.006^{*b}
NHR	0.27±0.014^{*b}	1.04±0.052^{*a}	0.69±0.05^{*b}	0.39±0.036^{*b}	0.41±0.024^{*b}	0.27±0.014^{*b}
MHR	0.014±0.0015^{*b}	0.032±0.003^{*a}	0.027±0.005^{*b}	0.017±0.001^{*b}	0.022±0.001^{*b}	0.017±0.003^{*b}
PLR	280.91±10.46	300.41±19.99	228.74±28.31	289.23±21.8	325.34±48.08	282.37±35.94

a*- mean difference is significant compared with Group- I at P. value < 0.001

b*- mean difference is significant compared with Group- II at P. value < 0.001

5.7. Effect of HEECLR on liver enzymes

Activities of AST, ALT, and ALP in the serum were increased significantly (P<0.001) in the cancer group (Group-II). In rats treated with different doses of HEECLR (250mg/kg, 500mg/kg & 1000mg/kg) (Group III-V respectively), the AST, ALT and ALP levels were found to be significantly (P<0.001) decreased. No Significant (P<0.05) difference in liver enzymes level was when the co-treatment group (Group-VI) compared with G-I.

Table 8: Effect of *C.longa* extract on liver enzymes

Group (G) No.	Results (M ±SD)		
	ALT U/L	ALP U/L	AST U/L
G-I	74.33±5.36^{*b}	223.75±17.29^{*b}	145.1±10.34^{*b}
G-II	188.83±17.93^{*a}	453.28±46.91^{*a}	232±24^{*a}
G-III	131.53±14.04^{*b}	338.28±25.47^{*b}	176.3±12.12^{*b}
G-VI	107.9±5.8^{*b}	247.8±15.28^{*b}	167.8±9.98^{*b}
Gr-V	79.96±6.89^{*b}	214.48±17.37^{*b}	158.83±11.95^{*b}
G-VI	91.06±7.12^{d}	242.06±17.13^{d}	156.33±19.62^{d}

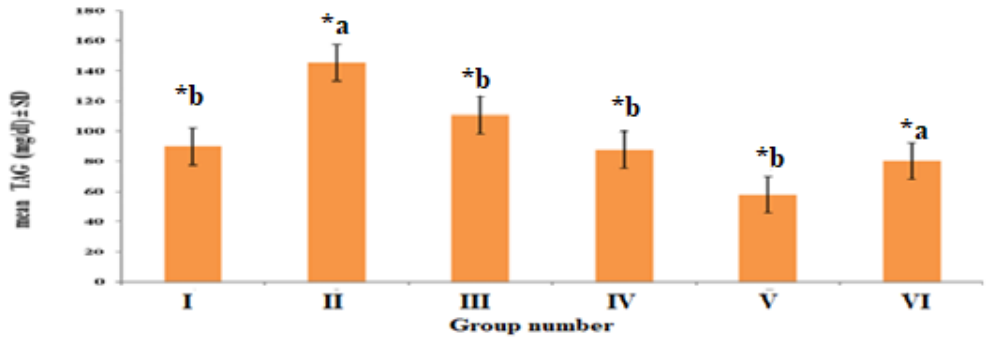
a*- mean difference is significant compared with Group- I at P. value < 0.001

b*- mean difference is significant compared with Group- II at P. value < 0.001

d - no significant mean difference compared with Group- I at P. value < 0.05

5.8. Effect of HEECLR on Triacylglycerol (TAG)

Figure 15 revealed a significant (P<0.001) TAG level increased in the cancer group (Group-II). Different doses of HEECLR treated groups (250, 500 and 1000mg/kg) were found to be significantly (P<0.001) decreased TAG level. No significant (P<0.05) difference on TAG level between Co-treatment (Group-VI) and Group -I.



a*- mean difference is significant compared with Group- I at P. value < 0.001

b*- mean difference is significant compared with Group- II at P. value < 0.001

d - no significant mean difference compared with Group- I at P. value < 0.05

Figure 36: Effect of HEECLR on TAG

5.9. Histopathological observation of colon in DMH-induced versus treated with HEECLR

All rats (6 rats) colon histological examination in the control group (G-I) shows normal colon showing full-thickness epithelium (mucosa, muscularis mucosa, submucosa, and muscularis propria) (figure 17-a) colonic mucosa revealing tubule-villous architecture with numerous goblet cells (figure 17- b). In cancer group (G-II) adenocarcinoma was observed in one rat with histological appearance of colonic carcinoma: well-differentiated adenocarcinoma with well-formed irregular glands lined by elongated, hyperchromatic nuclei and infiltrating the submucosa and necrotic debris, present in the gland lumen is typical (figure17-e). The remaining five rat's colon show adenocarcinoma with metastasis to the liver with histological appearance of colonic mucosa lined by dysplastic epithelium with malignant back to back glands in the lamina propria (figure17-f). In HEECLR 250 mg/kg treated group (G-III) adenocarcinoma were observed in 3 rats and adenocarcinoma with metastasis to the liver also seen on the remaining 3 rats. The histological examination description is similar to the above for both results. Adenocarcinoma (without metastasis) also seen in 500mg (6 rats) and 1000 mg/kg (5 rats) HEECLR treated the group with the same histological description in G-II. In one rat colon tubular adenoma seen with a histological observation of variably sized hyperplastic tubular glands lined by cytological atypical nuclei. Crypt dilation and rupture have seen near the basement membrane (figure 17-d). In DMH along with HEECLR or Co-treatment group (G-VI) chronic colitis or chronic inflammation with histological examination of colonic mucosa with transmural mononuclear inflammatory infiltrates mainly lymphocyte and plasma cells (figure 17-c).

Table 9: Histopathological examination result of colon

Group (G) No.	Histopathological examination result of colon					
	Normal	Chronic colitis	Tubular adenoma	Adenocarcinoma	Adenocarcinoma With liver metastasis	Total No. of rats
	No. of rats per groups					
G-I	6	-	-	-	-	6
G-II	-	-	-	1	5	6
G-III	-	-	-	3	3	6
G-IV	-	-	-	6	-	6
G-V	-	-	1	5	-	6
G-VI	-	6	-	-	-	6
Total No. of rats	6	6	1	15	8	36

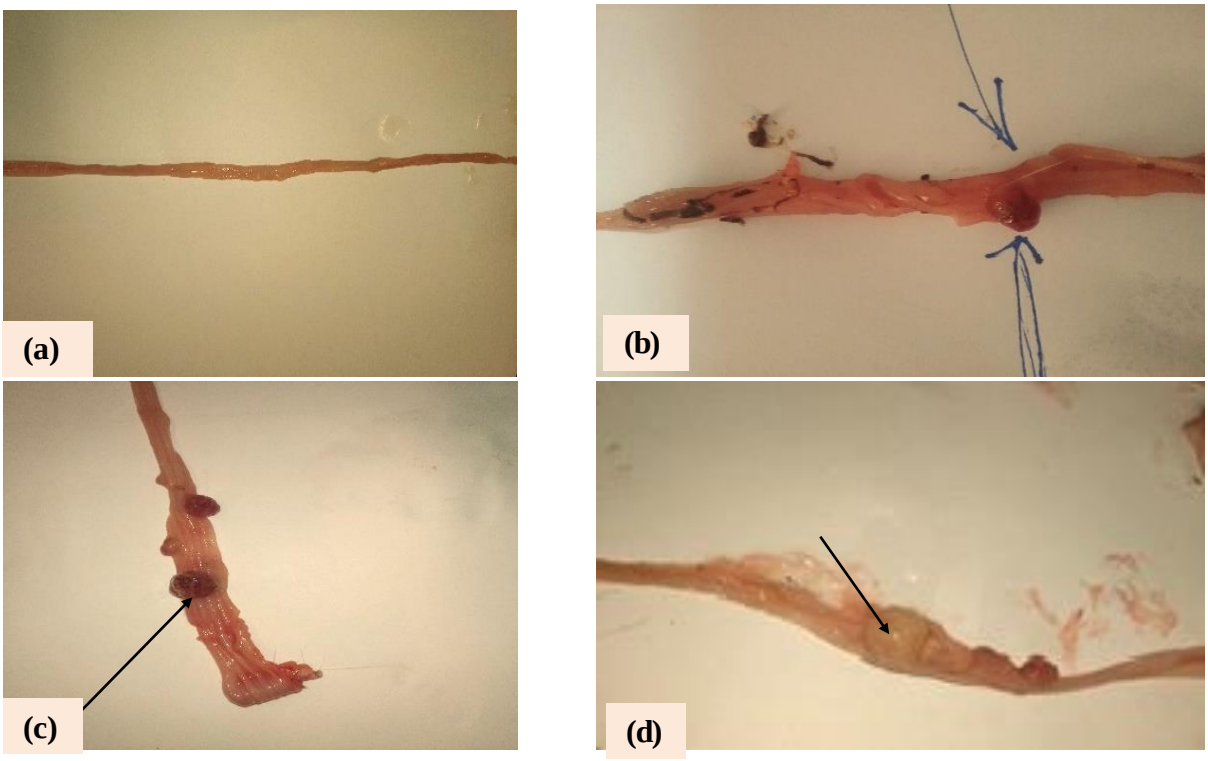


Figure 17: Macroscopic observation of colon:- (**a**) normal colon; (**c-d**) colon with visible mass

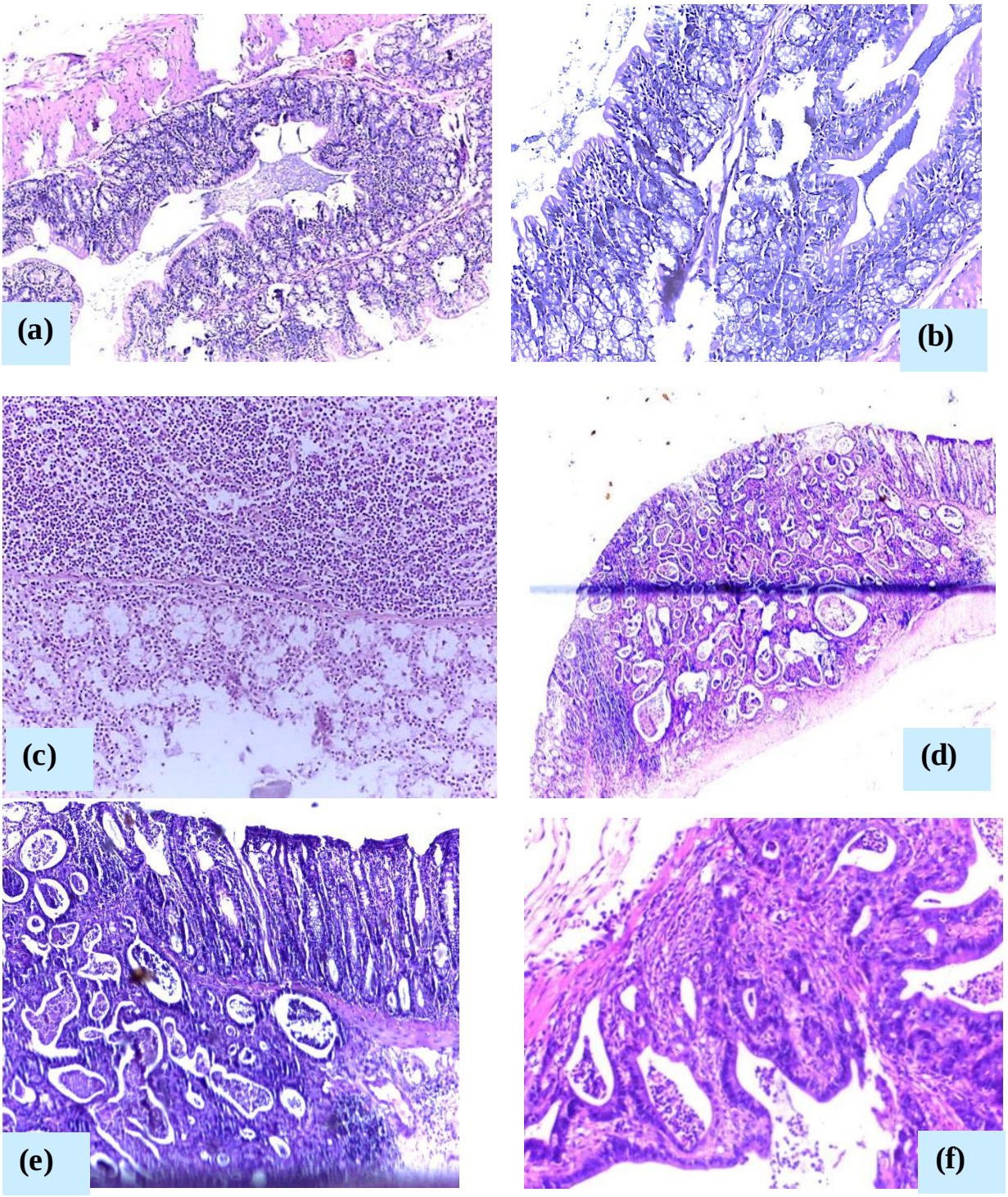


Figure 4: Photomicrographs showing hematoxylin and eosin-stained colon histopathological sections:- (**a**) control rat: normal colon structure(10x); (**b**) normal colon structure (40X); (**c**) chronic inflammation with lymphocyte infiltration (10X); (**d**) tubular adenoma (10X); (**e**) Adenocarcinoma(10X); (**f**) Adenocarcinoma with metastasis

5.10. Effects of HEECLR on Gross hepatic manifestation

The liver of the control group (G-I), cancer+ 500 mg/kg HEECLR (G-IV), Cancer + 1000 mg/kg (G-V) and Co-treatment group (G-VI) was normal with the smooth and red-brown surface. Fatty liver was seen in the cancer group (G-II) with the yellow-red color surface. The mass formation also was seen in the cancer group (G-II).

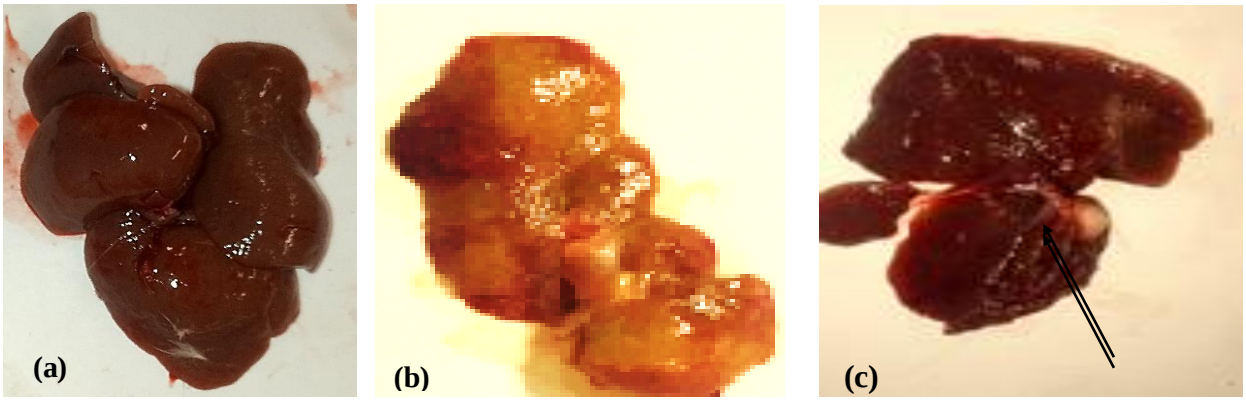


Figure 5: Macroscopic observation of liver:- (**a**) Normal liver; (**b**) fatty liver; (**c**)liver with visible mass.

5.11. Histopathological observation of liver in DMH-induced versus treated with HEECLR

As shown in table 10; control group (G-I), cancer + 500mg/kg HEECLR (G-IV), cancer + 1000mg/kg HEECLR (G-V), and Co-treatment group (G-VI) histological examination of liver reveals normal hepatocyte cell (polygonal cells) with abundant eosinophilic cytoplasm having centrally located nucleus with prominent nucleoli (figure 19-a). Fatty liver was also seen in cancer group (G-II) and cancer + 250 mg/kg HEECLR (G-III) with histological appearance of a mix of small and large fat droplets (seen as clear vacuoles) is most prominent around the central vein (figure 19-b & c). Secondary metastasis adenocarcinoma was seen in G-II and G-III with the appearance of secondary infiltrate adenocarcinoma; well-formed malignant glands infiltrate the liver eliciting a desmoplastic stromal reaction (Figure 19-e).

Table 10: Histopathological examination result of liver

Group No.	The histopathological examination result of liver			
	Normal	Fatty liver	Secondary metastasis adenocarcinoma	Total no. of rats
	No. of rats per groups			
G-I	6	-	-	6
G-II	-	1	5	6
G-III	-	3	3	6
G-IV	6	-	-	6
G-V	6	-	-	6
G-VI	6	-	-	6
Total no. of rats	24	4	8	36

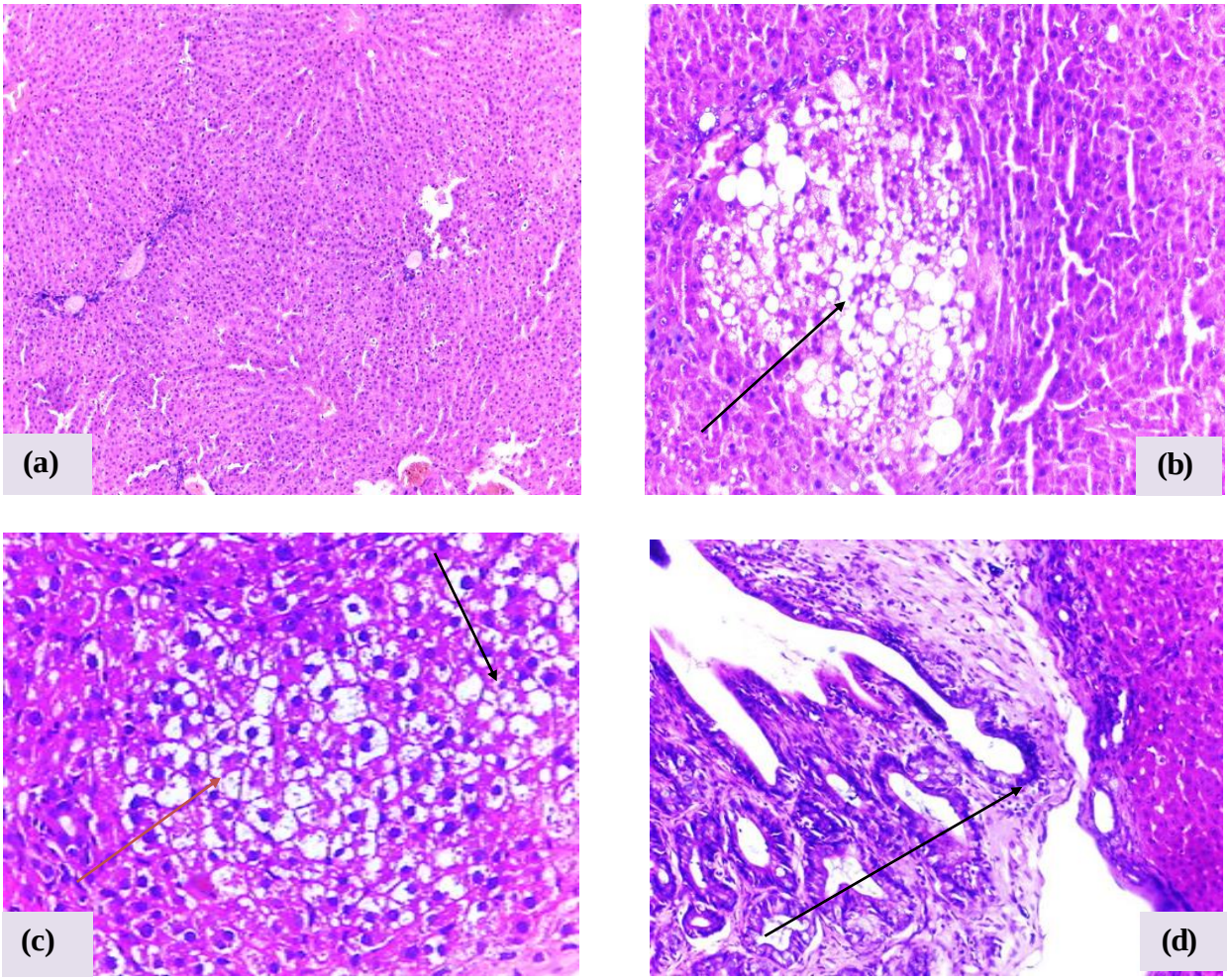


Figure 20: Photomicrographs showing H and E stained liver histopathological sections :-

(a) normal liver; **(b)** focal fatty change;**(c)** extensive fatty change;**(d)** secondary adenocarcinoma

5.12. Correlation between triacylglycerol level and liver enzymes; hematological parameters

Serum triacylglycerol level was positively correlated with ALT (r = 0.851, P< 0.01), ALP (r = 0.857, P< 0.01), AST (r = 0.72, P< 0.01), and total WBC (r = 0.835, P< 0.01) and neutrophil (r = 0.781, P< 0.01). Negative correlation was observed between triacylglycerol and lymphocyte (r = -0.695, P< 0.01); RBC (r = -0.711, P< 0.01), hemoglobin (r = -0.699, P < 0.01) and platelet (r = -0.814, P < 0.01).

Table 11: Pearson's analysis of correlation of triacylglycerol with WBC, Neutrophil, Lymphocyte, RBC, Hgb, Platelet, ALT, ALP, AST (p<0.01)

Parameters	Pearson (r)	P (two tail)
WBC	0.835	0.000
Neutrophil	0.781	0.000
Lymphocyte	-0.695	0.000
RBC	-0.711	0.000
Hgb	-0.699	0.000
Platelet	-0.814	0.000
ALT	0.851	0.000
ALP	0.857	0.000
AST	0.72	0.000

r **-** Pearson correlation coefficient

5.13. Correlation between hemoglobin and liver enzymes; WBC, neutrophil, lymphocyte, monocyte, platelet

Hemoglobin was positively correlated with platelet (r = 0.796, P< 0.01) and neutrophil (r = -0.736, P< 0.01). Negative correlation was observed between hemoglobin and WBC (r = -0.741, P< 0.01); Lymphocyte (r = 0.668, P < 0.01), Monocyte (r = -0.63, P < 0.01); ALT (r = -0.761, P < 0.01); ALP (r = -0.766, P<0.01) and AST (r=- 0.661, P<0.01).

Table 12: Pearson's analysis of correlation of Hemoglobin with liver enzymes; WBC, Neutrophil, Lymphocyte, Monocyte, Platelet ($p<0.01$)

Parameters	Pearson (r)	P (two tail)
WBC	-0.741	0.000
Neutrophil	-0.736	0.000
Lymphocyte	0.668	0.000
Monocyte	-0.63	0.000
Platelet	0.796	0.000
ALT	-0.761	0.000
ALP	-0.766	0.000
AST	-0.661	0.000

r- Pearson correlation coefficient

6. DISCUSSION

CRC is ranked as the fourth most lethal and commonly diagnosed cancer in the world according to the National Cancer Institute's latest report. Prolonged chronic inflammation is known to high risks of developing CRC. Oxidative stress due to ROS is an important trigger for cancer. Potential antioxidant compounds especially derivatives of medicinal plants have received great attention in the current research trend for CRC treatment. *C.longa* is a known medicinal plant used as medical treatments for various types of chronic illness including CRC. Its effect on health is generally centered upon secondary metabolites especially an orange-yellow colored, lipophilic polyphenol substance called "curcumin," which is acquired from the rhizomes of the herb. Curcumin is known to have antioxidant, anti-inflammatory, chemo-preventive, and hepatoprotective effects.

The phytochemical screening of HEECLR shows that the presence of saponins, tannins, flavonoids, alkaloids, phenols, and steroids. These results were supported by many previous research (Saxena and Sahu, 2012; Rajesh *et al.*, 2013; Pawar *et al.*, 2015). Saponins have many medicinal uses including anti-tumor, hepatoprotective, and anti-inflammatory activities (Moghimipour and Handali, 2015). Plants saponins can reduce the risk of colorectal cancer. Saponins could inhibit colorectal cancer cell proliferation and induce apoptosis. Tannins have been observed to have remarkable activity in cancer prevention. The anti-inflammatory effects of tannins help control irritating bowel disorders (Kim *et al.*, 2008). Flavonoids are water-soluble antioxidants. They scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals which prevent oxidative cell damage followed by the prevent cancer (Rahman, 2007; Lobo *et al.*, 2010).

In this study IC_{50} of the extract was 166.4 µg/mL. DPPH scavenging activity also appeared to depend on the phenolic concentration. Components such as phenolics, flavonoids, and tannins, identified from these plants might be responsible for their antioxidant activity (Sathyanarayanan *et al.*, 2018). Radical scavenging activity is also known to be related to the nature of phenolics contributing to their electron transfer/hydrogen donating ability (Loganayaki *et al.*, 2013).

In the present study, we found an alteration in the hematological parameters of rats was seen. The total white blood cell count was increased in the cancer group (G-II). Probable mechanisms between colon cancer and inflammation may be due to the presence of leukocytes in case cancer

may be interpreted as an aborted attempt of the immune system to reject the tumor. However, the increment of leukocyte indicates that leukocyte infiltration can promote tumor phenotypes, such as angiogenesis, growth, and invasion. This may be due to inflammatory cells that can influence cancer promotion by secreting cytokines, growth factors, chemokines, and proteases, which stimulate proliferation and invasiveness of cancer cells. Furthermore, leukocyte brings much of the cyclooxygenase-2 (COX-2) (Eiró and Vizoso, 2012). COX-2 may be linked to the pathogenesis of colon cancer. COX-2 protein is over-expressed and prostaglandin E2 levels are elevated in the portal vein and colonic mucosa in colorectal cancer (Lee *et al.*, 2006). COX-2 promotes angiogenesis by enhancing the production of the vascular endothelial growth factor (Gately and Li, 2004).

The neutrophils count was increased in the cancer group (G-II). Neutrophils are the most abundant leukocytes in the blood and are considered to be the first line of defense during inflammation and infections. The role of neutrophils has been described in various cancer types, including colorectal cancer (Ho *et al.*, 2014). In addition, neutrophils are also found infiltrating many types of tumors. Tumor-associated neutrophils (TANs) have relevant roles in malignant disease. Indeed, neutrophils may be potent antitumor effector cells. However, increasing clinical evidence shows TANs correlate with poor prognosis. In many patients with advanced cancer, elevated counts of neutrophils in the blood are found. How tumors induce neutrophilia is uncertain, but the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) is a possible mechanism in several types of cancer (Mc-Gary *et al.*, 1995). In addition, other cytokines such as granulocyte colony-stimulating factor (G-CSF), interleukin- (IL-) 1, and IL-6 produced by tumors seem to contribute to elevated neutrophil numbers in blood (Lechner *et al.*, 2010).The tumor microenvironment controls neutrophil recruitment and in turn TANs help tumor progression (Uribe-Querol and Rosales, 2015). Moreover, infiltrated neutrophils recruit to tumor foci to secret various forms of cytokines including CSF and neutrophil elastase (NE) (Wu *et al.*, 2011). CSF contributing to enhancing the tumor progression and responsible for the increase of neutrophils (Wu *et al.*, 2011). NE is a protease that can cause damage and then generate favorable environments for carcinogens tumor progression. Furthermore, NE can enzymatically degrade insulin receptor substrate-1 (IRS-1) and then increase the interactions of phosphatidylinositol 3-kinase (PI3K) and the potent mitogen platelet-derived growth factor receptor (PDGF), triggering to tumor cell proliferation (Houghton *et al.*, 2010).

Apart from that neutrophils can release ROS this results in a DNA base damages (Cadet and Wagner, 2013), as well as mutations (Cooke *et al.,* 2003), which are essential for cancer initiation, cell proliferation, cancer-favored inflammation, immune suppression (Uribe-Querol and Rosales, 2015).

The lymphocyte count was reduced in the cancer group (G-II). The possible mechanism for the depletion of lymphocytes count could be migration and infiltration of lymphocyte to cancer tissue (Cohen and Blasberg, 2017) and also cancer cells have immunosuppressive mechanisms to avoid T-cell responses (Rabinovich *et al.,* 2007). Low absolute lymphocyte count (LC), has been associated with inferior outcomes in various cancers, including colorectal cancer (Liang *et al*., 2016).

The monocyte count was increased in the cancer group (G-II). Chemokines which are produced by cancer cells, promote the recruitment of peripheral monocytes to the cancer microenvironment, thereby promoting the recruitment of monocytes from the bone marrow to peripheral blood (Shibutani *et al.,* 2017). They are important to tumor tissue and can differentiate into tumor-associated macrophages and their pro-tumor function is supporting tumor initiation, local progression, and distant metastasis (Richards *et al.*, 2013). Hypoxic areas of cancer cells release a high number of chemo-attractants that enhance macrophage migration to these hypoxic sites. Hypoxia also imprisons macrophages by decreasing their mobility; this terminates the macrophage response to chemo-attractants outside the hypoxic areas (Chanmee *et al.,* 2014). Besides tumor-associated macrophages, circulating monocytes have a role in suppressing adaptive immunity and promoting angiogenesis, invasion, and migration (Williams *et al*., 2016).

The administration of HEECLR in different doses (250, 500 and1000) mg/kg (G-III, G-IV, and G-V respectively) restored the alteration of white blood cells and its differentials (neutrophil, lymphocyte, and monocyte). The possible reason for this effect may be due to the anti-inflammatory and immune-stimulatory activities of *C.longa* (Chandrasekaran *et al*., 2013). The anti-inflammatory property of *C. longa* is due to the presence of curcuminoids (Bagad *et al.,* 2013). Curcumin has inhibited the tumor immunity via inhibition of the expression of indoleamine-2,3-dioxygenase (suppress the function of anti-tumor T cells) and also inhibit COX, PGE2 production, which exerts potent immunosuppressive effects in the tumor microenvironment (Basnet and Skalko-Basnet, 2011). Curcumin can down-regulate the activity

of COX-2, lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes; inhibit the production of the inflammatory cytokines TNF-α, IL, and monocyte chemoattractant protein (MCP) (Bagad *et al*., 2013).

The reduction of neutrophil count in HEECLR administered rat may be due to the presence of curcumin. Curcumin was previously reported to accelerate neutrophil apoptosis by increasing the cell surface expression of annexin-V and CD16 shedding (Antoine and Girard, 2013). Curcumin can interfere with neutrophil responses to various physiological stimuli and a part of its anti-inflammatory action is mediated via inhibition of neutrophil function in the cancer cells. Inhibition of neutrophil function by curcumin appears to be mediated via calcium-dependent mechanisms (Srivastava, 1989). Curcumin reduces neutrophil recruitment to the inflammatory sites by affecting chemokine gradient formation as well as by the direct effect of the compound on neutrophil polarization, chemotaxis, and chemo-kinesis (Larmonier *et al*., 2011).

In our study, HEECLR administration restored the lymphocyte count. Churchill *et al*. indicate that curcumin can augment tumor eradication by restoring T-cell responses (Churchill *et al*., 2000). Bhattacharyya et al showed the enhancement of the antitumor immune response of T cells in tumor-bearing hosts by the administration of curcumin (Bhattacharyya *et al*., 2010). This result was also supported by another study (Tuyaerts *et al*., 2018).

Our result also showed the reduction of monocyte in HEECLR administered rats. Curcumin could prevent production of interleukin-8 (IL-8), monocyte inflammatory protein-1 (MIP-1α), monocyte chemotactic protein-1 (MCP-1), IL-1β, tumor necrosis factor-α (TNF-α), or lipopolysaccharide (LPS)-stimulated monocytes and macrophages (Tuorkey, 2014) which are important for production, migration and infiltration of monocytes/macrophages.

The red blood cell count, hemoglobin, and hematocrit were reduced in the cancer group (G-II). This finding may be TNF-α inhibits hemoglobin production by down-regulation of GATA-1 (essential transcription factors in gene regulation during hematopoietic differentiation (Dicato *et al*., 2010; Moriguchi *et al*., 2010). TNF α has an inhibiting effect on erythroid differentiation and a decrease in hemoglobin synthesis by up-regulating transcription factors NF-kB and GATA-2 (Morceau *et al*., 2009). NF-kB canonical pathway (p50/p65) inhibits the expression of globin genes. During inflammation, IL-6 induces the liver to produce hepcidin. Hepcidin decreases iron absorption from the bowel and blocks iron utilization in the bone marrow (Dicato *et al*., 2010).

And also, cancer systemic iron availability is decreased through its sequestration within the macrophage. Tumor-associated macrophages (TAM) adopt the systemic circulated iron and donate iron to the tumor microenvironment (Jung *et al.,* 2019).

Moreover, elevated levels of reactive oxygen species (ROS) will attack erythrocytes lead to hemolysis and result in the reduction of hematocrit, hemoglobin & RBC (Mohanty *et al.,* 2014).

RBC, hemoglobin, and hematocrit increased in 500 mg /kg and 1000 mg/kg dose of HEECLR treated rats. The study done by Aggarwal *et al* suggests that curcumin has the ability to inhibit the production and the action of TNF-α (Aggarwal *et al.,* 2013). Kim *et al* and Jin *et al* showed that *C.longa* has an anti-inflammatory effect via inhibition of NF-κB and IL-6 (Jin *et al.,* 2007, Kim *et al.,* 2012) and also Laine *et al* reported that *Curcuma* decreases serum hepcidin levels in humans (Laine *et al.,* 2017).

Platelet and plateletcrit values significantly decrease in the cancer group (G-II). In contrast, most of the studies done by the previous researcher found that PC and PCT levels were higher in CRC patients (Plantureux *et al.,* 2018; Zhu *et al.,* 2018). The reason for the reduction of platelet count may be cancer cells induce and releasing of pro-metastatic platelet-derived microparticles which induce platelet aggregation (Jurasz *et al.,* 2004).

Administration of HEECLR in doses of 500 mg/kg and 1000 mg/kg increases the PC and PCT. This finding is supported by Hussain the study in aged rats shows curcumin administration caused an increase in platelet count (Hussain, 2015) and also for rats were treated by ar-turmerone (*Curcuma* rhizome derived component) inhibiting platelet aggregation, resulting in elevated levels of PC and PCT (Lee, 2006).

Neutrophil to Lymphocyte ratio (NLR) was increased in the cancer group (G-II). Most researchers found that prognostic and predictive role of neutrophil/lymphocytes ratio in metastatic colorectal cancer (Walsh *et al.,* 2005; Leitch *et al.,* 2007; Ozdemir *et al.,* 2014; Dell'Aquila *et al.,* 2018). The elevated level of NLR potential prognostic marker for inflammation in CRC.

In this study ALP, ALT, and AST elevated in the cancer group (G-II). These findings supported by the previous studies (Sharma and Sharma, 2011; Kawatra *et al.,* 2015; Sisein *et al.,* 2016). DMH damage plasma membranes of hepatic tissue, alter in detoxifying enzymes / anti-oxidant

system of the liver by influencing the hepatic GSH redox system (Anilakumar *et al.*, 2010; Sharma and Sharma, 2011). This is tangible evidence for exposure for DMH coupled with slow down the detoxification enzymes in the liver result in increasing cancer incidents.

HEECLR reduced the elevated level of ALT, ALP, and AST. The different kinds of researcher *C.longa* have the ability to protect the liver from different hepatotoxic compounds (Al-Rubaei *et al.*, 2014; Lee *et al.*, 2016; Mansour-Ghanaei *et al.*, 2019). The hepatoprotective capacity of *C.longa* by restoring hepatic GSH and hepatic GSH/GSS ratios result suppress oxidative stress (Lee *et al.*, 2016). Curcumin may have the ability to boost detoxification and prevent liver damage (Al-Rubaei *et al.*, 2014).

Cancer group rats were found that fatty infiltration. The rats were treated by (500 and 1000) mg/kg HEECLR found that normal liver. Curcumin might amend hepatic steatosis and block fatty liver disease progression through inhibiting fatty acids synthesis and biosynthesis of unsaturated fatty acids such as stearic acid, oleic acid and linoleic acid (Egashira *et al.*, 2012). It can improve mitochondrial activity, facilitate β-oxidation and decrease lipogenesis (Ferramosca *et al.*, 2017). In addition, oxidative stress and immune system disorder play important roles in the contribution of liver dysfunction such as NAFLD (Inzaugarat *et al.*, 2017). In this case, curcumin can improve anti-oxidant system and prevent NAFLD by decreasing the production of reactive oxygen species, the hepatic protein expression of oxidative stress, pro-inflammatory cytokines, and chemokines (Salomone *et al.*, 2016; Ferramosca *et al.*, 2017).

Our finding reveals that serum triacylglycerol levels significantly elevated in the cancer group (G-II). Coppola and his colleagues show the association between triglyceride and colorectal cancer (Coppola *et al.*, 2015). The study done by (Zhang *et al.*, 2014) abnormally altered levels of lipids including serum triacylglycerol in the serum of patients with colorectal cancer may be correlated with the occurrence and development of colorectal cancer. Moreover, Ulmer *et al* suggest that serum triacylglycerol concentrations pathogenesis of several cancers, the result of insulin resistance and increased circulating insulin/insulin-like growth factor-1 in CRC (Giovannucci, 2007; Ulmer *et al.*, 2009).

HEECLR reduced the serum triglyceride level. Our finding supported by the different researchers with different protocols shows the reduction of TAG by *C.longa* (Mohammadi *et al.*,

2013; Yang *et al.*, 2014; Qin *et al.*, 2017). *C.longa* has the ability boosting lipid metabolism via activating on PPAR-α and PPAR-γ (Sahebkar, 2014a; Sahebkar, 2014b; Sahebkar, 2014c).

Most of the rats with CRC shows metastasis to the liver. The previous researchers found that high metastatic tumor cells in the colon of nude mice have been shown to result in the rapid and efficient formation of metastases to the liver (100%) (Fu *et al.*, 1992; Furukawa *et al.*, 1993; Rashidi *et al.*, 2000). However, the administration of 500 mg/kg and 1000 mg/kg dose HEECLR totally prevent the metastasis. The previous studies reported that curcumin has the ability to prevent metastasis in different cancer (Kuttan *et al.*, 2007; Bandyopadhyay, 2014;Bachmeier *et al.*, 2018). Cortactin (cortical actin binding protein or CTTN, a monomeric protein located in the cytoplasm of cells), encoded by the CTTN/EMS1 gene, is a v-Src substrate localized with cortical actin at the plasma membrane and is up-regulated in several types of cancer (Wu *et al.*, 1991). The phosphorylated form of cortactin (pTyr421) plays a major role in cancer cell migration and invasion. It was shown that pTyr421-cortactin was up-regulated in colon cancer. Curcumin interacted with PTPN1 tyrosine phosphatases to rise its efficacy leading to the dephosphorylation of pTyr421-CTTN. Curcumin considerably reduced the pTyr421-CTTN in colon cancer cells. Altogether, Curcumin modulated the activity of PTPN1 phosphatase to reduce cortactin phosphorylation and interaction with CTNND1, and finally to reduce colon cancer cell migration (Radhakrishnan *et al.*, 2014). The other possible mechanism may be curcumin significantly inhibits colon cancer cell growth (Johnson *et al.*, 2009). Previous studies have revealed that NF-κB activation is critical for the proliferation, survival and metastasis of colon cancer cells (Chen *et al.*, 1999; Plummer *et al.*, 1999; Johnson *et al.*, 2009) therefore, inhibition of NF-κB activation is a potential antitumor strategy. Curcumin dose-dependently suppresses constitutive NF-κB activation in colon cancer cells which demonstrate that curcumin is a potent inhibitor of NF-κB activation (Singh and Aggarwal, 1995; Kumar *et al.*, 1998). According to Li and his colleague's study *C.longa* extract treatment could suppress colon tumor growth and inhibited liver or lung metastasis. Curcumin has been shown to inhibit cell growth and metastasis through suppression of STAT3, FAK, cofilin, Erk and Akt pathways (Li *et al.*, 2018).

Evaluation of HEECLR on the chemo-preventive effect of colorectal cancer induced by DMH was examined and the result shows that normal CBC, normal liver enzymes with chronic colitis. The result indicates that healthy liver prevents the development of CRC in rats. DMH itself is not

a carcinogen, it is metabolically activated into DNA reactive metabolites by phase I and phase II xenobiotic enzymes in order to exert lipid peroxidation and tumorigenic activity (Qi *et al.*, 2015).

The phase II enzymes, including glutathione S-transferase (GST) and DT-diaphorase (DTD) which facilitate detoxification and excretion of the carcinogen from the colonic lumen. detoxify the electrophilic intermediates(Sheweita and Tilmisany, 2003; Senapathy *et al.*, 2011). The increased levels of phase II enzymes are beneficial for chemoprotection or chemo-preventive against carcinogens. Thangaraj *et al.* findings showed a significant decrease in the phase II enzyme activities in the liver and colon of DMH induced cancer rats, which could be due to the utilization of more detoxifying enzymes to counteract DMH induced neoplastic transformation (Thangaraj *et al.*, 2018).

Curcumin also exerts a cytoprotective effect on non-cancer cells through the transcriptional induction of phase II enzymes. This latter effect may be critical to its chemopreventive activity (Hatcher *et al.*, 2008). Curcumin has been shown to elevate activities of phase II enzymes such as glutathione transferases (Susan and Rao, 1992), NAD(P)H:quinone reductase (QR) (Dinkova-Kostova and Talalay, 1999), and heme oxygenase (Balogun *et al.*, 2003; Scapagnini *et al.*, 2006), while inhibiting pro-carcinogen activating phase I enzymes, such as cytochrome P4501A1 (Ciolino *et al.*, 1998). The study done by Patel *et al* explains in Vitro chemo-preventive actions of curcumin on the inhibition of cell proliferation, Pro-apoptotic property, inhibition of angiogenesis, Inhibition of carcinogenesis, and Inhibition of COX-2 (Patel *et al.*, 2010). The other study done by Rao *et al* showed curcumin has been tested as a chemo-preventive agent against azoxymethane (AOM)-induced colon carcinogenesis in preclinical models, and dietary administration of curcumin inhibits the formation of aberrant crypt foci (ACF), putative pre-neoplastic lesions that occur in the colon of both animals and humans (Rao *et al.*, 1993).

Pearson correlation analysis of this study shows triacylglycerol has a positive correlation with WBC; Neutrophil and negative correlation with lymphocyte, RBC, hemoglobin, platelet. Shankar *et al.* found a positive association between Triglyceride and WBC on cardiovascular patients (Shankar *et al.*, 2007). Triglyceride levels are shown to be closely related to inflammation, including elevated WBC count (Ford *et al.*, 2004; Davidson *et al.*, 2006). However, recent evidence also suggests a direct role for triglycerides in the activation of leukocytes including neutrophil and free radical production (Van Oostrom *et al.*, 2004, Wanten

et al., 2002). Wanten *et al* reported that triglyceride stimulates neutrophils for the production of oxygen free radicals (Wanten *et al.,* 1999). The correlation seen between triacylglycerol with total WBC and its differentials maybe during chronic inflammation secretion of multiple cytokines (TNF-α, IL-1, IL-2, IL-6) increase serum triglyceride (Jung and Choi, 2014).

In this study, there is a negative correlation between TG and hemoglobin. In contrast, Choi *et al* study showed the positive correlation between TG and hemoglobin in iron deficiency anemia (Choi *et al*., 2001). Aye *et al* showed a positive correlation between TG and PC in polycystic ovarian syndrome patients (Aye *et al.,* 2014). The positive correlation between TG and ALT, ALP; AST. The correlation result of this study showed that triacylglycerol was positively correlated with the liver enzyme level. There is substantial scientific evidence that showed how lipids can affect liver function in preclinical and also in clinical settings (Bertolotti *et al*., 2014). Higher lipid levels including high triglyceride are likely to be susceptible to altered metabolism profile. A fatty liver with inefficient functioning could lead to a decrease in its ability to detoxification. This will eventually aggravate systemic oxidative stress. The decreased antioxidant capacity of the liver could be compromised with increasing lipid levels, resulting in more inflammation and reactive oxygen species in the body (Jung and Choi, 2014).

Moreover, the Pearson correlation analysis shows a negative correlation between hemoglobin and WBC, neutrophil, and monocyte, in addition, positive correlation also was seen between hemoglobin and lymphocyte; platelet. This correlation finding shows the consequence of inflammation on the hemoglobin levels. The study done by Maccio *et al* confirmed that the lowest Hb levels were associated with the highest values of inflammatory markers (Macciò *et al*., 2015). In Maccio *et al* study, ROS was negatively correlated with Hb, while GPx and SOD were positively correlated with Hb. ROS are able to inhibit the synthesis of EPO (Stenvinkel *et al*., 1998). Oxidative stress increases the fragility of red blood cells, decreases the rate of erythroid maturation and shortens erythrocyte lifespan (Millonig *et al*., 2012). Furthermore, in our study negative correlation between hemoglobin and ALT, AST; ALP was seen. The probable reason for this result may be liver damage bring by DMH administration bring dysregulation of hepcidin and iron dysregulation. This leads to a reduction in hemoglobin. The other possible mechanism for correlation between two variables may be iron accumulation in hepatocytes and Kupffer cells lead to an increase in ROS production and pro-inflammatory mediators' initiates' cell damage.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

DMH induced colorectal cancer rats were alter WBC, its differentials, RBC indices, and platelet indices. However, administration of HEECLR in doses of 500 mg/kg and 1000 mg/kg restores changes in those hematological parameters due to its immune-modulatory activity. DMH bring fatty change in liver and elevate liver enzymes. 500 and 1000 mg/kg HEECLR reduced elevated liver enzymes with normal liver appearance. DMH brings colorectal cancer with metastasis to liver although HEECLR prevents metastasis to the liver this shows the anti-metastasis property of *C.longa* but did not cure colorectal cancer. HEECLR has chemo-preventive effect on DMH administer rat the result showed normal CBC, normal liver enzyme but seen only chronic colitis. It indicates that HEECLR facilitates detoxification of the DMH in the liver. We conclude that HEECLR, prepared from *C.longa* grown in Ethiopia was found to have adequate secondary metabolites which are important for anti-inflammatory, anti-oxidant and anti-metastatic activities and also prevents colorectal cancer formation by its chemo-preventive action through boosting detoxification function of the liver.

7.2. Recommendation

The result presented in this study regarding Ethiopian harvested *C.longa* should be taken as basis for further investigation. Therefore, we recommend:

- Further experimental studies and clinical trials should be performed to determine effective therapeutic and preventive doses
- Emphasis should be given on hematological parameters and ratios (NLR, LMR, PLR, LHR, NHR, MHR) since they give important information about cancer progression.

8. LIMITATION OF STUDY

The major limitations of this study were:

- In order to compare the effectiveness of extract with the standard drugs, the rat group receiving anti-cancer drugs was not included in the study.
- Tests for oxidative stress and mitochondrial dysfunction were not performed
- Isolated curcumin was not tested separately for its individual effect
- The treatment period was short
- Except for triacylglycerol other lipid profiles were not performed

9. REFERENCES

Abdelaziz, E.Z., Hegazy, A.M.S., Badawy, A., Bayomy, N.A. and Rabat, A.E., 2016. Potential Anti-angiogenic Effect of a Combination of Boswellic Acids and Cisplatin against Chemically-induced Colon Cancer in Mice. *Journal of Chemical Biology &Therapeutics*, 1(2), pp.1-7.

Adewoyin, A.S., and Nwogoh, B. 2014. Peripheral blood film - a review. *Annals of Ibadan postgraduate medicine*, 12(2), pp.71-79.

Aggarwal, B.B., Gupta, S.C., and Sung, B. 2013. Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *British Journal of Pharmacology*, 169(8), pp.1672-92.

Aggarwal, B.B., Sundaram, C., Malani, N., & Ichikawa, H. 2007. Curcumin: The Indian Solid Gold. In: Aggarwal, B.B., Surh, Y.J. and Shishodia, S. eds., 2007. *The molecular targets and therapeutic uses of curcumin in health and disease* (Vol. 595). Springer Science & Business Media, pp. 1-74

Al-Eryani, L., Wahlang, B., Falkner, K.C., Guardiola, J.J., Clair, H.B., Prough, R.A. and Cave, M., 2015. Identification of environmental chemicals associated with the development of toxicant-associated fatty liver disease in rodents. *Toxicologic pathology*, *43*(4), pp.482-497.

Ali-Hussain, H.E. 2002. Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa, Linn*, and partially purified product from *Abroma augusta,Linn.* in streptozotocin-induced diabetes. Indian journal of clinical biochemistry: *Indianjournal of clinical biochemistry*, 17(2), pp. 33-43.

Al-Rubaei, Z.M., Mohammad, T.U., and Ali, L.K. 2014. Effects of local curcumin on oxidative stress and total antioxidant capacity in vivo study. *Pakistan Journal of Biological Sciences*, 17(12), pp.1237-1241.

Anilakumar, K.R., Khanum, F., and Bawa, A.S. 2010. Effect of coriander seed powder (CSP) on 1,2-dimethyl hydrazine-induced changes in antioxidant enzyme system and lipid peroxide formation in rats. *Journal of Dietary Supplements*, 7(1), pp. 9-20.

Antoine, F. and Girard, D. 2013. Mechanisms involved in curcumin-induced human neutrophil apoptosis: Evidence that curcumin activates the endoplasmic reticulum stress-induced cell apoptosis pathway. *SAGE Open Medicine*, 1(2050312113488104), pp. 1-7

Anyasor, G.N., Funmilayo, O., Odutola, O., Olugbenga, A., and Oboutor, E.M. 2014. Chemical constituents in n-butanol fractions of *Castus afer ker Gawl* leaf and stem. *Journal of intercultural ethnopharmacology*, 3(2), pp. 78-84.

Aye, M.M., Kilpatrick, E.S., Aburima, A., Wraith, K.S., Magwenzi, S., Spurgeon, B., Rigby, A.S., Sandeman, D., Naseem, K.M. and Atkin, S.L., 2014. Acute hypertriglyceridemia induces platelet hyperactivity that is not attenuated by insulin in polycystic ovary syndrome. *Journal of the American heart association*, 3(e000706), pp. 1-10.

Babu, P.S.,and Srinivasan, K. 1995. Influence of dietary curcumin and cholesterol on the progression of experimentally induced diabetes in albino rats. *Molecular and Cellular Biochemistry*, 152(1), pp.13-21.

Bachmeier, B.E., Killian, P.H., and Melchart, D. 2018. The Role of Curcumin in Prevention and Management of Metastatic Disease. *International journal of molecular sciences*, 19(6), pp.1-18.

Bagad, A. S., Joseph, J. A., Bhaskaran, N., and Agarwal, A. 2013. Comparative Evaluation of Anti-Inflammatory Activity of Curcuminoids, Turmerones, and Aqueous Extract of *Curcuma longa*. *Advances in pharmacological sciences*, 2013(805756), pp.1-7.

Bakker, E.R., Hoekstra, E., Franken, P.F., Helvensteijn, W., Van Deurzen, C.H., Van Veelen, W., Kuipers, E.J. and Smits, R., 2013. β-Catenin signaling dosage dictates tissue-specific tumor predisposition in Apc-driven cancer. *Oncogene*, *32*(38), pp. 4579-4585.

Balkwill, F., Charles, K. and Mantovani, A. 2005. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer cell*, 7(3), pp. 211-217.

Balogun, E., Hoque, M., Pengfei, G., Killeen, E., Green, C. J., Foresti, R., Jawed, A. and Motterlini, R. 2003. Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. *Biochemical Journal*, 371(3), pp.887-895.

Bandyopadhyay, D. 2014. Farmer to pharmacist: curcumin as an anti-invasive and antimetastatic agent for the treatment of cancer. *Frontiers in Chemistry*, 2(113), pp. 1-11.

Basnet, P. and Skalko-Basnet, N. 2011. Curcumin: an anti-inflammatory molecule from a curry spice on the path to cancer treatment. *Molecules*, 16(6), pp. 4567-4598.

Bekusova, V.V., Patsanovskii, V.M., Nozdrachev, A.D., Trashkov, A.P., Artemenko, M.R. and Anisimov, V.N., 2017. Metformin prevents hormonal and metabolic disturbances and 1,2-dimethylhydrazine-induced colon carcinogenesis in non-diabetic rats. *Cancer biology & medicine*, 14(1), pp.100-107.

Bertolotti, M., Lonardo, A., Mussi, C., Baldelli, E., Pellegrini, E., Ballestri, S., Romagnoli, D., and Loria, P. 2014. Nonalcoholic fatty liver disease and aging: epidemiology to management. *Worldjournal of Gastroenterology*, 20(39), pp.14185-14204.

Bhattacharyya, S., Hossain, D.M.S., Mohanty, S., Sen, G.S., Chattopadhyay, S., Banerjee, S., Chakraborty, J., Das, K., Sarkar, D., Das, T. and Sa, G., 2010. Curcumin reverses T cell-mediated adaptive immune dysfunctions in tumor-bearing hosts. *Cellular & molecular immunology*, 7(4), pp. 306-315.

Bhowmik, D., Jayakar, B., Chiranjib, K., Kumar, S. and Chandira, M. 2008. *Curcuma longa*: A Herbal and Traditional Medicine. *Archives of applied science research*, 1(2), pp.86-108.

Brown, M., Lipscomb, J. and Snyder, C. 2001. The burden of illness of cancer: Economic Cost and Quality of Life. *Annual review of Public Health*, 22, pp.91-113.

Bruening, W., Sullivan, N., Paulson, E.C., Zafar, H., Mitchell, M., Treadwell, J. and Schoelles, K., 2014. Imaging tests for the staging of colorectal cancer.

Byrne, K. and Dalgleish, A. 2001. Chronic immune activation and inflammation as the cause of malignancy. *British Journal of Cancer*, 85(4), pp.473-483.

Cadet, J. and Wagner, J.R. 2013. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harbor Perspectives in biology*, 5(2), pp.1-18.

Cassetta, L. and Pollard, J.W. 2018. Targeting macrophages: therapeutic approaches in cancer. *Nature Reviews Drug Discovery*, 17(12), pp.887-904.

Chakravarti, N., Myers, J.N. and Aggarwal, B.B. (2006). Targeting constitutive and interleukin-6-inducible signal transducers and activators of transcription 3 pathway in head and neck

squamous cell carcinoma cells by curcumin (diferuloylmethane). *International Journal of Cancer*, 119(6), pp.1268-1275.

Chandrasekaran, C.V., Kannan Sundarajan, J.R.E., Gururaja, G.M., Mundkinajeddu, D. and Agarwal, A., 2013. Immune-stimulatory and anti-inflammatory activities of *Curcuma longa* extract and its polysaccharide fraction. *Pharmacognosy Research*, 5(2), pp. 71–79.

Chanmee, T., Ontong, P., Konno, K. and Itano, N. 2014. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)*, 6(3), pp.1670-1690.

Chen, H., Zhang, Z.S., Zhang, Y.L. and Zhou, D.Y. 1999. Curcumin inhibits cell proliferation by interfering with the cell cycle and inducing apoptosis in colon carcinoma cells. *Anticancer research*, 19(5A), pp. 3675-3680.

Chen, Y., Wang, W., Zhang, X., Yu, X., Xi, K., Wen, Y., Wang, G., Feng, X. and Zhang, L., 2018. Prognostic significance of combined preoperative platelet-to-lymphocyte ratio and lymphocyte-to-monocyte ratio in patients undergoing surgery with stage IB non-small-cell lung cancer. *Cancer management and research*, 10, pp.5411-5422.

Cheng, C.C., Liao, P.N., Ho, A.S., Lim, K.H., Chang, J., Su, Y.W., Chen, C.G.S., Chiang, Y.W., Yang, B.L., Lin, H.C. and Chang, Y.C., 2018. STAT3 exacerbates survival of cancer stem-like tumorspheres in EGFR-positive colorectal cancers: RNAseq analysis and therapeutic screening. *Journal of biomedical science*, *25*(60), pp.1-12.

Choi, J.W., Kim, S.K. and Pai, S.H. 2001. Changes in Serum Lipid Concentrations during Iron Depletion and after Iron Supplementation. *Annals of clinical & laboratory Science*, 31(2), pp.151-156.

Christie, M., Jorissen, R.N., Mouradov, D., Sakthianandeswaren, A., Li, S., Day, F., Tsui, C., Lipton, L., Desai, J., Jones, I.T. and McLaughlin, S., 2013. Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/β-catenin signaling thresholds for tumorigenesis. *Oncogene*, *32*(39), pp. 4675-4682.

Churchill, M., Chadburn, A., Bilinski, R.T. and Bertagnolli, M.M. 2000. Inhibition of intestinal tumors by curcumin is associated with changes in the intestinal immune cell profile. *The journal of surgical research*, 89(2), pp.169-175.

Cichocki, J.A., Furuya, S., Luo, Y.S., Iwata, Y., Konganti, K., Chiu, W.A., Threadgill, D.W., Pogribny, I.P. and Rusyn, I., 2017. Nonalcoholic fatty liver disease is a susceptibility factor for perchloroethylene-induced liver effects in mice. *Toxicological Sciences*, *159*(1), pp.102-113.

Ciolino, H.P., Daschner, P.J., Wang, T.T. and Yeh, G.C. 1998. Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. *Biochemical pharmacology*, 56(2), pp. 197-206.

Cohen, I. J., and Blasberg, R. 2017. Impact of the Tumor Microenvironment on Tumor-Infiltrating Lymphocytes: Focus on Breast Cancer. *Breast cancer: basic and clinical research*, 11(1178223417731565), pp.1-12.

Colussi, D., Brandi, G., Bazzoli, F. and Ricciardiello, L. 2013. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *International journal of molecular sciences*, 14(8), pp.16365-16385.

Cooke, M.S., Evans, M.D., Dizdaroglu, M. and Lunec, J. 2003. Oxidative DNA damage: mechanisms, mutation, and disease. *The FASEB journal: official publication of American societies for Experimental Biology*, 17(10), pp.1195-1214.

Coppola, J.A., Shrubsole, M.J., Cai, Q., Smalley, W.E., Dai, Q., Ness, R.M., Fazio, S., Zheng, W. and Murff, H.J., 2015. Plasma lipid levels and colorectal adenoma risk. *Cancer Causes & Control*, *26*(4), pp.635-643.

Coussens, L. and Werb, Z. 2002. Inflammation and cancer. *Nature*, 420(6917), pp.860-867

Crispe, N. 2016. Hepatocytes as Immunological Agents. *Journal of immunology*, 196, pp.17-21.

Danciu, C., Vlaia, L., Fetea, F., Hancianu, M., Coricovac, D.E., Ciurlea, S.A., Şoica, C.M., Marincu, I., Vlaia, V., Dehelean, C.A. and Trandafirescu, C., 2015. Evaluation of phenolic profile, antioxidant and anticancer potential of two main representants of Zingiberaceae family against B164A5 murine melanoma cells. *Biological research*, *48*(1), pp.1-9.

Davidson, M.H., Bays, H.E., Stein, E., Maki, K.C., Shalwitz, R.A. and Doyle, R., 2006. Effects of fenofibrate on atherogenic dyslipidemia in hypertriglyceridemic subjects. *Clinical Cardiology: An International Indexed and Peer-Reviewed Journal for Advances in the Treatment of Cardiovascular Disease*, *29*(6), pp.268-273.

Dell'Aquila, E., Cremolini, C., Zeppola, T., Lonardi, S., Bergamo, F., Masi, G., Stellato, M., Marmorino, F., Schirripa, M., Urbano, F. and Ronzoni, M., 2018. Prognostic and predictive role of neutrophil/lymphocytes ratio in metastatic colorectal cancer: a retrospective analysis of the TRIBE study by GONO. *Annals of Oncology*, *29*(4), pp.924-930.

Demir, A.K., Demirtas, A., Kaya, S.U., Tastan, I., Butun, I., Sagcan, M., Sahin, S., Tasliyurt, T. and Yilmaz, A., 2015. The relationship between the neutrophil–lymphocyte ratio and disease activity in patients with ulcerative colitis. *The Kaohsiung journal of medical sciences*, *31*(11), pp.585-590.

Deshpande, U.R., Gadre, S.G., Raste, A.S., Pillai, D., Bhide, S.V. and Samuel, A.M., 1998. Protective effect of *Curcuma longa* (*Curcuma longa L.)* extract on carbon tetrachloride-induced liver damage in rats. *Indian journal of experimental biology*, *36*(6), pp.573-577.

DE-Souza, A., and Costa-Casagrande, T.A. 2018. Animal models for colorectal cancer. *Arquivos brasileiros de cirurgia digestiva* : *ABCD- Brazilian archives of digestive surgery*, 31(2), pp.1-4 .

Dicato, M., Plawny, L., and Diederich, M. 2010. Anemia in cancer. *Annals of Oncology*, 21(7), pp.167-172.

Dinkova-kostova, A.T. and Talalay, P. 1999. Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes. *Carcinogenesis*, 20(5), pp. 911-914.

Donatus, I.A., Sardjoko, N and Vermeulen, N.P. 1990. Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochemical Pharmacology*, 39(12), pp.1869-1875.

Egashira, K., Sasaki, H., Higuchi, S., and Ieiri, I. 2012. Food-drug Interaction of Tacrolimus with Pomelo, Ginger, and *Curcuma longa* Juice in Rats. *Drug metabolism and pharmacokinetics*, 27(2), pp. 242-247.

Eiro, N and Vizoso, F.J. 2012. Inflammation and cancer. *World journal of GastrointestinalSurgery*, 4(3), pp.62-72.

Elhassaneen, Y., Ragab, S. and Badawy, R. 2017. Antioxidant Activity of Methanol Extracts from Various Plant Parts and Their Potential Roles in Protecting the Liver Disorders Induced by Benzo(a)pyrene. *World Journal of Public health*, 2(1), pp.38-50.

Evani, S.J., Prabhu, R.G., Gnanaruban, V., Finol, E.A. and Ramasubramanian, A.K. 2013. Monocytes mediate metastatic breast tumor cell adhesion to endothelium under flow. *Federation of American Societies for Experimental Biology*, 27(8), pp.3017-3029.

Ferramosca, A., Di Giacomo, M., and Zara, V. 2017. Antioxidant dietary approach in treatment of fatty liver: new insights and updates. *World journal of gastroenterology*, 23(23), pp.4146-4157.

Ford, ES., Giles, WH., Mokdad, AH., and Myers, GL. 2004. Distribution and correlates of C-reactive protein concentrations among adult US women. *Clinical Chemistry*, 50(3), pp.574-581.

FU, X., Herrera, H., Kubota, T. and Hoffman, R. 1992. Extensive liver metastasis from human colon cancer in nude and seid mice after orthotopic onplantation of histologically-intact human colon carcinoma tissue. *Anticancer research*, 12(5), pp. 1395-1398.

Furukawa, T., Kubota, T., Watanabe, M., Kuo, T.H., Nishibori, H., Kase, S., Saikawa, Y., Tanino, H., Teramoto, T. and Ishibiki, K. 1993. A metastatic model of human colon cancer constructed using cecal implantation of cancer tissue in nude mice. *Surgery today*, 23(5), pp. 420-423.

Gately, S., and Li, WW. 2004. Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. *Seminars in Oncology*, 31(7), pp.2-11.

Gebreazgaabher, F.G. and Negash, Z.M. 2015. Analysis of Major Factors Affecting Production and Marketing of Korarima (*Aframomum Corrorima* (Braun) P.C.M. Jansen) in Ethiopia. *Journal of Economics and Sustainable Development*, 6(9), pp.72-79.

Giovannucci, E. 2007. Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. *The American journal of Clinical Nutrition*, 86(3), pp.836-842.

GLOBOCAN 2018. Global Cancer Facts & Figures 4th Edition ed. Atlanta: American Cancer Society.

Gothai, S., Muniandy, K., Gnanaraj, C., Ibrahim, I.A.A., Shahzad, N., Al-Ghamdi, S.S., Ayoub, N., Veeraraghavan, V.P., Kumar, S.S., Esa, N.M. and Arulselvan, P., 2018. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomedicine & Pharmacotherapy, 107*, pp.1514-1522.

Gun, S.Y., Lee, S.W.L., Sieow, J.L. and Wong, S.C. 2019. Targeting immune cells for cancer therapy. *Redox Biology*, pp.101-174.

Hagland, HR., Berg, M., Jolma, IW., Carlsen, A., & Soreide, K. 2013. Molecular Pathways and Cellular Metabolism in Colorectal Cancer. *Digestive surgery*, 30(1), pp.12-25.

Hamiza, O.O., Rehman, M.U., Khan, R., Tahir, M., Khan, A.Q., Lateef, A. and Sultana, S., 2014. Chemopreventive effects of aloin against 1, 2-dimethylhydrazine-induced preneoplastic lesions in the colon of Wistar rats. *Human & experimental toxicology*, *33*(2), pp.148-163.

Hamiza, O.O., Rehman, M.U., Tahir, M., Khan, R., Khan, A.Q., Lateef, A., Ali, F. and Sultana, S., 2012. Amelioration of 1, 2 Dimethylhydrazine (DMH) induced colon oxidative stress, inflammation and tumor promotion response by tannic acid in Wistar rats. *Asian Pacific Journal of Cancer Prevention*, *13*(9), pp.4393-4402.

Hatcher, H., Planalp, R., Cho, J., Torti, F. and Torti, S. 2008. Curcumin: from ancient medicine to current clinical trials. *Cellular and molecular life sciences*, 65(11), pp. 1631-1652.

Hegde, K., Haniadka, R., Alva, A., Periera-Colaco, M.M. and Baliga, M.S., 2012. *Curcuma longa* (*C.longa L.*) the Golden Curry Spice as a Nontoxic Gastroprotective Agent. In: Watson, R.R. and Preedy, V.R. eds. A Review. *Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease: Bioactive Foods in Chronic Disease States*, Elsevier, pp.337-348.

Hewlings, S.J. and Kalman, D.S., 2017. Curcumin: a review of its' effects on human health. *Foods*, *6*(10), pp. 1-11.

Ho, A.S., Chen, C.H., Cheng, C.C., Wang, C.C., Lin, H.C., Luo, T.Y., Lien, G.S. and Chang, J., 2014. Neutrophil elastase as a diagnostic marker and therapeutic target in colorectal cancers. *Oncotarget*, *5*(2), pp.473-480.

Houghton, A.M., Rzymkiewicz, D.M., Ji, H., Gregory, A.D., Egea, E.E., Metz, H.E., Stolz, D.B., Land, S.R., Marconcini, L.A., Kliment, C.R. and Jenkins, K.M., 2010. Neutrophil elastase–mediated degradation of IRS-1 accelerates lung tumor growth. *Nature medicine*, *16*(2), pp.219-223

https://www.roche.com/med-colorectal-cancer. [online]: (Accessed date: 28 July 2019)

https://www.zetpilnutrition.com/trying-to-capitalize-on-the-power-of-curcumin.[online]: (Accessed date: 30September 2019)

https://www.hopkinsmedicine.org/gastroenterology_hepatology/_pdfs/small_large_intestine/sporadic_nonhereditary_colorectal_cancer.pdf (Accessed date: 17 December 2019)

Huang, C., Ju, D., Chang, C., Reddy, P. and Velmurugan, B. 2017. A review on the effects of current chemotherapy drugs and natural agents in treating non–small cell lung cancer. *BioMedicine*, 7(4), pp.12-23.

Hussain, M. 2015. Comparative Study on Hematological Changes in Adult and Aged Rats after Curcumin Administration. *Journal of Egyptian Society for Physiological Sciences,* 34(3), pp.357-366.

Hussain, S., Hofseth, L. and Harris, C. 2003. Radical causes of cancer. *Nature Reviews Cancer*, 34, pp.357-366.

Hussein, S.A., El-Senosi, Y., Ragab, M.R. and Hammad, M. 2014. Hypolipidemic effect of curcumin in hyper-cholesterolemic rats. *Benha Veterinary Medical journal*, 27, (2), pp.277-289.

IARC (International Agency for Research on Cancer). 2014.*World cancer report*. Geneva, Switzerland: World health organization.

IARC(International Agency for Research on Cancer). 2018. *Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018*. Geneva, Switzerland: World health organization.

Ida, S., Watanabe, M. and Baba, H. 2015. Chronic inflammation and gastrointestinal cancer. *Journal of Cancer Metastasis and Treatment*, 1(3), pp.138-143.

Iijima, Y. and Joh, A. 2014. Pigment Composition Responsible for the Pale-Yellow Color of Ginger (*Zingiber officinale*) Rhizomes. *Food Science and Technology Research*, 20 (5), pp. 971-978.

Inzaugarat, M.E., De Matteo, E., Baz, P., Lucero, D., García, C.C., Ballerga, E.G., Daruich, J., Sorda, J.A., Wald, M.R. and Cherñavsky, A.C., 2017. New evidence for the therapeutic potential of curcumin to treat nonalcoholic fatty liver disease in humans. *PLoS One*, *12*(3), pp.1-15.

Jagetia, G.C. and Rajanikant, G.K. 2015. Curcumin Stimulates the Antioxidant Mechanisms in Mouse Skin Exposed to Fractionated γ-Irradiation. *Antioxidants*. 4(1), pp.25-41.

Janeen, T., Hillyar, C., Nibber, A.K., Ovadje, P., Hrkal, P., andNibber, T. 2016. The truth about curcumin.*Canada,Advanced orthomolecular research*, pp.1-30.

Jin, C.Y., Lee, J.D., Park, C., Choi, Y.H. and Kim, G.Y. 2007. Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. *Acta Pharmacologica Sinica*, 28(10), pp.1645-1651.

Johnson, R. L., and Fleet, J.C.the 2013. Animal models of colorectal cancer. *Cancer metastasis reviews*, 32(0), pp.39-61.

Johnson, S.M., Gulhati, P., Arrieta, I., Wang, X., Uchida, T., Gao, T. and Evers, B.M., 2009. Curcumin inhibits proliferation of colorectal carcinoma by modulating Akt/mTOR signaling. *Anticancer research*, *29*(8), pp.3185-3190.

Jung, M., Mertens, C., Tomat, E. and Brüne, B. 2019. Iron as a Central Player and Promising Target in Cancer Progression. *International journal of molecular science*, 20(273), pp.1-18.

Jung, U. and Choi, M.S. 2014. Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. *International Journal of Molecular Sciences*, 15(4), pp.6184-6223.

Jurasz, P., Alonso-Escolano, D. aand Radomski, M.W. 2004. Platelet- cancer interactions: mechanisms and pharmacology of tumour cell-induced platelet aggregation. *British journal of pharmacology*, 143(7), pp.819-826.

Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y., Oikawa, S. and Murata, M., 2016. Nitrative and oxidative DNA damage in infection-related carcinogenesis in relation to cancer stem cells. *Genes and environment*, *38*(1), pp.1-12.

Kawatra, S., Taranalli, A., Gaidhani, S., Khanduri, S. and Reddy, E.J. 2015. Protective effect of *Lantana camara L*. Against 1,2-Dimethyl hydrazine induced colon cancer in rats. *World journalof pharmacy and pharmaceutical sciences,* 4(10), pp.845-860.

Khater, S., El-Belbasi, H. and F.Dowidar, M. 2013. Hepatoprotective Activity of Curcumin against Rifampicin-Induced Genotoxicity in Experimental Mice. *Journal of American Science* 9(4), pp.279-288.

Kim, J.H., Gupta, S.C., Park, B., Yadav, V.R. and Aggarwal, B.B. 2012. *Curcuma longa* (*C.longa*) inhibits inflammatory nuclear factor (NF)-κB and NF-κB-regulated gene products and induces death receptors leading to suppressed proliferation, induced chemo-sensitization, and suppressed osteoclastogenesis. *Molecular Nutrition & Food Research,* 56(3), pp.454-65.

Kim, J.Y., Park, K.W., Moon, K.D., Lee, M.K., Choi, J., Yee, S.T., Shim, K.H. and Seo, K.I., 2008. Induction of apoptosis in HT-29 colon cancer cells by crude saponin from Platycodi Radix. *Food and Chemical Toxicology*, *46*(12), pp.3753-3758.

Kisková, T. and Kassayová, M. 2019. Resveratrol Action on Lipid Metabolism in Cancer. *International Journal of Molecular Sciences*, 20(2704), pp.1-20.

Kiso, Y., Suzuki, Y., Watanabe, N., Oshima, Y. and Hikino, H. 1983. Antihepatotoxic principles of *Curcuma longa* rhizomes. *Planta medica*, 49(3), pp.185-187.

Kocaadam, B. and Sanlier, N. 2015. Curcumin, an Active Component of *Curcuma longa* (*C.longa),* and Its Effects on Health. *Critical reviews in food science and nutrition*, 57(13), pp. 2889-2895

Kolligs, F. 2016. Diagnostic and Epidemiology of Colorectal Cancer, *Visceral medicine*, 32, pp. 158-164

Kumar, A., Dhawan, S., Hardegen, NJ., and Aggarwal, BB. 1998. Curcumin (Diferuloylmethane) inhibition of tumor necrosis factor (TNF)-mediated adhesion of monocytes to endothelial cells by suppression of cell surface expression of adhesion molecules and of nuclear factor-kappaB activation. *Biochemical pharmacology*, 55(6), pp.775-783.

Kumar, N. and Sakhya, S.K. 2013. Ethnopharmacological properties of *Curcuma longa*: *A Review international journal of pharmaceutical science and research*, 4(1), pp.103-112.

Kuttan, G., Hari-Kumar, K.B., Guruvayoorappan, C. & Kuttan, R. (2007). Anti-tumor, Anti-invasion, & Anti-metastatic effects of Curcumin. In: Aggarwal, B.B., Surh, Y.J. and Shishodia,

S. eds. *The molecular targets and therapeutic uses of curcumin in health and disease* (Vol. 595). Springer Science & Business Media, pp. 173-184

Lainé, F., Laviolle, B., BardouJacquet, E., Fatih, N., Jezequel, C., Collet, N., Ropert, M., Morcet, J., Hamon, C., Reymann, J.M. and Loréal, O., 2017. Curcuma decreases serum hepcidin levels in healthy volunteers: a placebo controlled, randomized, doubleblind, crossover study. *Fundamental & clinical pharmacology*, *31*(5), pp.567-573.

Landskron, G., De-La-Fuente, M., Thuwajit, P., Thuwajit, C2., and Hermoso, MA. 2014. Chronic Inflammation and Cytokines in the Tumor Microenvironment. *Journal of immunologyresearch*, 2014 (149185), pp.1-19

Larmonier, C.B., Midura-Kiela, M.T., Ramalingam, R., Laubitz, D., Janikashvili, N., Larmonier, N., Ghishan, F.K. and Kiela, P.R., 2010. Modulation of neutrophil motility by curcumin: implications for inflammatory bowel disease. *Inflammatory bowel diseases*, *17*(2), pp.503-515.

Lechner, M.G., Liebertz, D.J. and Epstein, A. L. 2010. characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. *Journal of immunology*, 185(4), pp. 2273-84.

Lee, H.S.2006. Antiplatelet property of *Curcuma longa L.* rhizome-derived ar-turmerone. *Bioresource technology*, 97(12), pp.1372-1376.

Lee, H.Y., Kim, S.W., Lee, G.H., Choi, M.K., Jung, H.W., Kim, Y.J., Kwon, H.J. and Chae, H.J., 2016. *Curcuma longa* extract and its active compound, curcumin, protect against chronic CCl_4-induced liver damage by enhancing antioxidation. *BMC complementary and alternative medicine*, *16*(1), pp.1-9.

Lee, W., Loo, C., Bebawy, M., Luk, F., Mason, R. and Rohanizadeh, R. 2013. Curcumin and its Derivatives: Their Application in Neuropharmacology and Neuroscience in the 21st Century. *Current neuropharmacology*, 11(4), pp.338-378.

Lee, Y.J., Lee, H.R., Nam, C.M., Hwang, U.K. and Jee, S.H. 2006. White blood cell count and the risk of colon cancer. *Yonsei medical journal*, 47(5), pp.646-656.

Leitch, E.F., Chakrabarti, M., Crozier, J.E.M., McKee, R.F., Anderson, J.H., Horgan, P.G. and McMillan, D.C., 2007. Comparison of the prognostic value of selected markers of the systemic inflammatory response in patients with colorectal cancer. *British journal of cancer*, *97*(9), pp.1266–1270.

Li, C.F., Li, J., Bai, P. and Lv, Y.M. 2011. Characteristic analysis of plasma lipid metabolism level in patients with colorectal adenoma. *Journal of Peking university. Health science*,43(3), pp.432-435.

Li, J., Yang, X., Ma, J., Gong, F. and Chen, Q. 2018. Relationship of Red Blood Cell Distribution Width with Cancer Mortality in Hospital. *BioMed Research International*, 2018 (8914617), pp.1-9

Li, M., Yue, G.G.L., Tsui, S.K.W., Fung, K.P. and Lau, C.B.S. 2018. *Curcuma longa* extract, with absorbable curcumin, has potent anti-metastatic effect in vitro and in vivo. *Phytomedicine*, 46, pp.131-141.

Liang, L., Zhu, J., Jia, H., Huang, L., Li, D., Li, Q. and Li, X. 2016. Predictive value of pretreatment lymphocyte count in stage II colorectal cancer and in high-risk patients treated with adjuvant chemotherapy. *Oncotarget*, 7(1), pp. 1014-1028.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, 4(8), pp.118-126.

Loganayaki, N., Siddhuraju, P. and Manian, S.2013. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora L*. and *Ceiba pentandra L*. *Journal of Food Science & Technology,* 50(4),pp.687-95.

Long, J., Zhang, C. J., Zhu, N., Du, K., Yin, Y., Tan, X. and Qin, L. 2018. Lipid metabolism and carcinogenesis, cancer development. *American journal of cancer research*, 8, pp.778-791.

Lou, X.L., Sun, J., Gong, S.Q., Yu, X.F., Gong, R. and Deng, H., 2015. Interaction between circulating cancer cells and platelets: clinical implication. *Chinese Journal of Cancer Research*, *27*(5), pp.450-460.

Lowhan, A writer/producer. 2003. Produced by Orgeon Public Broadcasting; Anneberg Foundation; Corporation for Public Broadcasting; Rediscovering biology: molecular to global perspectives. S. Burlington, VT:Annenberg/CPB.

Macciò, A., Madeddu, C., Gramignano, G., Mulas, C., Tanca, L., Cherchi, M.C., Floris, C., Omoto, I., Barracca, A. and Ganz, T., 2015. The role of inflammation, iron, and nutritional status in cancer-related anemia: results of a large, prospective, observational study. *haematologica*, *100*(1), pp.124-132.

Maeda, H. and Khatami, M., 2018. Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. *Clinical and translational medicine*, 7(1), pp.1-20.

Mansour-Ghanaei, F., Pourmasoumi, M., Hadi, A. and Joukar, F., 2019. Efficacy of curcumin/*Curcuma longa* on liver enzymes in patients with non-alcoholic fatty liver disease: a systematic review of randomized controlled trials. *Integrative Medicine Research*, 8(1), pp.57-61.

Mantovani, A., Allavena, P., Sica, A. and Balkwill, F., 2008. Cancer-related inflammation. *Nature*, 454(24), pp.436-444.

Mcgary, C.T., Miele, M.E. and Welch, D.R. 1995. Highly metastatic 13762NF rat mammary adenocarcinoma cell clones stimulate bone marrow by secretion of granulocyte-macrophage colony-stimulating factor/interleukin-3 activity. *American Journal of Pathology*, 147, pp. 1668-81.

Megaraj, V., Ding, X., Fang, C., Kovalchuk, N., Zhu, Y. and Zhang, Q.Y., 2014. Role of hepatic and intestinal p450 enzymes in the metabolic activation of the colon carcinogen azoxymethane in mice. *Chemical research in toxicology*, *27*(4), pp.656-662.

Mehrotra, R., Pandya, S., Chaudhary, A., Singh, H., Jaiswal, R., Singh, M., Gupta, S. & Singh, M. 2009. Lipid profile in oral submucous fibrosis. *Lipids in Health and Disease*, 8, pp.1-7.

Millonig, G., Ganzleben, I., Peccerella, T., Casanovas, G., Brodziak-Jarosz, L., Breitkopf-Heinlein, K., Dick, T.P., Seitz, H.K., Muckenthaler, M.U. and Mueller, S., 2012. Sustained

submicromolar H_2O_2 levels induce hepcidin via signal transducer and activator of transcription 3 (STAT3). *Journal of Biological Chemistry*, 287(44), pp.37472-37482.

Moghimipour, E. and Handali, S.2015. Saponin: properties, methods of evaluation and applications. *Annual Research & Review in Biology*, 5(3), pp.207-220.

Mohammadi, A., Sahebkar, A., Iranshahi, M., Amini, M., Khojasteh, R., Ghayour-Mobarhan, M., and Ferns, GA.2013. Effects of supplementation with curcuminoids on dyslipidemia in obese patients: a randomized crossover trial. *Phytotherapy research*, 27(3), pp. 374-379.

Mohanty, J., Nagababu, E. and Rifkind, J.M., 2014. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. *Frontiers in physiology*, 5(84), pp. 1-6.

Morceau, F., Dicato, M. and Diederich, M., 2009. Pro-inflammatory cytokine-mediated anemia: regarding molecular mechanisms of erythropoiesis. *Mediators of inflammation*, 2009, pp. 1-11.

Moriguchi, T., Suzuki, M., Engel, J.D. and Yamamoto, M., 2010. GATA1 and GATA2 function in hematopoietic differentiation. In: Kondo, M. eds.*Hematopoietic stem cell biology*. Humana Press.pp. 117-142.

Muhidin, S.O., Magan, A.A., Osman, K.A., Syed, S. and Ahmed, M.H., 2012. The relationship between nonalcoholic fatty liver disease and colorectal cancer: the future challenges and outcomes of the metabolic syndrome. *Journal of obesity*, 2012, pp.1-8.

Mujeeb, F., Bajpai, P. and Pathak, N., 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed researchinternational*, 2014, pp.1-11.

Nagpal, M. and Sood, S., 2013. Role of curcumin in systemic and oral health: An overview. *Journal of natural science, biology, and medicine*, *4*(1), pp. 3-7.

Nasri, H., Sahinfard, N., Rafieian, M., Rafieian, S., Shirzad, M. and Rafieian-Kopaei, M., 2014. *Curcuma longa*: A spice with multifunctional medicinal properties. *Journal of Herbal medicine Pharmacology*, 3(1), pp. 5-8

OECD (Organization for Economic Co-operation and Development) Guideline for testing of chemicals. 2001.

Oghenejobo, M., Opajobi, O.A., Bethel, O.U.S. and Uzoegbu, U., 2017. Antibacterial Evaluation, Phytochemical Screening and Ascorbic Acid Assay of *Curcuma longa* (*C.longa*). *MOJ Bioequivalence & Bioavailability*, *4*(2), pp.232-239.

Ozdemir, Y., Akin, M.L., Sucullu, I., Balta, A.Z. and Yucel, E., 2014. Pre-treatment neutrophil/lymphocyte ratio as a prognostic aid in colorectal cancer. *Asian Pacific Journal of CancerPrevention*, 15(6), pp.2647-2650.

Ozgehan, G., Kahramanca, S., Kaya, I.O., Bilgen, K., Bostanci, H., Guzel, H., Kucukpinar, T.H. and Kargici, H., 2014. Neutrophil-lymphocyte ratio as a predictive factor for tumor staging in colorectal cancer. *Turkish journal of medical sciences*, *44*(3), pp.365-368.

Pandey, A., Gupta, R.K. and Srivastava, R., 2011. Curcumin-the yellow magic. *Asian Journal of Applied Sciences*, *4*(4), pp.343-354.

Park, E.J., Jeon, C.H., Ko, G., Kim, J. and Sohn, D.H., 2000. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *Journal of Pharmacy and Pharmacology*, *52*(4), pp.437-440.

Park, J. and Conteas, C.N., 2010. Anti-carcinogenic properties of curcumin on colorectal cancer. *World journal of gastrointestinal oncology*, *2*(4), pp.169-176.

Parsa, N., 2012. Environmental factors inducing human cancers. *Iranian journal of public health*, *41*(11), pp.1-9.

Patel, V.B., Misra, S., Patel, B.B. and Majumdar, A.P., 2010. Colorectal cancer: chemo-preventive role of curcumin and resveratrol. *Nutrition and cancer*, *62*(7), pp.958-967.

Pawar, M.A., Patil, S.S. and Nagrik, D.M., 2015. Phytochemical and physicochemical investigation of *Curcuma longa Linn*. rhizome. *International Journal of Chemical and Physical Sciences*, *4*, pp.458-463.

Pearce, A., Sharp, L., Hanly, P., Barchuk, A., Bray, F., De Camargo Cancela, M., Gupta, P., Meheus, F., Qiao, Y.L., Sitas, F. and Wang, S.M.2018. Productivity losses due to premature mortality from cancer in Brazil, Russia, India, China, and South Africa (BRICS): a population-based comparison. *Cancer epidemiology*, *53*, pp.27-34.

Peethambaran, C., Hailemichael, G., Tessema, H. and Kifelew, H. 2016. Ethiopia Adores *Curcuma longa* in Hearth and Fields. *Spices India English*. 29, pp.18-23.

Perrone, D., Ardito, F., Giannatempo, G., Dioguardi, M., Troiano, G., Lo Russo, L., De Lillo, A., Laino, L. and Lo Muzio, L., 2015. Biological and therapeutic activities, and anticancer properties of curcumin. *Experimental and therapeutic medicine*, *10*(5), pp.1615-1623.

Perse, M. and Cerar, A., 2005. The dimethylhydrazine induced colorectal tumours in rat-experimental colorectal carcinogenesis. *Radiology and Oncology*, *39*(1), pp. 61-70.

Perse, M. and Cerar, A., 2010. Morphological and molecular alterations in 1, 2 dimethylhydrazine and azoxymethane induced colon carcinogenesis in rats. *BioMed Research International*, 2011, pp. 1-14.

Pizzorno, J. & murray, M., 1999. Detoxification. In: Pizzorno, J. & murray, M. (eds.) *Textbook of Natural Medicine,*pp. 438-450

Plantureux, L., Mège, D., Crescence, L., Dignat-George, F., Dubois, C. and Panicot-Dubois, L., 2018. Impacts of cancer on platelet production, activation and education and mechanisms of cancer-associated thrombosis. *Cancers*, *10*(11), pp.1-23.

Plummer, S.M., Holloway, K.A., Manson, M.M., Munks, R.J., Kaptein, A., Farrow, S. and Howells, L., 1999. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-κB activation via the NIK/IKK signalling complex. *Oncogene*, *18*(44), pp.6013- 6020.

Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C. and Ramirez-Tortosa, M., 2016. Curcumin and health. *Molecules*, *21*(3), pp.1-22.

Qi, G., Zeng, S., Takashima, T., Nozoe, K., Shobayashi, M., Kakugawa, K., Murakami, K., Jikihara, H., Zhou, L. and Shimamoto, F. 2015. Inhibitory effect of various breads on DMH-induced aberrant crypt foci and colorectal tumours in rats. *BioMedical research international*, 2015,pp. 1-8.

Qin, S., Huang, L., Gong, J., Shen, S., Huang, J., Ren, H. and Hu, H., 2017. Efficacy and safety of *Curcuma longa* and curcumin in lowering blood lipid levels in patients with cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Nutrition journal*, *16*(1), pp.68.

Rabinovich, G.A., Gabrilovich, D. and Sotomayor, E.M., 2007. Immunosuppressive strategies that are mediated by tumor cells. Rabinovich, G.A., Gabrilovich, D. and Sotomayor, E.M., 2007. Immunosuppressive strategies that are mediated by tumor cells. *Annual Review of Immunology*, *25*, pp.267-296.

Radhakrishnan, V.M., Kojs, P., Young, G., Ramalingam, R., Jagadish, B., Mash, E.A., Martinez, J.D., Ghishan, F.K. and Kiela, P.R., 2014. pTyr421 cortactin is overexpressed in colon cancer and is dephosphorylated by curcumin: involvement of non-receptor type 1 protein tyrosine phosphatase (PTPN1). *PLoS One*, *9*(1), p.e85796.

Rahman, K., 2007. Studies on free radicals, antioxidants, and co-factors. *Clinical interventions in aging*, *2*(2), pp.219.

Rajesh, H., Rao, S.N., Megha, R., Prathima, K., Rejeesh, E. and Chandrashekar, R., 2013. Phytochemical analysis of methanolic extract of *Curcuma longa Linn* rhizome. *International Journal of Universal Pharmacy and Bio Sciences*, *2*(2), pp.39-45.

Rao, C.V., Simi, B. and Reddy, B.S., 1993. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. *Carcinogenesis*, *14*(11), pp.2219-2225.

Rashidi, B., Sun, F., Jiang, P., An, Z., Gamagami, R., Moossa, A. and Hoffman, R. 2000. A nude mouse model of massive liver and lymph node metastasis of human colon cancer. Anticancer research, 20, pp.715-722.

Reyes-Gordillo, K., Shah, R., Lakshman, M.R., Flores-Beltran, R.E. and Muriel, P., 2017. Hepatoprotective properties of curcumin. In: *Liver Pathophysiology* Academic Press, pp. 687-704.

Richards, D.M., Hettinger, J. and Feuerer, M., 2013. Monocytes and macrophages in cancer: development and functions. *Cancer microenvironment*, 6(2),pp.179-191.

Robinson, M.W., Harmon, C. and O'Farrelly, C., 2016. Liver immunology and its role in inflammation and homeostasis. *Cellular & molecular immunology*, 13(3), pp.267.

Rosenberg, D.W., Giardina, C. & Tanaka, T. 2009. Mouse models for the study of colon carcinogenesis. *Carcinogenesis*,30(2), pp.183-96.

Roy, A., Ahuja, S. & Bharadvaja, N. 2017. A Review on Medicinal Plants against Cancer. *Journal of Plant Sciences and Agricultural Research*, 2 (1:008), pp.1-5.

Sahebkar, A., 2014. Are curcuminoids effective C-reactive proteinlowering agents in clinical practice? Evidence from a meta-analysis. *Phytotherapy research*, *28*(5), pp.633-642.

Sahebkar, A., 2014. Curcuminoids for the management of hypertriglyceridaemia. *Nature Reviews Cardiology*, *11*(2), pp.123.

Sahebkar, A., 2014. Low-density lipoprotein is a potential target for curcumin: Novel mechanistic insights. *Basic & clinical pharmacology & toxicology*, *114*(6), pp.437-438.

Saifi, M.A., Alyousif, M.S. and Ahmed, M., 2015. Biochemical investigations on the protective role of curcumin in liver damage by chloroquine. *International Journal of Pharmacology*, *11*(7), pp.870-873.

Sakarkar, D.M. and Deshmukh, V.N., 2011. Ethnopharmacological review of traditional medicinal plants for anticancer activity. *International Journal of PharmTech Research*, *3*(1), pp.298-308.

Salomone, F., Godos, J. and Zelber-Sagi, S., 2016. Natural antioxidants for non-alcoholic fatty liver disease: molecular targets and clinical perspectives. *Liver International*, *36*(1), pp.5-20.

Santoshkumar, J., Mariguddi, D.D. and Manjunath, S., 2016. Comparative study of hypolidemic effects of ethanolic extract of rhizomes of *Curcuma longa* (*C.longa*) versus pioglitazone in alloxan induced diabetic rats. *International Journal of Pharmacology*, *5*(1), pp.5-11.

Sathyanarayanan, S., Chandran, R., Thankarajan, S., Abrahamse, H. and Thangaraj, P., 2018. Phytochemical composition, antioxidant and anti-bacterial activity of *Syzygium calophyllifoliumWalp*. fruit. *Journal of food science and technology*, *55*(1), pp.341-350.

Saxena, J. and Sahu, R., 2012. Evaluation of phytochemical constituent in conventional and non-conventional species of *curcuma*. *International Research Journal of Pharmacy*, 3(8), pp.203-204.

Scapagnini, G., Colombrita, C., Amadio, M., D'agata, V., Arcelli, E., Sapienza, M., Quattrone, A. and Calabrese, V. 2006. Curcumin activates defensive genes and protects neurons against oxidative stress. *Antioxidants & redox signaling*, 8, pp.395-403.

Schrijvers, D., 2011. Management of anemia in cancer patients: transfusions. *The oncologist*, *16*(3), pp.12-18.

Seifu, E. and Tassew, A., 2014. Small-scale milk processing, utilization and marketing of traditional dairy products in Bahir Dar Zuria and Mecha districts, northwestern Ethiopia. *Journal of Food Technology Research*, *1*(3), pp.122-132.

Senapathy, J. G., JayanthI, S., Viswanathan, P., Umadevi, P. and Nalini, N. 2011. Effect of gallic acid on xenobiotic metabolizing enzymes in 1, 2-dimethyl hydrazine induced colon carcinogenesis in Wistar rats–a chemopreventive approach. *Food and chemical toxicology*, 49, pp.887-892.

Shakeri, F., Soukhtanloo, M. and Boskabady, M.H., 2017. The effect of hydro-ethanolic extract of *Curcuma longa rhizome* and curcumin on total and differential WBC and serum oxidant, antioxidant biomarkers in rat model of asthma. *Iranian journal of basic medical sciences*, *20*(2), p.155.

Shang, S., Hua, F. and Hu, Z.W., 2017. The regulation of β-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget*, 8(20), pp.33972-33989.

Shankar, A., Mitchell, P., Rochtchina, E. and Wang, J.J., 2007. The association between circulating white blood cell count, triglyceride level and cardiovascular and all-cause mortality: population-based cohort study. *Atherosclerosis*, *192*(1), pp.177-183.

Sharma, A. and Sharma, K.K., 2011. Chemoprotective role of triphala against 1,2-dimethylhydrazine dihydrochloride induced carcinogenic damage to mouse liver. *Indian Journal of Clinical Biochemistry*, *26*(3), pp.290-295.

Sharma, R.A., McLelland, H.R., Hill, K.A., Ireson, C.R., Euden, S.A., Manson, M.M., Pirmohamed, M., Marnett, L.J., Gescher, A.J. and Steward, W.P., 2001. Pharmacodynamic and pharmacokinetic study of oral *Curcuma* extract in patients with colorectal cancer. *Clinical Cancer Research*, *7*(7), pp.1894-1900.

Sheel, R., Nisha, K. and Kumar, J., 2014. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. *IOSR Journal of Applied Chemistry*, *7*(1), pp.10-3.

Shen, H., Lipka, S., Kumar, A. and Mustacchia, P., 2014. Association between nonalcoholic fatty liver disease and colorectal adenoma: a systemic review and meta-analysis. *Journal of gastrointestinal oncology*, *5*(6), pp.440.

Shen, Y., Wang, C., Ren, Y. and Ye, J., 2018. A comprehensive look at the role of hyperlipidemia in promoting colorectal cancer liver metastasis. *Journal of Cancer*, *9*(16), pp.2981.

Sheweita, S. and Tilmisany, A. 2003. Cancer and phase II drug-metabolizing enzymes. *Currentdrug metabolism*, 4, pp.45-58.

Shibutani, M., Maeda, K., Nagahara, H., Fukuoka, T., Nakao, S., Matsutani, S., Hirakawa, K. and Ohira, M., 2017. The peripheral monocyte count is associated with the density of tumor-associated macrophages in the tumor microenvironment of colorectal cancer: a retrospective study. *BMC cancer*, *17*(1), p.404.

Shrivastava, S., Singh, N., Nigam, A.K., Chandel, S.S., Shrivastava, R. and Kumar, S., 2017. Comparative study of hematological parameters along with effect of chemotherapy and radiotherapy in different stages of breast cancer.*International Journal of Research in Medical Sciences*, *5(1)*, pp.311-315.

Singh, S. and Aggarwal, B.B. 1995. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane). *Journal of biological chemistry*,270(42), pp.24995-5000.

Sisein, EA., Diepreye, E ., and AL., O.R.E., 2016. *Garcinia kola* extract (kolaviron) prevents DMH induced liver damage in wistar rats by restoring antioxidants. *Galore International Journalof Health Sciences*, 1(1), pp.18-24.

Soler-rivas, C., Espín, J.C. and Wichers, H.J. 2000. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochemical Analysis*, 11(5), pp.330-338.

Song, Y., Huang, Z., Kang, Y., Lin, Z., Lu, P., Lin, Q., Cai, Z., Cao, Y. and Zhu, X., 2018. Clinical Usefulness and Prognostic Value of Red Cell Distribution Width in Colorectal Cancer. *BioMed research international*, 2018(9858943), p.7

Soni, K.B., Rajan, A. and Kuttan, R., 1992. Reversal of aflatoxin induced liver damage by *Curcuma longa* and curcumin. *Cancer Letters*, *66*(2), pp.115-121.

Srivastava, R., 1989. Inhibition of neutrophil response by curcumin. *Agents and actions*, *28*(3-4), pp.298-303.

Stenvinkel, P., Heimbürger, O., Lönnqvist, F. and Bárány, P., 1998. Does the ob gene product leptin stimulate erythropoiesis in patients with chronic renal failure? *Kidney international*, *53*(5), pp.1430-1431.

Susan, M. and Rao, M. 1992. Induction of glutathione S-transferase activity by curcumin in mice. *Arzneimittel-Forschung*, 42, pp.962-964.

Tanaka, T., 2009. Colorectal carcinogenesis: Review of human and experimental animal studies. *Journal of carcinogenesis*, *8*, pp.5-5.

Tariq, K. and Ghias, K., 2016. Colorectal cancer carcinogenesis: a review of mechanisms. *Cancer biology & medicine*, *13*(1), p.120.

Tayyem, R.F., Heath, D.D., Al-Delaimy, W.K. and Rock, C.L., 2006. Curcumin content of *Curcuma longa* and curry powders. *Nutrition and cancer*, *55*(2), pp.126-131.

Thangaraj, K., Natesan, K., Palani, M. and Vaiyapuri, M. 2018. Orientin, a flavanoid, mitigates 1,2 dimethylhydrazine-induced colorectal lesions in Wistar rats fed a high-fat diet. *Toxicology reports*, 5, pp.977-987.

Tomeh, M.A., Hadianamrei, R. and Zhao, X., 2019. A review of curcumin and its derivatives as anticancer agents. *International journal of molecular sciences*, *20*(5), p.1033.

Tuasha, N., Petros, B. and Asfaw, Z., 2018. Plants Used as Anticancer Agents in the Ethiopian Traditional Medical Practices: A Systematic Review. *Evidence-Based Complementary and Alternative Medicine*, 2018(6274021), p.28.

Tuorkey, M., 2014. Curcumin a potent cancer preventive agent: Mechanisms of cancer cell killing. *Interventional Medicine and Applied Science*, *6*(4), pp.139-146.

Tuyaerts, S., Rombauts, K., Everaert, T., Van Nuffel, A.M.T. and Amant, F., 2018. A phase 2 study to assess the immunomodulatory capacity of a lecithin-based delivery system of curcumin in endometrial cancer. *Frontiers in nutrition*, *5*, p.138.

Ulmer, H., Borena, W., Rapp, K., Klenk, J., Strasak, A., Diem, G., Concin, H. and Nagel, G., 2009. Serum triglyceride concentrations and cancer risk in a large cohort study in Austria. *British journal of cancer*, *101*(7), p.1202.

Ung, V.Y., Foshaug, R.R., MacFarlane, S.M., Churchill, T.A., Doyle, J.S., Sydora, B.C. and Fedorak, R.N., 2010. Oral administration of curcumin emulsified in carboxymethyl cellulose has a potent anti-inflammatory effect in the IL-10 gene-deficient mouse model of IBD. *Digestive diseases and sciences*, *55*(5), pp.1272-1277.

Uribe-Querol, E. and Rosales, C., 2015. Neutrophils in cancer: two sides of the same coin. *Journal of immunology research*, *2015* (983698), p.21.

Usman, H., Abdulrahman, F.I. and Usman, A., 2009. Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines*, *6*(3), pp.289-95.

Vachharajani, V., Wang, S.W., Mishra, N., El-Gazzar, M., Yoza, B. and McCall, C., 2010. Curcumin modulates leukocyte and platelet adhesion in murine sepsis. *Microcirculation*, *17*(6), pp.407-416.

Vallianou, N.G., Evangelopoulos, A., Schizas, N. & Kazazis, C. 2015. Potential Anticancer Properties and Mechanisms of Action of Curcumin. *Anticancer Research*,35, pp.645-652.

Van Oostrom, A.J.H.H.M., Rabelink, T.J., Verseyden, C., Sijmonsma, T.P., Plokker, H.W.M., De Jaegere, P.T. and Cabezas, M.C., 2004. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis*, *177*(1), pp.175-182.

Vimalkumar, C.S., Hosagaudar, V.B., Suja, S.R., Vilash, V., Krishnakumar, N.M. and Latha, P.G., 2014. Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Oleadioica Roxb*., infected with the rust fungus *Zaghouania oleae* (EJ Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and phytochemistry*, *3*(4), pp.69-72.

Wahlang, B., Beier, J.I., Clair, H.B., Bellis-Jones, H.J., Falkner, K.C., McClain, C.J. and Cave, M.C., 2013. Toxicant-associated steatohepatitis. *Toxicologic pathology*, *41*(2), pp.343-360.

Walsh, S.R., Cook, E.J., Goulder, F., Justin, T.A. and Keeling, N. J. 2005. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *Journal of surgical oncology*, 91(3), pp.181-184.

Wanten, G.J., Naber, A.H., Kruimel, J.W., Tool, A.T., Roos, D. and Jansen, J.B., 1999. Influence of structurally different lipid emulsions on human neutrophil oxygen radical production. *European journal of clinical investigation*, *29*(4), pp.357-363.

Wanten, G.J.A., Janssen, F.P. and Naber, A.H.J., 2002. Saturated triglycerides and fatty acids activate neutrophils depending on carbon chain length. *European journal of clinical investigation*, *32*(4), pp.285-289.

Watanabe, K., Biesinger, J., Salmans, M.L., Roberts, B.S., Arthur, W.T., Cleary, M., Andersen, B., Xie, X. and Dai, X., 2014. Integrative ChIP-seq/microarray analysis identifies a CTNNB1 target signature enriched in intestinal stem cells and colon cancer. *PloS one*, *9*(3), p.e92317.

Watt, DG., Martin, JC., Park, JH., Horgan, PG., and McMillan, DC. 2015. Neutrophil count is the most important prognostic component of the differential white cell count in patients undergoing elective surgery for colorectal cancer. *The American journal of surgery*, 210(1), pp.24-30.

WHO, 1997. Guidelines for the assessment of herbal medicines. In: *Quality assurance of pharmaceuticals: a compendium of guidelines and related materials*., 1, pp.31–37.

WHO, 2005. National policy on traditional medicine and regulation of herbal medicines.*Report of WHO global survey.*http://whqlibdoc.who.int/publications/2005/9241593237.pdf

WHO, 2018. Cancer .https://www.who.int/news-room/fact-sheets/detail/cancer

Williams, C.B., Yeh, E.S. and Soloff, A.C., 2016. Tumor-associated macrophages: unwitting accomplices in breast cancer malignancy. *NPJ breast cancer*, *2*, p.15025.

Wojtukiewicz, M.Z., Sierko, E., Hempel, D., Tucker, S.C. and Honn, K.V., 2017. Platelets and cancer angiogenesis nexus. *Cancer and Metastasis Reviews*, *36*(2), pp.249-262.

Wu, H.O.N.G., Reynolds, A.B., Kanner, S.B., Vines, R.R. and Parsons, J.T., 1991. Identification and characterization of a novel cytoskeleton-associated pp60src substrate. *Molecular and cellular biology*, *11*(10), pp.5113-5124.

Wu, L., Saxena, S., Awaji, M. and Singh, R.K., 2019. Tumor-associated neutrophils in cancer: going pro. *Cancers*, *11*(4), p.564.

Wu, Q., Hu, T., Zheng, E., Deng, X. and Wang, Z., 2017. Prognostic role of the lymphocyte-to-monocyte ratio in colorectal cancer: an up-to-date meta-analysis. *Medicine*, *96*(22), p.e7051.

Wu, Y., Zhao, Q., Peng, C., Sun, L., Li, X.F. and Kuang, D.M., 2011. Neutrophils promote motility of cancer cells via a hyaluronanmediated TLR4/PI3K activation loop. *The Journal of pathology*, *225*(3), pp.438-447.

WWW.WCRF.ORG. 2018. Colorectal cancer statistics [Online].

Yang, M.H., Rampal, S., Sung, J., Choi, Y.H., Son, H.J., Lee, J.H., Kim, Y.H., Chang, D.K., Rhee, P.L., Kim, J.J. and Rhee, J.C., 2013. The association of serum lipids with colorectal adenomas. *The American journal of gastroenterology*, *108*(5), p.833.

Yang, Y.S., Su, Y.F., Yang, H.W., Lee, Y.H., Chou, J.I. and Ueng, K.C., 2014. Lipid lowering effects of curcumin in patients with metabolic syndrome: a randomized, double blind, placebo controlled trial. *Phytotherapy research*, *28*(12), pp.1770-1777.

Youssef, K.M., Ezzo, A.M., El-Sayed, M.I., Hazzaa, A.A., EL-Medany, A.H. and Arafa, M., 2015. Chemopreventive effects of curcumin analogs in DMH-Induced colon cancer in albino rats model. *Future Journal of Pharmaceutical Sciences*, *1*(2), pp.57-72.

Zhang, X., Zhao, X.W., Liu, D.B., Han, C.Z., Du, L.L., Jing, J.X. and Wang, Y., 2014. Lipid levels in serum and cancerous tissues of colorectal cancer patients. *World Journal of Gastroenterology: WJG*, *20*(26), p.8646.

Zhou, H., SBeevers, C. and Huang, S., 2011. The targets of curcumin. *Current drug targets*, *12*(3), pp.332-347.

Zhu, X., Cao, Y., Lu, P., Kang, Y., Lin, Z., Hao, T. and Song, Y., 2018. Evaluation of platelet indices as diagnostic biomarkers for colorectal cancer. *Scientific reports*, *8*(1), p.11814.

Zingg, J.M., Hasan, S.T. and Meydani, M., 2013. Molecular mechanisms of hypolipidemic effects of curcumin. *Biofactors*, *39*(1), pp.101-121.