ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF MEDICINE
DEPARTMENT OF ANATOMY

Evaluation of acute and subacute dermal toxicity of aerial part *Cymbopogon nardus* formulation on hematological, clinical chemistry and histopathology of skin, kidneys and liver of Albino Wistar rats

BY:- DAGIM TSEGAYE (BSc)

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Thesis Submission Form

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LIST OF ABBERVIATIONS and ACRONYMS

AAU Addis Ababa University

CHS Collage of Health Science

EDTA Ethylenediaminetetraacetic Acid

EPA Environmental Protective Agency

EPHI Ethiopian Public Health Institute

HCT Hematocrit

HGB Hemoglobin

L Lymphocyte

LD_{50} Lethal Dose kill 50 % of the animals

MCHC Mean corpuscular hemoglobin concentration

MCV Mean corpuscular volume of male

OECD Organization of Cooperative Economic Development

PLT Platelet

RBC Red blood cell

SEM Standard Error of Mean

TM Traditional Medicine

WBC White blood cell

WHO World Health Organization

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SUMMARY

Background, Traditional medicine is the culmination of generations of indigenous system of medicine practitioners' therapeutic experiences. Although over 2,000 plants have been identified in Africa and are utilized to cure a wide range of ailments, only few of these plants have had their safety thoroughly investigated. Ethiopians have historically utilized plants as medicine, although little is known about the characteristics and range of these traditional practices.

Objective, The aim of this study was to Evaluate of acute and subacute dermal toxicity of aerial part *Cymbopogon nardus* formulation on hematological, clinical chemistry and histopathology of skin, kidneys and liver of Albino Wistar rats.

Methodology, The plant material was harvested about 270 kilometers south of Addis Ababa, in the Wondogenet region surrounding Shashemene town. The Armaeur Hansen Research Institute's (AHRI) traditional and modern medicine research directorate provided the formulation. Essential oil extraction was undertaken by taking the fresh plant material and subjecting it to hydro-distillation for 2 hours. The plant used Clevenger-Arm equipment to carry out the hydro-distillation process. A round-bottom flask weighing 500g of plant material was set on a heating mantle, and the flask was attached to the Clevenger-Arm apparatus after two liters of water were added. The final yield of the oil weighed was 4 ml and the formulation was done with some additives containing 2% of the oil of *cymbopogon nardus* and 98% of petrolatum stored in refrigerator at 2-8ºC until it is used for the experiment.

For acute dermal toxicity test five female rats of age 8-12 week were used , For subacute dermal test 10 animals (5 female and 5 male) with healthy skin were used at each dose level. The females were nulliparous and non-pregnant. Three dose were used, with a control and, petrolatum was used as a vehicle

Result, The dermal mean lethal dose LD_{50} of the C.nardus formulation was found to be above 2000mg/kg but, no mortality sign was observed during the 14 days of study. 28 days dermal application of *C.nardus* formulation did not produce any toxicity on the behavioral, physical, biological, hematological analytes and gross pathology of the rats at treated dose compared to the control groups.

Conclusion, C. nardus formulation has no dermal toxicity and therefore presents a low toxicological risk. This study concludes that topical application of Rats do not experience systemic toxic responses or acute or subacute negative skin effects from the C. nardus formulation.

Key word, *Cymbopogon nardus* , acute, subacute dermal, sub chronic , toxicity.

1. INTRODUCTION

1.1. BACKGROUND

The utilization of medicinal plants as a form of medical treatment can be traced back to the earliest periods of human history. It stands as a foundational practice in the realm of healthcare, predating the advent of modern pharmaceuticals. Even in contemporary times, herbal or plant-based medicine remains a primary method of treatment for a significant portion of the global population, constituting approximately 80% of the populace (1).

In this approach, various parts of specific plants, such as the roots, leaves, fruits, flowers, or seeds, are commonly employed in the preparation of beneficial substances. These plant ingredients are processed and refined in a variety of ways, such as compressing them into tablets or pills, infusing them into teas, extracting them into different forms like tinctures, or combining them with ointments and lotions. It is indisputable that plant-based components play a significant role in modern medicine, with many traditional treatments having their origins in herbal remedies. Surprisingly, at least one active ingredient in a significant number of contemporary prescription drugs—roughly 25%—can be linked to a plant-derived source. This enduring relationship between human health and medicinal plants highlights the enduring value and efficacy of plant-based medicine throughout human civilization (2) .

Any culturally-derived ideas and practices used to promote health as well as prevent, identify, treat, or alleviate physical and mental illness are referred to as traditional medicine furthermore, traditional medicine is the culmination of generations of indigenous medicine practitioners' therapeutic experiences (1). More than 2,000 plants have been found in Africa and are used as treatments for a variety of illnesses, but only a small number of these plants have been examined for their safety (3). About 80% of the population in Ethiopia relies on traditional medicine (4).

The oil extracts derived from medicinal plants have emerged as versatile resources applicable across a spectrum of sectors encompassing pharmaceuticals, biomedicine, cosmetics, food, veterinary, and agriculture. These extracts serve as potent substitutes or adjuncts, contributing to enhanced efficacy in various applications. Particularly, the utilization of these oils for both prevention and treatment of a wide array of ailments has witnessed a notable surge in popularity. Notably, diseases transmitted by mosquitoes, such as Malaria, filariasis, yellow fever, dengue, and Japanese encephalitis, represent a significant global health concern, causing substantial

morbidity and mortality in both human and animal populations. This underscores the urgent need for effective mosquito repellents. However, existing literature highlights the adverse effects associated with synthetic repellents, revealing instances of skin eruptions, contact urticaria, and even toxic encephalopathy in children, prompting a reevaluation of their use and encouraging exploration of natural alternatives derived from medicinal plants (5).

Cymbopogon nardus oil extracts, also known as citronella grass, have received widespread attention for their medicinal benefits and numerous applications in traditional and modern medicine. *Cymbopogon nardus* is well-known for its natural insect repellent properties, making it an important ingredient in mosquito repellents and similar products. This plant's essential oil is known to include chemicals such as citronellal and geraniol, both of which have high insecticidal qualities. Citronella oil has a wide range of potential medical applications because of its antifungal, antibacterial, and anti-inflammatory properties in addition to its insect repellent properties (6). It has been used to treat a variety of diseases, including fevers, headaches, muscle aches, and digestive difficulties. Furthermore, citronella oil is frequently used in aromatherapy and relaxation treatments, helping to reduce stress and improve mental well-being (7). The varied therapeutic advantages of *Cymbopogon nardus* oil extracts highlight its importance in both traditional and modern medical treatments.

However, despite the fact that *Cymbopogon nardus* possessed well-grounded activity pertaining to topical application, the toxicity profile, especially, the dermal toxicity is yet not well studied. Hence, this study tried to establish the sub-acute and dermal toxicity profile of *Cymbopogon nardus* formulation.

1.2. Statement of problem

Plants that are consumed by humans contain a large number of compounds that have been proven to be harmful (8). When it comes to quantity, almost all substances may be classified as poisons; that is, they are dangerous at certain doses but not harmful at lower ones. Between these two thresholds, there are a number of possible outcomes that might occur, from moderate chronic poisoning over time to immediate death (8).

Ethiopia has a long history of using plants as medicine, but understanding of the scope and qualities of traditional healing methods is limited (4). Therefore the aim of this study is to assess the dermal toxic effects of leaves of *Cymbopogon nardus* formulation *on* some blood parameters and histopathologic profile of liver, skin and kidney on the rat model. There is no dermal toxicity profile on cymbopogon nardus formulation,the aim of this study will be to fill this gap.

1.3. Significance of the study

Since ancient times, aromatic herbs have been utilized for their therapeutic benefits.. However, there are several references in the literature to the harmful consequences of synthetic repellents, such as skin rashes, contact dermatitis, or toxic encephalopathy in children (5). More than 2,000 plants have been found in Africa and are used as treatments for a variety of illnesses, but only a small number of these plants have been examined for their safety (3).

In many parts of our nation, the *Cymbopogon nardus* plant is one of the most widely utilized traditional plants. On the other hand, society's knowledge of acute and subacute skin toxicity profiles is somewhat limited. This study aims to assess the dermal toxicity profile of a formulation of *Cymbopogon nardus.*

By creating ointments that repel mosquitoes, the data gathered from this study may be utilized as proof in the fight against malaria and other diseases spread by mosquitoes. Researchers might also use it as a baseline for more research in the field.

2. LITRATURE REVIEW

2.1 Cymbopogon nardus (C. nardus)

The genus Cymbopogon, a fascinating botanical group, encompasses an impressive array of 45 species of grasses categorized under the Poaceae family. Originating from India and tropical Asia, these grasses have established their significance in various domains due to their distinct properties. The Cymbopogon genus is noted for its perennial nature, often showcasing robust growth, reaching heights of up to 2.5 meters. This remarkable growth is facilitated by the presence of numerous stiff leafy stems, emerging from short rhizomatous roots, ensuring the plant's resilience and vitality. One intriguing aspect of Cymbopogon is its growth cycle, which typically spans several months. The initial harvest of these plants is typically observed around 4 to 6 months after planting. Subsequently, a structured harvest schedule is established, with subsequent harvests occurring at intervals of 2 to 3 months. The harvesting process itself is meticulous, involving cutting the plant at approximately 20 cm above the ground level. This careful approach to harvesting not only encourages regrowth but also ensures the preservation of the plant for future harvests, contributing to sustainable utilization. The careful management of Cymbopogon species underscores their importance and the strategic methods employed to maximize their yield and utility in various applications (9).The better weather for growing lemongrass is a warm and humid climate full sun and 250-330 cm of rainfall per year, evenly distributed in most areas of the year. The temperature range is 20-30 °C (9).

According to M,Raitha 2014, Many names for this plant exist in different countries, including Lemongrass in India, Capim-Cidaro in Brazil, Lemongrass in Egypt, Citronella in the USA, Sakumau in Malaysia, Takhari in Thailand, Cymbopogon in Italy, and Tej-Sar in Ethiopia. In addition to being used in various household products like spices, food, and beverages, vanilla lemongrass essential oil has a distinct lemon flavor and is used in the perfume, soap, and cosmetic industries to treat fever and infections. It also possesses anti-inflammatory, antibacterial, and antioxidant qualities (10).

Taxonomy of *C.nardus* plant for the study.

1. Kingdom- Plantae
2. Order-Poates
3. Family- *Poaceae*

4. Genus- *Cymbopogon*

5. Species- *Cymbopogon nardus*

Many people use lemongrass oil (LO) as a pesticide and preservation. According to studies, LO has antifungal and antibacterial qualities. In 1948, the US EPA first registered citronella oil as an insect repellent. It is the most common active ingredient found in "natural" or "herbal" insect repellents. Originally extracted from the grass plant Cymbopogon nardus, oil of citronella.

Lemongrass is a medicinal plant that increases herbal resistance against pathogenic illness, according to studies by Wifek et al. (2016) on the plant's qualities, uses, and active components. Lemongrass essential oil contains astringent, antibacterial, analgesic, antipyretic, antiseptic, and carminative qualities (11). A research done in the Ivory Coast revealed that the essential oil of C. nardus includes compounds including limonene and citronellol, with the majority chemical molecule being citronellal (42.20%) (12).

According to research by Seyoum et al. (2002), the usage of repellent plants is a deeply embedded part of African tradition and culture. To reduce the amount of mosquitoes that enter at night, residents in Kenya burn or spray a variety of plants, but people in Eretria just hang them about their beds, doors, and windows (13).

Burning dried repellant herbs is a widespread practice in Ethiopia to keep mosquitoes and insects away (7). Most of the time, performed by using the traditional charcoal stove (thermal expulsion) in the early evenings. Due to their lower toxicity to mammals and non-target organisms than synthetic repellents, plant-based repellents have recently seen a rise in popularity among health-conscious customers. As a result, further ethnobotanical research is required in order to develop risk-reduced, environmentally friendly insecticides and repellents from the traditionally used repellent plants due to the rapidly increasing demand and decreasing availability (7).

There has been a push for citronella candles as a useful tool for keeping mosquitoes away from home. In order to prevent bites from Aedes species mosquitoes, one study examined the effectiveness of commercially available 3% citronella candles, 5% citronella incense, and plain candles in a field setting. Comparing subjects near the citronella candles to controls without any protection, there was a statistically significant 42% reduction in bites (p. 42). However, there were 23% fewer bites when regular candles were used. The effectiveness of ordinary candles and

citronella incense was the same. Simple candles may have this effect because they act as a "decoy" source of moisture, warmth, and carbon dioxide (14).

Mosquito repellents are widely utilized as malaria prevention measures. Since mosquito repellents are available in a wide range of commercial formulations, including solutions, lotions, gels, creams, sprays, and sticks, they are now the most effective therapy. We use repellent to prevent mosquitoes from landing on our skin, clothing, or other surfaces; nevertheless, it may just act as a deterrent rather than an insecticide. (15,16).

2.2. Toxicology

Toxicology is the discipline of science that deals with poisons. A poison is any material that has a negative impact when given to a live creature, whether on purpose or accidentally. Although more descriptive, broader definitions of toxicology, including "the study of the detection, occurrence, qualities, effects, and control of dangerous chemicals," do not overcome the problems, Rarely, if ever, can toxicity be described as a single molecular event; rather, it is a series of processes that begin with exposure, continue with distribution and metabolism, interactions with cellular macromolecules (often DNA or proteins), and the manifestation of a toxic end point. Toxins are macromolecules found in cells (often proteins or DNA) and the manifestation of a hazardous end point Excretion might reduce the severity of this process (8).

Testing for the toxicity of novel chemicals is essential to the drug-development process. Preclinical toxicity testing is used to determine the detrimental effects of an experimental chemical on several biological systems, taking into account factors such as species, organs, and dosage. The following methods can be used to determine a substance's toxicity: (a) investigating unintentional exposures to the material; (b) in vitro studies utilizing cells or cell lines; and (c) in vivo exposure on experimental animals. The numerous experimental animal models and techniques used to assess compounds for toxicity are the primary subjects of this review. According to Parasuraman(2011), pre-clinical toxicity testing helps to calculate the "No Observed Adverse Effect Level," which is needed to initiate the clinical evaluation of investigational products (17).

2.3. Blood and its components

Blood is crucial for the supply of nutrients, hormones, metabolic excretion, immune system functions, and homeostatic reactions (18). For the objectives of illness diagnosis, prevention, and

treatment, its parameters are the most highly accurate, sensitive, and dependable that researchers utilize and rely on (19).

Erythrocytes, leukocytes, and platelets are components that make up the specialized connective tissue known as blood. According to Junqueira and Carneiro (2005), these produced components are suspended in plasma, a fluid component of the extracellular matrix (20). A typical adult rat's total blood volume is between 5.6 to 7.1 milliliters per 100 grams of body weight (21) , or around 6 liters for an adult human (22).

Study on Aqueous Leaf Extract of Acalypha wilkesiana on Hematological Parameters (2013) stated that Hematological markers, including Hgb, RBC, HCT, MCV, MCHC, WBC, L, and PLT, are valuable indicators for evaluating the potential for toxicity of plant extracts in live organisms. For instance, the body's ability to operate normally is threatened when blood cells are harmed or destroyed (23).

The erythrocytes are nucleus-free biconcave disks that, in a healthy state, never exit the circulatory system. 7–13 million red blood cells per milliliter. They are round with prominent, smooth borders on stained smears. Rat erythrocytes have a diameter of 4–7 μm and resemble human erythrocytes in appearance. Human erythrocytes have a diameter of 6–8 μm, which is comparable to the size of a resting lymphocyte nucleus, and a thickness of 1.5–2.5 μm. A central pallor region makes up around one-third of the cell's diameter (24).The main job of erythrocytes is to transport hemoglobin, which is responsible for carrying carbon dioxide and oxygen Rat erythrocytes only live 61 days on average, compared to 120 days in humans (25).

The body's defense system is made up of leukocytes, or white blood cells, which are mobile units that are primarily directed toward areas of severe inflammation and infection. Upon reaching their objective, leukocytes move between the capillary endothelial cells and venules in order to depart from the blood vessel. Once within the connective tissue gaps, they perform their regular protective role against foreign body (20,26)..

According to study done in (1997), a dose-dependent rise in the WBC count suggests the existence of physiologically active herbal components that boost the immune system by raising the quantity of defensive WBC (27).

Leukocytes are classified as granulocytes (having both specific and azurophilic granules) and agranulocytes (having just azurophilic granules but without the specific ones) based on the kind of granules in their cytoplasm and the form of their nucleus. Neutrophils, eosinophils, and

basophils are among the granulocytes; lymphocytes and monocytes are among the agranulocytes (20,25). These cells work in coordination to give the body strong defenses against bacterial, viral, and parasite diseases (28).

White blood cells, or leukocytes, are the body's defense system. Most of them are sent straight to areas of severe inflammation and infection. Leukocytes travel from the blood stream between the endothelial cells of the venules and capillaries, penetrate the gaps in the connective tissue, and perform their protective function against foreign bodies once they reach their target (20,26). There are five types of WBC that constitute different concentrations. These include neutrophils (40–75%), eosinophils (1-6%), basophils (1%), monocytes (2-10%), and lymphocytes (20–40%) in humans (27).

Neutrophils, also known as polymorphonuclear neutrophilic granulocytes, are the most common leukocyte in humans. Their primary function is to phagocytose tissue debris and combat and eliminate invading bacteria, viruses, and other dangerous agents. They frequently arrive first at an injury or inflammatory site (25,26). Rat neutrophil nuclei exhibit hyper segmentation, making up 14–20% of all leucocytes (29).

Mature eosinophils have a distinctive bi lobed nucleus and are roughly the size of neutrophils. Numerous big, elongated granules distinctive to retractile are their primary distinguishing feature and they are efficient against certain parasitic worms and phagocytize antigen-antibody complexes. (20). Rat eosinophil nuclei have a band-like structure that can spiral into a ring. 1–4% of all leucocytes are made up of it (21).

According to Ebo et al. (2008), basophils are granulocytes with a segmented nucleus (30). After the first exposure to antigen, they are crucial in the release of histamine and other inflammatory mediators (31).

WBCs include lymphocytes, which the body uses to respond to foreign substances once it has consumed them. Certain medicinal plants have been reported to have the ability to reduce blood lymphocyte counts, which implies that the drug may include biologically active ingredients with anti-lymphocytic effects (32).

Platelets are non-nucleated, disc-shaped cell fragments that are produced by the bone marrow and used in blood clotting and homeostasis. According to Taylor et al. (1997), there are 150,000–400,000 platelets per cubic millimeter of blood in humans (21).

Platelets are the main cells in charge of controlling bleeding, and under normal conditions, when they become activated in response to bleeding, the clotting process begins. Their cell membranes include phospholipids that trigger the clotting factors in blood plasma, resulting in fibrin threads that strengthen the platelet plug. These fibrin threads make up the majority of the clot. Serotonin, a neurotransmitter released by platelets adhering to one another to form a blood clot, also increases blood vessel constriction, restricting blood flow to the injured area (33).

The liquid known as plasma has a pale straw color and is 90% water with the remaining 10% being a mixture of low- and high-molecular-weight substances in solution or suspension. In essence The extracellular fluid of bodily tissue and plasma, an aqueous solution of inorganic salts, are continuously exchanging. plasma, the liquid medium in which components are formed, transports nutrients from their place of synthesis or absorption to various parts of the body. Additionally, it carries metabolic waste products that excretory organs remove from the blood (20). According to Bernstein (1993), Plasma proteins consist of three main types: albumin, globulins (α, β, and γ-globulins), and fibrinogen. Blood collected from the body and put into a test tube that hasn't been coated with an anticoagulant, such as heparin, can clot (34).

Blood's parameters remain the most accurate, sensitive, and reliable that researchers use for disease diagnosis, prevention, and treatment (19). Blood is necessary for the delivery of nutrients, hormones, metabolic excretion, immunological processes, and homeostatic responses (18).

2.4. Structure, Function and Histopathology of Liver

The biggest gland and the body's second-largest organ after the epidermis is the liver which weighs around 1.5 kg and makes up 2% of an adult human's body weight however, in mice the proportion varies according on the body weight of the mice (35).The caudate lobe, left lateral lobe, median lobe, and right lobe are the four separate lobes that make up the mouse liver, and they vary in size. The superior and inferior caudate lobes, which make up the caudate lobe, together account for almost 10% of the liver's total weight. The inferior omentum completely encloses the superior caudate lobe, which is joined to the left lateral lobe by a thin interlobular ligament (36).

The inferior part of the caudate lobe is located beyond the stomach and is connected to the spleen and pancreas. It is enveloped in a fibrous capsule that is attached to the dorsal wall of the minor omentum. The left lateral lobe, which is dorsocaudal to the median lobe, cranio-ventral to the caudate lobes, and connected to the superior caudate lobe by interlobular ligaments, accounts for around 34% of the liver's total weight (37).

About 26% of the liver's total weight is made up of the median lobe, which is anchored under the diaphragm by the falciform ligament, which runs from the xiphoid and diaphragm to the liver and forms the interlobular fissure. Furthermore, the left portion of the median lobe, which makes up around one-third of the entire structure, is smaller than the right section. The two halves of the median lobe are divided by a deep cleft. The liver lobe on the right side of the inferior vena cava is called the right superior and inferior lobes, and it is divided into two separate sections (36). The right inferior lobe, which has a pyramidal shape and a tip pointing to the inferior vena cava, makes up 14% of the total liver weight and is dorsally attached to the diaphragm. The right superior lobe has a wide base that extends to the paracaval region of the liver, and it makes up approximately 16% of the total weight of the liver (37). Rat livers and human livers differ greatly, although their microscopic characteristics are essentially the same. The lobules of the liver are anatomically arranged into polygons and encircled by connective tissue. Rat liver sections allow for the easiest appreciation of this lobular arrangement. The hepatocytes' central venous cord forms the lobule's core, while the adjacent sinusoids spread radially between it and the lobule's perimeter. At the tips of the lobules are portal triads, which are made up of branches of the portal vein, the hepatic artery, and the bile duct or ducts (38).

The hepatocytes produce bile, which is then released into the bile canaliculi From the hepatic cords to the portal triad at the hepatic lobules' border, bile flows the major blood arteries that supply the liver of rats start and proceed similarly to those of humans (38). A lesser portion (20–30%) of the liver's blood is supplied by the hepatic artery, whereas the majority (70–80%) of the liver's blood (originating from the stomach, intestines, and spleen) comes through the portal vein (36). The primary job of liver is to cleanse the body, remove toxins and chemicals from the bloodstream, and convert them into products that can be easily expelled by the bile or urine. However, the liver is damaged when these toxins build up in the body more quickly than the liver can handle them. Moreover, several of the current chemotherapeutic medicinal herbs are toxic and capable of harming hepatocytes or liver cells (39).

2.5. Structure, Function and Histopathology of Kidney

Kidneys are located retroperitoneal in mice, one on each side of the spinal column, at the level of the T12 through L3 vertebrae. A kidney cross section may reveal two distinct sections: the inner medulla and the outer cortex further, Convoluted tubules, Bowman's capsule, and the start of collecting ducts comprise the cortex, which is covered in fibrous connective tissue (40). Renal pyramids are the mass of pyramidal structures that make up the medulla. These structures are composed of the loop of Henle, collecting ducts, and associated vasculatures. Nephrons and collecting tubules, which are produced from distinct embryological primordia, comprise the tubule, which functions as the kidney's functional unit (34).

The cortex, medulla, and collecting system are the three components that make up each kidney. The granular-appearing cortex is the outer pale zone of the kidney that has been hemisected. The medulla has several pyramidal structures. The cortical parenchyma that fills the spaces between neighboring pyramids is what gives rise to Bertin's columns. A renal lobe is composed of both Bertin columns and the sub capsular cortex, which are located in the medullary pyramid (34).
The collecting system is made up of the two or three main branches, or major calyces, that each renal pelvis has. A medullary papilla is typically assigned to each of the three or four minor calyces that result from the division of each main calyx. All pyramids have a base at the corticomedullary border and a tip that extends toward the renal pelvis to generate papillae. Rat kidneys are referred to be "unipapillate" because they only have one renal pyramid, unlike human kidneys. The renal pelvis is directly around the rat's unipapillate (40)kidney. The rat kidneys' general and microscopic appearance, however, is similar to that of the human kidney.

The kidney's functional unit is the nephron. An adult rat kidney has around 30,000 nephrons, while a human kidney has between 0.6 and 1.4 × 106 nephrons. Every nephron is composed of tubules that have dilated ends called Bowman's capsules, which contain the glomerulus. The human kidney's glomerulus typically has a diameter of 200 μm, while the rat kidney's is 120 μm. However, glomeruli vary significantly in size and number according to birth weight, gender, and age. Juxtamedullary glomeruli are larger than glomeruli in the superficial cortex. This is not how the human kidney works (34).

Proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and the loop of Henle with ascending and descending limbs make up the renal tubules. Compared to the DCT, the PCT is a longer, more intricate tubule. According to Junqueira and Carneiro (2012), the luminal surfaces of PCT cells are covered with many long microvilli that provide a noticeable brush barrier for reabsorption. The U-shaped loop of Henle is situated between the proximal and distal convoluted tubules. Its ascending and descending limbs are made of simple epithelia, which are squamous farther down in the medulla and cuboidal closer to the cortex (20),Compared to PCT cells, the simple cuboidal cells that make DCT are smaller, flatter, and do not have a brush border. The first straight segment of the distal tubule interaction with the vascular pole of the renal corpuscle of its parent nephron at the point where it comes into contact with the arterioles is part of the specialized structure known as the juxtaglomerular apparatus (41).

The kidney's function is to selectively take in ions, most often Na+ and K+, in order to regulate the fluid's osmotic balance. The tubular anatomy of the kidney is aberrant in nephronic syndrome, especially in the proximal convoluting tubules, where the tubular cells have pink cytoplasmic granules and seem larger, with slightly enlarged nuclei. These electrolyte concentrations are elevated in the blood (serum) in this condition. The thick ascending limb, thin descending limb, loop, and thick descending limb of the Henle were shown to be less impacted by herbal medication, suggesting that the major focus of toxicity is convoluting and collecting tubules. Thus, convoluting tubule cells may have much expanded cells; thus, their lumens (42,43).

Pathological changes to the kidney's tubular structure can cause tubular cells' cell membranes to burst or leak, which raises certain enzyme levels in blood that are normally concentrated in the liver and kidney. Urine and creatinine are examples of waste products that enter the circulation and are removed by the kidney. An elevation in the blood levels of these waste products indicates impaired renal function due to pathological alterations to the kidney tubular structure (43). Moreover, the toxicity of prescribed medications causes kidney tubular cells to undergo cytoplasmic vacuolation (41).

The majority of research has shown that exposure to various hazardous compounds causes a noticeable alteration in the cellular component of the proximal convoluting tubules' fine structure Furthermore, widespread interstitial fibrosis and severe tubular loss, most frequently in the outer cortex, have been reported to result from the use of several traditional medicinal herbs in the treatment of renal tubular injury (42).

2.6. Structure, Function and Histopathology of Skin

Covering the whole exterior surface and acting as a significant physical barrier against the environment, the skin is the largest and most important organ of protection. Its duties encompass controlling body temperature as well as defense against infections, microbes, UV rays, trauma, and poisons. In addition, immunologic surveillance, sensory perception, the regulation of insensible fluid loss, and overall homeostasis are all influenced by the skin., the skin is also incredibly adaptable, having varying thicknesses and specific roles in various body locations (44,45).

The epidermis, dermis, and hypodermis are the three main layers of skin, arranged from superficial to deep. It is possible to further split each layer into its component sections. The skin's topmost layer is called the epidermis. It is composed of five layers that go from deep to superficial: stratum basale/germinativum, which is the basal layer; stratum spinosum, which is the prickle cell layer; stratum granulosum, which is the granular layer; stratum lucidum, which is the clear layer; and stratum corneum, which is the cornified layer (44).

The following cells make up the epidermis: Keratinocytes are basal stem cell-derived squamous epithelial cells that constantly develop from the basal to the corneum layer and desquamate. The melanin that gives skin its color and shields it from UV rays is produced by melanocytes. Langerhans cells: cells that deliver antigens Méchanoreceptors, or Merkel cellsWe locate the derm next to the epidermis. This area is irregularly structured and primarily composed of connective tissue. It is located deep within the stratum basale's. In adults, type I collagen makes up around 85% of the collagen fibers that give tenacity, with type III collagen making up the remaining portion. Apart from collagen, the dermis also has elastic fibers that help the skin to rebound. At the dermo-epidermal junction, type IV collagen is mostly found enveloping Schwann cells and vascular endothelium. Collagen fibers of types V, VI, and VII make up relatively little of the dermal structure(45).

An injury to the skin by any toxins or physical damage triggers a chain of events that include damage to the epidermis and dermis, blood loss, and contamination of the tissues beneath moreover, a complex collection of overlapping dynamic interaction mechanisms, including as haemostasis, inflammation, proliferation, and remodelling, are involved in the conservative evolutionary process of skin wound healing in addition to this, the release of different growth factors, cytokines, and low molecular weight substances from the damage site kickstarts the healing procedure shortly after injury (46).

3. OBJECTIVE

3.1. General objective

- ❖ To evaluate acute and subacute dermal toxicity of aerial part *Cymbopogon nardus* formulation on hematological, clinical chemistry and histopathology of skin, kidneys and liver of Albino Wistar rats.

3.2. Specific objectives

- ✓ To determine the LD_{50} of *Cymbopogon nardus* formulation on the Albino Wistar rats.
- ✓ To determine the effect of 28-day topical application of cymbopogon *nardus* formulation on blood parameters of Albino Wistar rats.
- ✓ To investigate the effect of 28-day topical application of cymbopogon *nardus* formulation on the microstructure of liver of Albino Wistar rats.
- ✓ To determine the effect of 28-day topical application of *cymbopogon nardus* formulation on the microstructure of kidney of Albino Wistar rats.
- ✓ To determine the effect of 28-day topical application of *cymbopogon nardus* formulation on the microstructure of skin of Albino Wistar rats.

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4. METHOD AND MATERIAL

4.1. Study Design:

Laboratory based experiment.

4.2. Study Setting:

AAU, School of Medicine, Histology and Pathology Core Laboratories, College of Health Sciences (CHS), and Armaeur Hansen Research Institute (AHRI), Traditional and Modern Medicine Research Directorate.

4.3. Study area

The study was conducted at Addis Ababa University, College of Health Science, School of Medicine, Department of Anatomy (Histology Laboratory), and AHRI.

4.4. Collection of plant material

The *C. nardus* leaves were gathered in the Wondogenet area near Shashemene town, which is around 270 kilometers south of Addis Ababa. Then, a taxonomist from EPHI recognized and verified the leaves.

4.5. Characteristics of the formulation

The formulation was taken from the EPHI. To extract essential oils, fresh plant material was subjected to a three-hour hydro-distillery process. The hydro-distillation process was separately performed for medicinal plant using Clevenger-Arm apparatus. Two liters of water were added to the 500g of plant material in a round bottom flask that was placed on a heating mantle, and the flask was connected with the Clevenger-Arm apparatus. The condenser was left open to water flow. The excess water was returned to the flask and the volatile oils were transported with the steam during boiling into the graded distillate receiving tube. Anhydrous sodium sulfate was used to further purify the extracted oil from the aqueous solution after it was collected in a sterile container. After that, the essential oil was weighed and kept between 2 and 8 OC in the refrigerated until the experiment's usage (47). The final yield of the oil weighed was 4 ml, and the formulation, done with some additives, contained 2% of the oil of *Cymbopogon nardus* and 98% of petrolatum. It was stored in the refrigerator at 2–8 ^{O}C until it was used for the experiment.

Table 1. C. nardus formulation preparation

Essential oil *of C. nardus*	*2.00% (v/v) in 70% ethanol*
White petrolatum	*97.50 % (v/v)*
Jatropha curcas fixed oil	*0.50 %*
2 % Tween-80	*To make 50 ml solution*

4.6. Experimental Animals

At the beginning of the first dosage, the rats utilized in this investigation were between 8 and 12 weeks old, with females weighing 250–300g and being nulliparous and not pregnant. The rats came from the EPHI. The rats were kept in stainless steel cages in an environmentally controlled space with a temperature of 22–23 OC and a relative humidity of at least 30% but not more than 70% (402, OECD 2017). Artificial lighting was used in the space, with a 12-hour cycle of light and dark. The rats were given unlimited access to food (Standard pellets) and water during the adaption phase.

According to OECD guidelines 402 (2017), Using group cages, the animals were acclimated to the laboratory environment for five days before the trial began. For the purpose of the study, animals were chosen at random and given unique identifying tags. All of the test animals' fur was plucked from their dorsal and flank areas the day before the test chemical was administered (i.e. 10% of the total body surface area) by closely clipping. Care was taken to avoid abrading the skin, which could alter its permeability (48).

4.7. Grouping and dosing of Experimental Animals

According to OECD guideline 402 for acute dermal toxicity, five experimental animals (female) were used and administered 2000mg/kg as a starting dose (limited dose) because, in 99% of cases of acute dermal toxicity, the starting dose is relatively higher than the oral dose of 2% (v/v) of the therapeutic dose formulation of Cymbopogon nardus. For subacute dermal toxicity, 10 animals (5 females (nulliparous nonpregnant pregnant) and 5 males) with healthy skin were used at each dose level. Experimental animals, each male and female, were allocated into four groups consisting of three test groups and one control group. The dosage for the first test groups was determined based on the findings from the acute toxicity result, and it was 100mg/kg, 200mg/kg, and 400 mg/kg, the control group was taking the vehicle (petrolatum).

4.8. Cage Side Observations

Before and after dosage, each animal in its cage was closely monitored for changes in its physical characteristics, including its eyes, skin, and hair, as well as any autonomic symptoms like salivation, diarrhea, or urine, and CNS affects like tremors (48).

4.9. Acute Dermal Toxicity Study

The Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals 402 (OECD 2017) and the guidelines of the World Health Organization (WHO) (WHO 2000) were followed in the acute toxicity test. By adding the acclimatization time, the research duration grew to 20 days from 14 days. Five days before the trial began, the animals were housed in groups to get them ready to the lab environment.

Rats in a group were given cutaneous dosages of the extract, which were determined by calculating each animal's doses based on their average body weight. The *C.nardus* formulation was held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. In order to preserve the gauze dressing and test chemical and make sure the animals couldn't consume it, the test site was also coated in an appropriate way. Following dosage, animals were watched as often as possible for the first thirty minutes, then once a day for the next twenty-four hours. During the first two to six hours following the start of the exposure period and every day after that, for a total of fourteen days, careful attention was paid to the animals. Observations of lethargy, drowsiness, coma, salivation, convulsions, tremors, and diarrhea were noted (48).

The rats were weighted and scarified at the end of the experiment (15 days in total), and their internal organs, including the liver and kidney, were grossly seen after death.

4.10. Subacute dermal toxicity

The subacute toxicity test was according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (410) (OECD 1981). It was 28 days of study. For a duration of 28 days, treatment groups of experimental animals received one dose per day of the test drug administered topically in graded amounts, while control groups received the vehicle (petrolatum). The animals were watched every day during the application period to look for indications of toxicity. The animals who make it through the test are slaughtered and necropsied (49).

4.11. Weight Measurement

Before the C. nardus formulation was given, each animal in the experimental and control groups had their individual weight recorded. This weight was subsequently measured once a week. After the test was over, the animals that had survived were weighed and painstakingly killed by intraperitoneal injection of 150 mg/kg pentobarbital while sedated. The kidney and liver were carefully removed, and an electronic balance was used to weigh them (Metteler AE 160). In order to compare the weights of the experimental and control groups, the weight difference and relative organ weight were computed using the animal's weight as well as the weights of the liver and kidney.

4.12. Gross Pathology

Every significant pathogenic alteration was noted for every animal in every group. In this instance, it's critical to note any physical variations that could occur throughout the various animal groups. During a gross pathological examination of the target organs, changes in the organs' color and texture, any necrosis or spots on each organ, and changes in the target organs' size, such as any expansion, were all closely examined (48,49).

4.13. Blood Collection for Hematological and Biochemical Analyses

Using 5-milliliter heparinized syringes, rapid heart punctures were carried out to draw out 2-4 milliliters of blood, which were then collected in test tubes containing and without anticoagulant EDTA, following an intraperitoneal injection of 150 mg/kg of pentobarbital. Under an auto-haematology analyzer system, hematological parameters such as hemoglobin concentration (HC), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and lymphocyte and platelet count (PLC) were then examined. (Sysmex XT-1800i, Japan). Blood samples were placed in a test tube without EDTA, allowed to clot, and then centrifuged at 3000 revolutions per minute for approximately 20 minutes to extract serum. The serum was then used to analyze biochemical parameters, such as urea, creatinine, AST, ALP, ALT, and ALB, using a clinical chemistry analyzer (Bio Technique, Italy). A comparison was then made between tretment and the control groups.

4.14. Animal Dissection, Tissue Collection and light microscope imaging

At the end of of the trial, each group's animals were sacrificed. after withdrawing blood under intraperitoneal injection of 150 mg/kg of pentobarbital. Immediately after euthanasia, After the

abdomen was opened, the kidneys and liver were extracted. these organs were swiftly weighed on an electronic balance, cleaned of any surrounding tissues, and placed on clean paper. We measured the weight of the organs. Sections of skin, liver, and kidney measuring 3-5 mm in thickness were randomly removed and preserved for histological examination.

Stained tissue slides of the skin, liver, and kidney were viewed using a light microscope (MC 80 DX Microscope Camera, Carl Zeiss, Germany) at various magnifications (x10, x40, and x100 objectives) for histological studies. An experienced pathologist conducted this study at the EPHI and AAU. Following the analysis of the histology slides from every group, a digital camera-equipped microscope was used to obtain photomicrographs of a few samples of the skin, liver, and kidney from the rats that were treated with extract and the rats that were not. Direct saving of the photomicrographs was done using a portable USB flash drive that was put within the microscope. Sections of skin, liver, and kidney tissue were photomicrographed at magnifications of x40 and x10. The treatment and control groups were compared based on observations.

4.15. Ethical Consideration

Every experiment was carried out in accordance with the strictest guidelines for the humane and compassionate use of animals in biomedical research, having been approved by the relevant authorities at the School of Medicine, AAU, and AHRI. The study's animal subjects didn't have to endure any needless suffering or unpleasant environments. (*OECD, 2008*). All animals received an intraperitoneal injection of 150 mg/kg pentobarbital to minimize pain and suffering during any surgical intervention, and the surgery was performed by a person with the necessary training. The animals were housed in a suitable habitat and shielded from infections.

4.16. Statistical Analysis

The Statistical Package for Social Science (SPSS) software, version 24, was used to enter and analyze data. The mean (μ) and standard error of mean (SEM) were used to display the statistical data. To examine significant statistical differences between the experimental groups, one-way analysis of variance (ANOVA) with post Hoc test was used at the $P<0.05$ level of significance. The liver and kidney histology results were presented in a qualitative manner.

5.RESULT

5.1.Acute Toxicity Study

The acute toxicity of the C. nardus formulation on the behavior of rats was carried out by dermal application of a single dose of 2000mg/kg (the limit dose) given to five rats. At dosages of 2000 mg/kg, no sign of toxicity was seen during the 14-day acute toxicity study period. This included changes in skin and fur, eyes, respiratory effects, autonomic symptoms including salivation, diarrhea, and urine, and CNS effects like tremors. This result indicated that the dermal lethal dose (LD50) is higher than 2000mg/kg. Since no deaths were reported at the limit dose, the formulation's LD50 was determined to be higher than 2000 mg/kg. Gross pathological examinations of the treated rats' liver and kidneys revealed no unusual alterations in size, shape, color, or texture.

Table 2. Experimental animal preparation and dosing for subacute study

Group	1st dose(G1)	2nd dose(G2)	3rd dose(G3)	Control(G4)
dose	100mg/kg	200mg/kg	400mg/kg	
Female rats	28g	56g	112g	Vehicle(petrolatum)
Male rats	30g	60g	120g	Vehicle (petrolatum)

5.2.Subacute Toxicity study

5.2.1.General observations and effect of C.nardus formulation on the behavior of rats

Among the male rats treated with the formulation at all three doses and the groups of female rats treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg, no signs of toxicity were seen during the course of the 28-day study period. During the whole trial time, no deaths were reported.

5.2.2.Effect of C.nardus formulation *on weight*

The findings indicate that during the course of the trial, the body weight of all animals, both control and treated, increased. Nonetheless, this rise was not statistically significant ($P > 0.05$) to be linked to the product. (ANOVA) test revels p value for mean weight gain difference for male was (0.96), for female rats was (0.71). p value for mean relative organ weight for kidney and liver of male was 0.18 ,0.528 and female rats was 0.217 0,071 respectively.

The weight of the kidney and liver was measured, and the organs' mean relative weight was examined. Between the experimental and control groups, there were no statistically significant

variations in the relative means of liver and kidney weight. The overall appearance of the internal organs did not change, as far as was noticed. In contrast to the control group of rats, the experimental group's organs did not exhibit any aberrant alterations in size, shape, color, or texture, nor did they exhibit any signs of organ necrosis.

Table 3. Effect of 28 days dermal application of C.nardus formulation on body weight difference and relative organ of male rats.

Group	Dose in mg/kg (n=5)	Body weight gain	Relative organ weight	
			liver	kidney
I	100mg/kg	39.8 ± 3.09	2.65 ± 0.06	0.30 ± 0.0118
II	200mg/kg	41.4 ± 5.20	2.64 ± 0.10	0.32 ± 0.128
III	400mg/kg	37.4 ± 8.04	2.72 ± 0.04	0.34 ± 0.012
IV	Control	40.0 ± 3.89	2.69 ± 0.05	0.32 ± 0.009
P-value		**0.962**	**0.187**	**0.528**

The values are expressed as mean ± SEM, $P<0.05$

Table 4. Effect of 28 days dermal application of C.nardus formulation on body weight difference and Relative organ weight of female rats.

Group	Dose in mg/kg	Body weight gain	Relative organ weight	
			Liver	kidney
I	100mg/kg	22.6 ± 3.90	2.49 ± 0.077	0.031 ± 0.004
II	200mg/kg	23.8 ± 3.1	2.38 ± 0.035	0.335 ± 0.01
III	400mg/kg	27.0 ± 3.04	2.62 ± 0.118	0.339 ± 0.014
IV	Control	22.6 ± 1.56	2.58 ± 0.075	0.343 ± 0.005
p-value		**0.71**	**0.07**	**0.217**

The values are expressed as mean ± SEM, $P<0.05$

5.2.3.Effect of C.nardus formulation on food and water intake

Tables 5 and 6 show the weekly mean food and water consumption. The treated groups' average food consumption was discovered to be comparable to that of the control groups. Nonetheless, male rats in the 200 and 400 mg/kg treatment groups showed a non-significant increase in water consumption over the course of the study's 28 days.

Table 5. Effect of 28 day dermal application of C.nardus formulation on food intake of male rats.

Food intake	Unit	Dose,			Control	P=value
		100mg/kg	200mg/kg	400mg/kg		
1st week	Gram	159 ± 7.54	174 ± 7.60	156 ± 8.21	160 ± 9.48	**0.95**
2nd week	Gram	160 ± 8.39	148 ± 7.69	158 ± 6.43	147 ± 6.56	**0.14**
3rd week	Gram	165 ± 10.9	156 ± 9.31	166 ± 9.31	177 ± 13.5	**0.87**
4th week	Gram	159 ± 9.67	153 ± 7.94	133 ± 5.47	171 ± 10.0	**0.75**

The values are expressed as mean ± SEM n=5 P<0.05

Table 6. Effect of 28 day dermal application of C.nardus formulation on water intake of male rats

water intake	unit	Dose			Control	P-value
		100mg/kg	200mg/kg	400mg/kg		
1st week	ml	89 ± 4.34	98 ± 2.09	100 ± 5.58	91 ± 4.14	**0.88**
2nd week	ml	92 ± 2.62	101 ± 6.35	101 ± 5.03	85 ± 3.59	**0.14**
3rd week	ml	93 ± 4.7	116 ± 7.8	117 ± 8.2	85 ± 2.00	**0.17**
4th week	ml	92 ± 3.19	108 ± 4.46	114 ± 6.92	86 ± 3.1	**0.37**

The values are expressed as mean ± SEM n=5, P<0.05

Table 7. Effect of 28 day dermal application of C.nardus formulation on food intake of female rat.

Food intake	unit	Dose			control	p-value
		100mg/kg	200mg/kg	400mg/kg		
1st week	Gram	126 ± 5.42	116 ± 7.80	110 ± 6.26	116± 13.4	**0.12**
2nd week	Gram	137 ± 7.21	108 ± 7.69	108 ± 4.46	100 ± 4.98	**0.88**
3rd week	Gram	131 ± 11.0	108 ± 6.97	126 ± 10.9	125 ± 11.6	**0.28**
4th week	Gram	123 ± 6.59	113 ± 10.3	120 ± 6.87	140 ± 5.58	**0.06**

The values are expressed as mean ± SEM n=5, P<0.05

Table 8. Effect of 28 day dermal application of C.nardus formulation on water intake of female rats.

water intake	Unit	Dose			Control	p-vaue
		100mg/kg	200mg/kg	400mg/kg		
1st week	Ml	73 ± 2.1	96 ± 7.67	76 ± 3.38	76 ± 3.04	**0.17**
2nd week	Ml	74 ± 3.30	101 ± 2.09	82 ± 1.15	72 ± 2.93	**0.64**
3rd week	Ml	80 ± 4.2	93 ± 3.46	85 ± 2.07	78 ± 2.82	**0.26**
4th week	Ml	86 ± 4.08	97 ± 2.57	86 ± 2.52	93 ± 7.91	**0.15**

The values are expressed as mean ± SEM n=5, P<0.05

5.2.4. Effect of C.nardus formulation on hematological parameters

Tables 9 and 10 show the sub-acute impact of the C. nardus formulation on blood hematological parameters for male and female rats. The male and female experimental groups' evaluated hematological parameters did not show a statistically significant difference from the control group.

Table 9. Effect of 28 day dermal application of C.nardus formulation on Hematologic parameters of male rats.

Hematologic Parameters	units	Dose			Control	p-value
		100mg/kg	200mg/kg	400mg/kg		
RBC	10^6/µl	6.138 ± 0.19	6.22 ± 0.22	6.07 ± 0.27	6.21 ± 0.26	**0.91**
WBC	10^3/µl	2.52 ± 0.36	3.46 ± 0.65	3.56 ± 0.41	3.71 ± 0.49	**0.12**
HGB	g/dl	15.13 ± 0.20	15.4 ± 0.56	15.3 ± 0.29	15.45 ± 0.24	**0.86**
HTC	%	37.92 ± 0.54	37.9 ± 0.83	37.1 ± 0.81	38.28 ± 0.63	**0.60**
MCV	Fi	62.42 ± 0.71	62.8 ± 0.76	63.9 ± 0.49	64.48 ± 0.49	**0.11**
MCHC	g/dl	37.0 ± 0.29	37.7 ± 0.38	36.9 ± 0.45	38.1 ± 0.28	**0.73**
LYM	%	80.7 ± 3.2	87.9 ± 0.48	87.5 ± 0.85	88.4 ± 0.338	**0.38**
PLT	10^3/µl	648.1 ± 38.1	645 ± 21.1	689 ± 20.1	679. ± 58.38	**0.67**

The values are expressed as mean ± SEM, n=5 , P<0.05

Table 10. Effect of 28 day dermal application of C.nardus formulation on hematological parameters of female rats.

Hematologic Parameters	units	Dose			Control	P-value
		100mg/kg	200mg/kg	400mg/kg		
RBC	10^6 /µl	7.17 ± 0.14	7.19 ± 0.17	7.01 ± 0.21	7.04 ± 0.29	**0.96**
WBC	10^3/ µl	2.42 ± 0.39	3.31 ± 0.45	3.84 ± 0.28	3.71 ± 0.49	**0.77**
HGB	g/dl	16.8 ± 0.18	16.6 ± 0.77	16.3 ± 0.35	16.4 ± 0.26	**0.86**
HTC	%	40.5 ± 0.55	40.5 ± 1.87	38.8 ± 0.75	39.1 ± 0.72	**0.71**
MCV	Fi	58.1 ± 0.466	57.8 ± 0.39	57.0 ± 0.31	57.2 ± 0.17	**0.35**
MCHC	g/dl	37.4 ± 0.37	37.0 ± 0.44	36.8 ± 0.33	37.7 ± 1.09	**0.77**
LYM	%	75.8 ± 2.59	83.4 ± 3.44	81.9 ± 3.72	81.8 ± 4.16	**0.06**
PLT	10^3/ µl	667 ± 23.8	639 ± 24.6	624 ± 39.29	621. ± 26.97	**0.79**

The values are expressed as mean ± SEM, n=5 , P<0.05

5.2.5.Effect of c.nardus formulation on Clinical parameters

Regarding the male and female groups that received C. nardus at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, there was also not a significant difference in the levels of Alanine Amino Transferase (ALT), Aspartate transfarase (AST), creatinine, and urea as compared to the control group as shown in Table.11 (for the male) and Table.12 (for the female).

Table 11. Effect of 28 day dermal application of C.nardus formulation on Clinical parameters of male rats.

Clinical parameters	Dose			control	p-value
	100mg/kg	200mg/kg	400mg/kg		
ALP(IU/L)	103±6.22	108±5.07	101±3.49	108±5.89	**0.56**
AST(IU/L)	95±5.44	96±1.14	88.4±5.1	97±3.21	**0.42**
ALT(IU/L)	27.7±1.53	30.0±2.23	30.8±2.41	30.3±1.10	**0.68**
ALB(g/dl)	4.07±0.16	3.84±0.26	3.70±0.23	4.14±0.13	**0.58**
Creatinine(mg/dl)	0.44±0.062	0.42±0.031	0.45±0.049	0.48±0.41	**0.82**
Urea(mg/dl)	18.6±1.64	19.1±1.58	18.4±0.75	18.3±1.13	**0.62**

The values are expressed as mean ± SEM, n=5 , P<0.05

Table 12. Effect of 28 day dermal application of C.nardus formulation on Clinical parameters of female rats.

Clinical parameters	Dose			Control	P-value
	100mg/kg	200mg/kg	400mg/kg		
ALP(IU/L)	103. ± 3.47	102 ± 2.89	103 ± 6.12	99.2 ± 3.13	**0.87**
AST(IU/L)	96.0 ± 3.37	91 ± 4.76	83.8 ± 3.96	94.6 ± 5.50	**0.25**
ALT(IU/L)	30.3 ± 1.01	30.2 ± 1.15	30.4 ± 2.52	29.9 ± 1.22	**0.99**
ALB(g/dl)	3.90 ± 0.25	3.98 ± 0.32	4.1 ± 0.17	4.0 ± 0.31	**0.58**
Creatinine(mg/dl)	0.41 ± 0.038	0.45 ± 0.031	0.37 ± 0.03	0.41 ± 0.05	**0.62**
Urea(mg/dl)	18.6 ± 0.97	18.9 ± 0.92	17.56 ± 1.13	17.3 ± 1.13	**0.61**

The values are expressed as mean ± SEM, n=5 , $P<0.05$

5.2.6.The effect of 28 day dermal application of C.nardus on the gross pathology of organs

The gross pathology of internal organs such as the kidney and liver did not show any discernible change in appearance. Rats in the experimental group did not exhibit any organ necrosis, nor did they differ abnormally from rats in the control group in terms of size, color, form, or texture.

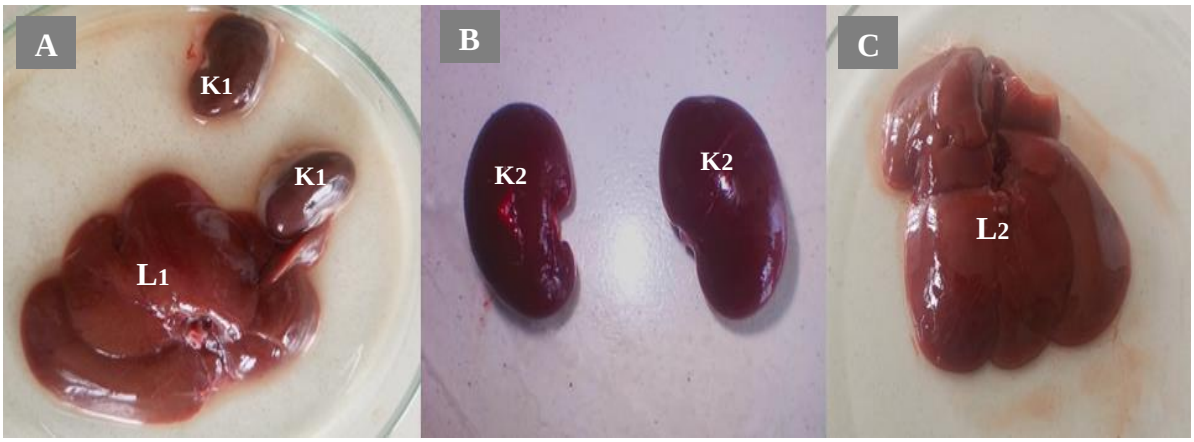

Figure 1. Image taken to evaluate the effect of 28 days *C.nardus* formulation on gross phathology of female rats. **A**: liver (L_1) and Kidney (K_1) taken from high dose. **B:** Kidney (K_2) taken from control group. **C:** liver (L_2) taken from control group.

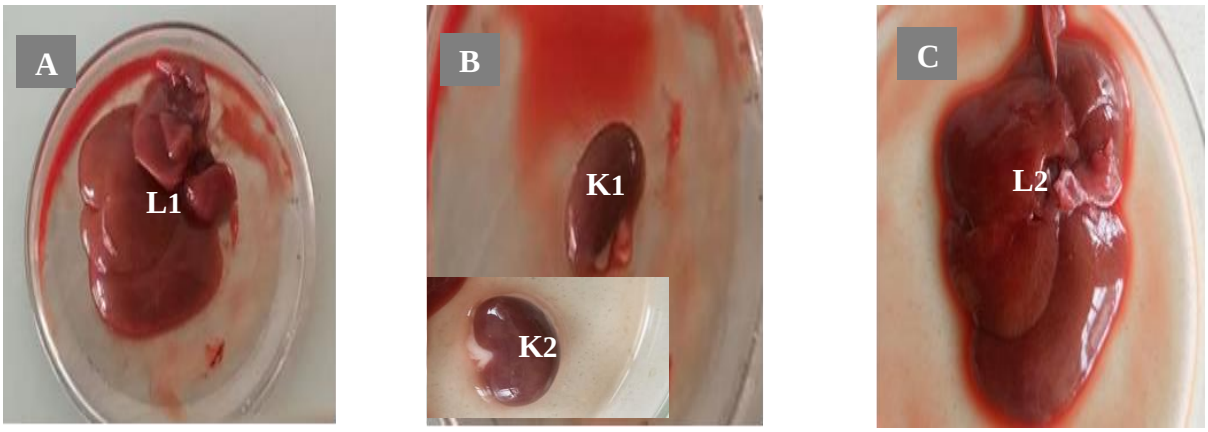

Figure 2. . Image taken to evaluate the effect of 28 days C.nardus formulation on the gross organ pathology of male rats. **A:** liver (L_1) taken from high dose. **B:** Kidney (K_1) taken from high dose and K2 taken from control group. **C:** liver (L_2) taken from control group.

5.2.6.Effect of C.nardus formulation on histopathology of liver

Histopathological analysis of the liver sections in the control group revealed that the hepatic sinusoids (S) and central vein (CV), which are bordered by endothelial cells (EC) and have typical radiating hepatocytes, appeared normal. The interlobular bile ductule, the branches of the hepatic artery, and the branch of the hepatic portal vein all showed normal appearances. (Figure A&C) shows Male and female Rats treated with C.nardus formulation at higher doses of 400mg/kg respectively. The male and female rats in the control group, as shown in (Figures B and D), had normal-appearing central veins (CV) and hepatic sinusoids (S), which are lined with endothelial cells (E) and have typical radiating hepatocytes.

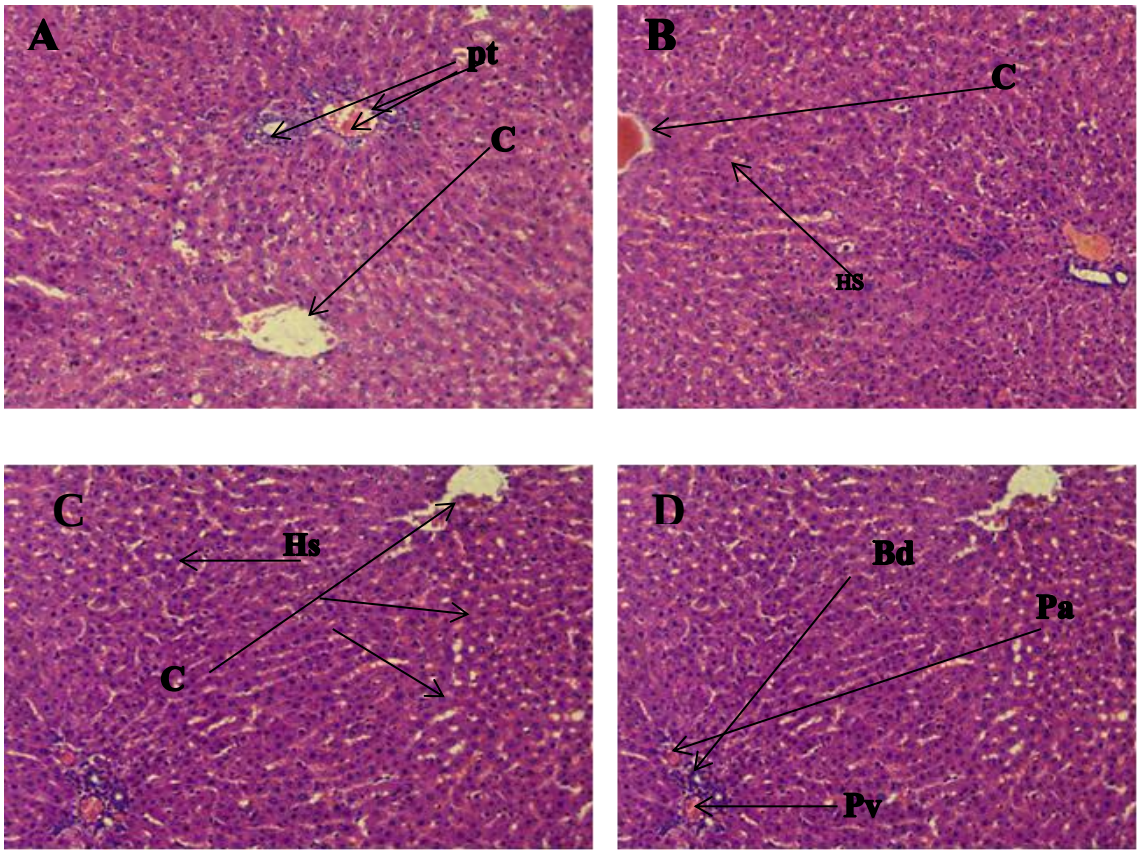

Figure 3. Image taken from microscope to evaluate the effect of 28 days dermal application of C.nardus on histopathology of liver on Albino wistar rats. **A**:(male rat tested with high dose) showing **cv**:central vein, **pt:**portal triad.. **B:** (male rat from control group) showing **cv:**central vein and **pt:**portal triad. **C:**(female rat tested with high dose) showing **Hs** hepatic sinusoid, radiating hepatocytes **D:** showing **pt**:portal triad, **Bd:** bileduct **Pa:** hepatic artery **Pv:** hepatic vein

5.2.7.Effect of C.nardus formulation on histopathology of kidney

Rats given large dosages of C. nardus formulation did not exhibit any appreciable microscopic alterations in their kidney sections as compared to controls (Figure B&D), according to histopathological investigations (Figure A&C). The kidney sections of the treated rats showed the following: normal glomerulus (G), Bowman's capsule lined with upper visceral layer/podocytes (P) and lower parietal layer/squamous cells (SC), urine space (US), proximal convoluted tubules (PCTs) lined by simple cuboidal epithelium with brush border, distal convoluted tubules (DCTs) lined by simple cuboidal epithelium with more nuclei per cross-section,

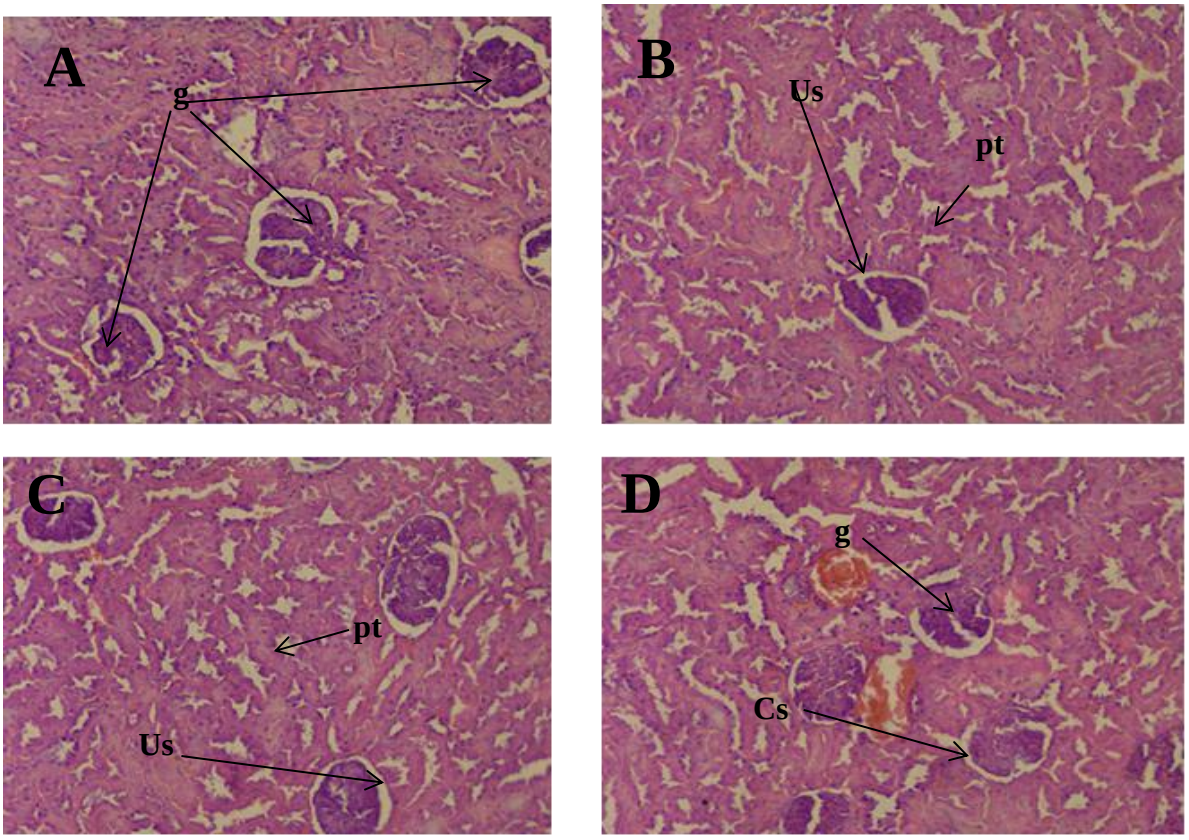

Figure 4. Image taken from the microscope to evaluate the effect of 28 days dermal application of C. nardus formulation on kidney of Abino wistar rats. **A:** male rat tested with high dose showing **g**:Glomerulus, **B:** male rat from control group showing **Us :** Urinary space and **Pt :**proximal c/ tubules tubules **C:** female rat tested with high dose showing **Ca**:cortical area and **ma**:medullary area **D:** female rat from control group ,**pt:** proximal c/tubules **Cs**:capsular space

5.2.8.Effect of C.nardus formulation on histopathology of skin

Histopathological analyses of the skin sections of rats given large dosages of the C. nardus formulation (Figures A & C) revealed no appreciable microscopic alterations in comparison to the control group (Figures B & D). Sections of the treated rats' skin showed normal glands, an abnormal connective tissue arrangement, the dermis, and the epithelial layer.

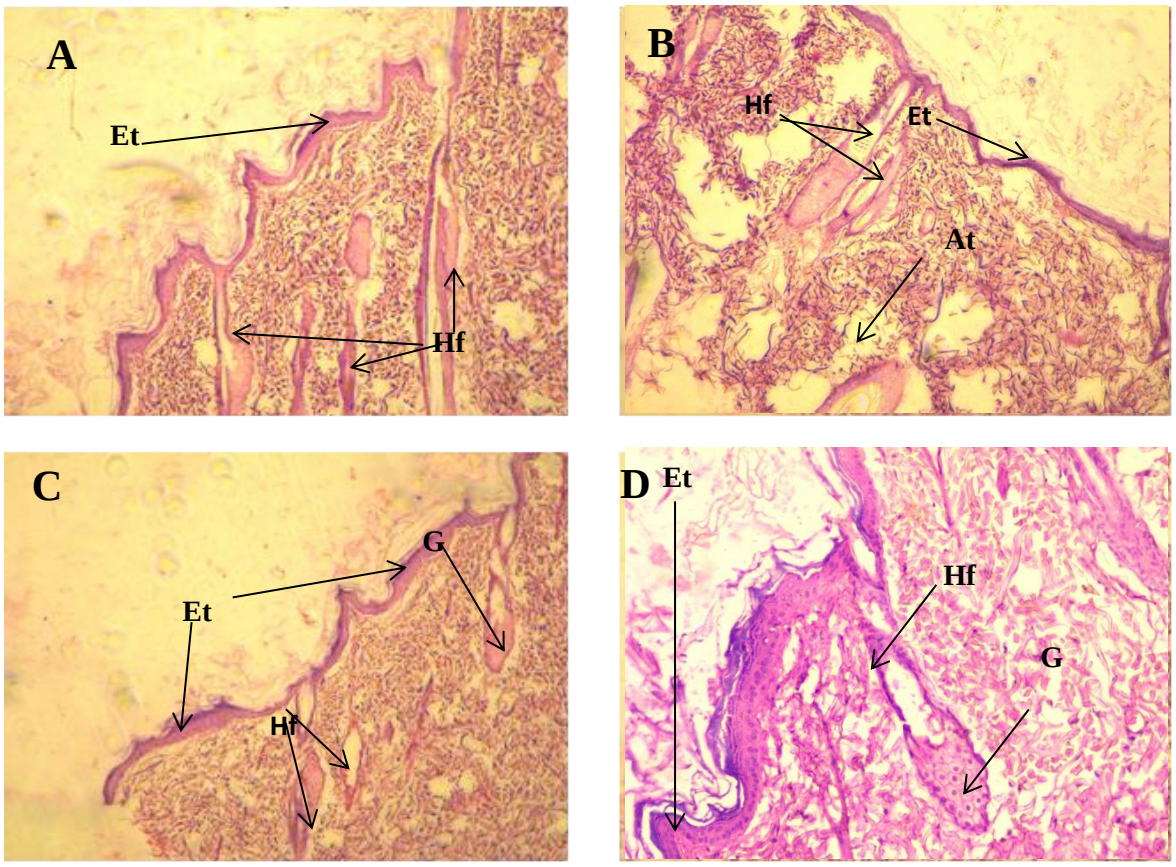

Figure 5. Image taken from microscope to evaluate the 28 days dermal application of C.nardus formulation on the skin of Albino wistar rats. **A:** male rat tested with high dose showing **Sg**: sebaceous gland, **At**:Adipocyte tissue and **Hf**:hair follicles **B:** female rat from control group)showing **Et:**Epitheliual tissue and **Hf**: hair follicles. **C:** female rat tested with high dose**) (**muscle tissue) and Adipocyte tissue **D:** male rat from control group showing epithelial tissue and irregular arrangement of connective tissue.

6.DISCUSSION

Traditional medicine (TM) refers to any , beliefs, and practices that are inherent to various cultures, that are utilized to maintain health as well as to prevent, diagnose, treat, or improve physical and mental illness (1). *Cymbopogon nardus (*Lemongrass oil or teji sar) is employed widely as a preservative and pesticide. It is the most common active ingredient found in "natural" or "herbal" insect repellents. Originally extracted from the grass plant *Cymbopogon nardus* (1).
The test for acute dermal toxicity was carried out according to the World Health Organization's Guidelines (WHO 2000) and the Organization of Economic Cooperation and Development's Guidelines for Testing of Chemicals (OECD 2017). The study of the acute oral toxicity of the C. nardus formulation during the 14 consecutive days did not show any mortality or signs of toxicity. It gave an LD_{50} estimated to be higher than 2000 mg/kg. This result is in line with the study of acute dermal toxicity, LD_{50} of N,N-diethylphenylacetamide (DEPA), a new multi insect repellent was 3200mg/kg (50). The formulation under test is categorized above the fifth class of toxicity, or in the class of compounds with a low acute dermal toxicity, in accordance with OECD guideline 402 and the General Harmonized System (GHS) of the United Nations (United Nations, 2011). This result is in line with the acute dermal toxicity study of polyherbal essential oil against blackflies with doses of 5%, 10%, and 20% (51).

According to the Organisation of Economic Co-operation and Development (OECD) guideline for testing of chemicals 410 (OECD 1981), daily administration of *C. nardus formulations at* 100, 200, and 400 mg/kg during the 28 consecutive days did not show any mortality or signs of toxicity. This result is in line with the acute and subacute dermal toxicity study of *M.malabathricum leaf ethanolic extract* with dose of 2.5% 5% 10% (52) However, the results showed a non-significant increase ($p > 0.05$) in mean water consumption, while the average food consumption of the treated groups of male and female rats was similar to the control ones. According to Dawoud, 2015 studies, the insignificant increase in mean water consumption could be explained by the progressive weight gain in animals and therefore is not linked to the formulation (53).

During evaluation of body weight of the treated and control groups results showed a non-significant increase in the bodyweight of treated animals compared to control ones. These results show that the formulation did not have a toxic effect on the body weight of treated rats compared

to the control ones, during the study period up to 400 mg/kg. this result is in line with the 28 day dermal toxicity study of the same plant oil in Burkinafaso (54).

For the evaluation of the effect of the *Cymbopogon nardus formulation* on the organs liver,kidney and skin a macroscopic examination of the organs was performed; the findings revealed no lesions, changes in color, or changes in shape in the test groups' organs as compared to the control groups in both the male and female rats. However, compared to the controls, there was a non-significant rise in the relative organ weights of the animals treated with the formulation. This finding indicates that the C. nardus formulation, up to 400 mg/kg, had no harmful effects on the relative organ weights of the rats treated in our sub-acute cutaneous toxicity investigation. Other study has also demonstrated that the animal essential organs lack abnormalities and histological indicators, *M.malabathricum leaf ethanolic extract* with dose of 2.5% 5% 10% (52).

Certain toxins can disrupt biochemical parameters and hematological once within the body, allowing for the identification of the intoxicated organs. Indeed, essential oils with plant origin have been linked to a number of adverse effects, including hepatotoxicity, nephrotoxicity, and cardiotoxicity (55). However, in this 28-day dermal toxicity study, there was no significant change in ALP (alkaline phosphate), ALT (Alanin transaminase), AST (Aspartate transaminase), ALB (albumin), creatinine, and urea levels in both male and female treatment and control groups. This result is in line with the study done in Ethiopia Evaluation of acute and sub-acute toxicity skin irritation test on cymbopogon citrus (56). There was also no significant change in RBC (red blood cells). WBC (white blood cell), HGB (haemoglobin), HTC (hematocrit), MCV (mean capsular volume), MCHC (mean capsular haemoglobin volume), LYM (lymphocyte), and PLT (platelet) that received C. nardus formulation at doses of 100mg/kg, 200mg/kg, and 400 mg/kg when compared with the control group. This result agrees with the 28-day dermal toxicity study of bensulfuron-methyl with doses of 250, 500, and 1000mg/kg (57).

7.CONCLUSIONS

- The dermal LD_{50} of the C.nardus formulation was found to be above 2000mg/kg
- The C.nardus formulation did not produce toxicity on the behavior and gross pathology of the acute as well as sub-acute study of rats at treated doses.
- The C.nardus formulation had no negative effects on the tested dosages' body weight, hematological, or biochemical markers.
- There were no signs of toxicity observed in the kidney ,skin and liver sections of treated rats.
- C.nardus formulation has a low dermal toxicity and therefore presents a less toxicological risk in the event of exposure.

8.RECOMMENDATION

Based on the result presented the following recommendation are made:-

- ✓ Well-designed research on the extract's sub chronic and chronic toxicity has to be conducted, taking into account factors such as body weight, hematological and biochemical parameters, and the histology of the liver and kidney.
- ✓ well-designed subacute, sub chronic, and chronic toxicity investigations must to be conducted to examine the impact of the extract on the liver and kidney at the ultramicroscopic level.
- ✓ Additional research on subacute, sub chronic, and chronic toxicity should be conducted on several other organs, including the heart, brain, lungs, pancreas, stomach, and intestines, among others.

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10. Appendices

10.1. Appendix I: preparation of working chemicals and solutions

10% Neutral Buffered Formalin

40% formaldehyde……100ml

Sodium hydrogen phosphate monohydrate…… ……4gm

Disodium hydrogen phosphate anhydrous 6.5gm

Distilled water…….900ml

Harris' hematoxylin (H)

Hematoxylin crystals……2.5gm

Absolute ethanol…… ……25ml

Potassium alum……50gm

Mercuric oxide…… ……1.25gm

Glacial acetic acid……20ml

Distilled water……500ml

1% Acid alcohol

70% Ethanol…….250ml

Hydrochloric acid (Concentrated)…….2.5ml

1% Alcoholic Eosin (E)

Eosin Y, water soluble……1gm

95% Ethanol……100ml

Glacial acetic acid…… ……..0.5ml

Bluing solution

Sodium bicarbonate…… ……2.5gm

Distilled water……1000ml

10.2. Appendix II: Tissue processing techniques and procedures

Fixation

Neutral buffered formalin, 10%.....24hrs

Washing

Running tap water……..….24hrs

Dehydration

Ethanol, 70%.....2hrs

Ethanol, 90%.....2hrs

Absolute Ethanol I, 99.9%.....1 1/2hrs

Absolute Ethanol II……1 1/2hrs

Absolute Ethanol III…….1 1/2hrs

Absolute Ethanol IV……..…….....overnight

Clearing

Xylene I……1 1/2hrs

Xylene II……..1 1/2hrs

Infiltration (in hot oven, 60oC)

Paraffin wax I (56oC)…… 1 1/2hrs

Paraffin wax I (56oC)…… 1 1/2hrs

Paraffin wax I (56oC)…… ……..…overnight

10.3. Appendix III Routine Hematoxylin and Eosin (H &E) staining procedures

Deparaffinization

Xylene I…….……..5min

Xylene II…….….5min

Rehydration

Absolute alcohol I…….……..…….4 min

Absolute alcohol II…….……..4 min

95% Ethanol……...……..….3 min

70% Ethanol…… …….……..3 min

Rinse in distilled water…….……5 min

Stain in Hematoxylin…… ……15 min

Rinse in running tap water.....…...5 min

Decolorized in acid alcohol.....1-3 sec

Rinse in running tap water…… …….……..…….....5 min

Immerse in sodium bicarbonate solution……..…… ……3-6 sec

Rinse in running tap water…….…..….…….…...5 min

Counterstain in Eosin……..……1 min

Dehydration

70% Ethanol……2 min

95% Ethanol……..……..2 min

Absolute Alcohol II……..…….….2 min

Absolute Alcohol I……..…….….2 min

Clearing

Xylene II……4 min

Xylene I……4 min

Mounting

DPX as mounting media