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Evaluation of Acute Toxicity and Anti-Rabies Activity of *Convolvulus Kilimandschari, Kalanchoe Lanceolate and Stephania Abyssinica,* Traditionally Used for the Treatment of Rabies in Arsi Zone, Oromia Regi onal State, Ethiopia.

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This is to certify that the thesis prepared by Anberber Alemu entitled:

Evaluation of Acute Toxicity and Anti-Rabies Activity of *Convolvulus Kilimashari,Kalanchoe Lanceolata* and *Stephania Abyssinica*, Traditionally Used For The Treatment of Rabies In Arsi Zone Oromiya Regional State, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Medical Laboratory Sciences (Diagnostic and public health microbiology)complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

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Abbreviations

ALB2	Albumin
ALP	Alkalaine Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BILD	Direct bilirubine
BILT	Total Bilirubine
Ca	Calcium
CDC	Centers for Disease Control and Prevention
CHOL2	Cholesrol
CK	*Concolvulus Kilimandschari*
Cl	Chlorine
CNS	Central Nervous System
CREA	Creatinine
CSF	Cerebra Spinal Fluid
EPHI	Ethiopian Public Health Institute
FAT	Fluorescent Antibody Test
FITC	Fluorescein Isothiocyanate
GLP	Good Laboratory Practice
GLUC	Glucose
HDL	High DensityLioprotin
K	Potassium
KL	*Kalancheo Lanceolate*
MOI	Multiplicity of Infection
MST	The mean survival time
Na	Sodium
NC	Nucleiccapsid

Abbreviations (Continued)

NTV	Nerve Tissue Vaccine
OECD	Organization of Economic Corporation and Development's
OIE	Office International des Epizooties
PBS	Phosphate Buffer Saline
PEP	Post Exposure Prophylaxis
PHO2	Phosphres
PV	Pasteur Virus
RABV or RV	Rabies Virus
SA	*Stephania Abyssinica*
TMMRD	Traditional and Modern Medicine Research Directorate
TMPs	Traditional Medicinal Plants
VDPD	Vaccine and Diagnostics Production Directorate.
WHO	World Health Organization

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ABSTRACT

Background; -Rabies is viral zoonotic disease progressive neurological infection which causes encephalitis in all warm-blooded animals. Dogs are the greatest reservoir host and transmit the disease to other animals and man. It is 100% fatal after the on-set of clinical manifestation of the disease. Plants are widely used conventionally for the treatment of rabies and contribute to the development of modern drug and treatment of this disease.

Objective: -The objectives of this study was to assess the acute toxicity and anti-rabies activity effect of *C. kilimandschari, K. lanceolate and S. abyssinica,* traditionally used for the treatment of human rabies.

Methods: - An experimental study was conducted between February, 2020 and June, 2021. *In vivo* acute toxicity and anti-rabies activity test was done in 78 Swiss albino mice. Extract was prepared from the powdered study plant using 70 % hydro-ethanol and concentrated by using rotary evaporator. Blood samples were collected for the assessment of biochemical parameters. Brain samples were collected for confirmatory test of rabies virus. Data was analyzed using SPSS version 26 for windows software, Statistical analysis was undertaken by one-way analysis of variance (ANOVA).

Results; -In this experiment, 20,15and 30gm crude extract / yield/ obtained from 100gm powdered 70%hydro ethanol extracted of *C. kilimandschari, K. lanceolate and S. Abyssinia* respectively. The crude extract of S. Abyssinica did exhibit sign of toxicity at dose 550mg/kg treatment groups of mice, The LD50 of Abyssinica was 560mg/kg.*C. Kilimandschari and K. lanceolate* did not exhibited any sign of toxicity at all doses levels. The level of serum enzyme ALPand CHO of mice treated with *C. Kilimandschari* were highly reduced. However, ALT, AST, BILD, BILT, Urea and K were increased in treated mice with *K. lanceolate* compared to negative control. All plant crude extract did show significantly($p<0.05$) increase in the survival period of time compared to positive control group of mice and they have anti-rabies activity in all dose of crude extracts.

Conclusion: - All plant extracts had demonstrated significant anti-rabies virus survival rate as compared to positive control groups. However, there is a need of large scale and detail studies should be designed.

Key words: Acute toxicity, Anti-rabies activity, *Stephania Abyssinica, Convolvulus Kilimandschari* and *kalanchoe Lanceolate*.

1. Introduction

1.1Background

Rabies is an important zoonotic viral disease with progressive neurological infection which causes encephalitis in all warm-blooded animals [1]. Rabies was primary identified in Egypt about 2300 BC and in ancient Greece, where it was well defined by Aristotle [2]. The first rabies epidemic in Ethiopia was recorded August 1903 in Addis Ababa [3]. Louis Pasteur in 1881 demonstrated the neurotropism of the virus. The first effective human rabies vaccine was developed in 1885by Louis Pasteur and Emile Roux which was an extremely effective rabies vaccine that provides immunity to rabies [4]. Rabies virus is neurotropic, negative sense, non-segmented, single-stranded RNA virus that belongs to the order of Mononegaviral, family of Rhabdoviridae and genus of *Lyssavirus* [5].

Entirely all warm-blooded animals are vulnerable to rabies and can spread of Rabies virus. Rabies virus has various mammalian reservoir host species from the orders Carnivora and Chiroptera including dog, cat, fox, wolves, raccoons, jackal, skunk, coyote, and bats. Dogs are the greatest important reservoir host and transmit the disease to other animals and man [6].Rabies virus is transmitted through non-bite breathe in rabies virus elements, organ and cornea transplants, exposed of open part of body, scratches and mucous membranes through saliva and brain tissue from a rabid animal containing RABV [**7**].The duration of a non-clinical incubation period of rabies ranges from 1-2 months but may vary from a week to several months, even years [8].Mortality in RABV exposure depends on the severity of bite, site of bite and the concentration of virus in the saliva [9].

The highest risk factor is bites on the hands, neck, face and head mainly with bleeding with shorter incubation period of rabies due to the decreased length and greater number of neurons. In general site of exposure and dose of RABV can persist in the muscle for long period, which might give a chance for post-exposure treatment and clearance of RABV by the immune system of the host [10] .It is 100% fatal due to involvement of nervous system after the on-set of clinical manifestation of the disease [11].In several developing nations, humans death due to rabies infection is under-reported because of cultural beliefs, inadequate rabies diagnostics and underprivileged of knowledge on the means of transmission and prevention of the rabies virus [12]. The genome of rabies virus is around 12 kb in size, which encodes five structural proteins

these are, nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) [13]. The RABV genome is composed of N, P and L proteins, which forms ribonucleoprotein complex that is responsible in replication of the virus in the cytoplasm of host cells. The Glycoprotein is the structural protein of the rabies virus that expressed on the viral surface, it is responsible for the viral pathogenicity and induces protective immunity against rabies [13, 14]. It is estimated that at least 59,000 human mortality happen each year globally, in Asia an estimated 35,172 and in Africa an expected 21,476 people died due to rabies annually.99% of deaths due to rabies cases in humans are dog bites in developing countries of Africa and Asia [15]. The mortality of rabies is preventable with preexposure prophylaxis and immediately provision of rabies post-exposure prophylaxis wound treatment, rabies immunoglobulin (RIG), and mass vaccinations of dog [16**].** The most reliable diagnosis of rabies can be done during the clinical period of the disease or after death on CNS tissues. Brainstem is the most important region for diagnostic tests [17].

The clinical sign of rabies is confused with other neurological signs caused by other neurotropic etiological agents. Detection of rabies virus using Reverse transcriptase and polymerase chain reaction (RT-PCR) and nucleic acid amplification are diagnosis techniques for the detection of RABV antemortem confirmation of rabies in clinical samples [18]. The direct fluorescent antibody technique (DFAT) is a golden standard test for diagnosis of rabies and approved by WHO.Because of short duration, low cost and high sensitivity. specific, cheap and gives reliable results providing results within few hours in more than 95-99% of rabies cases [19]. In addition to DFAT, mouse inoculation test is carried out especially in developed countries, which is also a highly sensitive method [20].

Traditional medicine is an ancient medical practice which exists, in the communities before the advent of modern health science. It is based on indigenous theories, beliefs and experience th at are conserved down from generation to generation [21]. Medicinal plants are help for treatment and prevention of for different types of diseases because of the presence of phytochemical ingredients naturally occurring in different parts of medicinal plants, Chlorophyll, proteins and common sugars are primary constituents and flabnoid, alkaloids and phenol steroids and terpenoid are an example of secondary compounds found in medicinal plants [22]. Terpenoids was

confirmed many significant pharmacologic activities such as anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities [23].

Medicinal plants play a greatest role in the advancement of modern studies on medication and by Serving as a starting point for the development of modern drug [24]. Plants have contributed to modern medicine, an estimated of 25% of modern drugs contain one or more biochemicals obtained from plants [25]. Currently, the important of remedial plants has been receiving attention worldwide because of various positive advantage such as affordability, accessibility, cost effectiveness, cultural believe, safe, recognition of new drug discovery and treatment of different kinds of ailment from medicinal plants as well as increasing drug resistant of micro-organism towards allopathic medicines, failure and deleterious adverse effects of allopathic medicines[26].Toxicity study is important to evaluate the antagonistic consequence of bio active substance on human or animals prior to the application of medicinal plant extracts for clinical use [27].

1.2. Statement of the Problem

Rabies is endemic above 150 countries around the world and kills more than 59,000 people annually; mostly in the world's poorest and most vulnerable communities.About 40% of the dead are children under15 ages existing in Asia and Africa [15]. Still rabies is one neglected disease tropical diseases with a huge burden and the major public health problem that affects human, domestic and wild animals [12]. In Ethiopia the number of recorded and undocumented human rabies cases has indicated that rabies occurs frequently in the community and has shown no reduction for over twenty years in the country due to poor dog managements and vaccination [15].

In most of developing countries the anti-rabies activity of medical plants has not been well studied, evaluated and documented. In Africa the traditional knowledge on consumption of medicinal plants was undocumented science [23]. Most of the Ethiopian indigenous medicinal plants knowledge is accessible in pastoral societies also information is still secretly in the hands of traditional healers. The knowledge is based on theories, beliefs and experience of the healers and has been passed from one group to other by orally within families. Most of the traditional healers lacks knowledge on the dosage and safety while prescribing traditional medicine to their patients.About 90% of Africa's population and 80% Ethiopia's population of the health care system of rural population still depends on indigenous system of traditional medicines for their primary health care. In Ethiopia, several traditional medicinal plants are used for the treatment of rabies in human and animals in different ethnic groups reported by different investigators [28], Due to inaccurate diagnoses and treatment of traditional healers, local community noticed some death cases occur. There is an absence of published evidences, adverse fatal side effects and cases of rabies deaths after traditionally treated with medicinal plants were the most problems reported by EPHI and some health facilities in Ethiopia [29]. Several traditional herbs have been formulated by traditional healers to treat human and animals' rabies. The quality and efficacy of medicinal plant used for treatment of rabies in humans is complicated.

1.3. Significance of the Study

Medicinal Plants play a crucial role in the development of up-to-date medicine studies by helping as an initial point for the growth of new drugs. This study helps to identify the medicinal plant materials with the potential constituents applicable to modern medicine and important to provide information throughout the world, nation and at the level of communities. Further, it will help other researchers as reference to conduct research activity in investigating the biomolecules which are present in different parts of plant as Medicine. The study will be helpful to traditional healers to promote indigenous knowledge in collaboration with EPHI technical backup partnership to protect the benefit of healer's ownership and patent right of the product. Most of the Ethiopian indigenous medicinal plant's knowledge is presented in pastoral societies and information is still secretly in the hands of traditional healers.

2. Literature Review

Medicinal plants

Medicinal plants have a long history use to humans for thousands of years to treat medical illness and prevent many types of diseases. The use of the whole plant part for treatment causes hazardous effect on human and animals. Because the presence of different types of secondary metabolites found in the medicinal plants change in the plant's compounds in different climates, development of synergistic compounds, changes secondary metabolites, storage and preparation of plant parts [30].

Application of ethno-botany can principal to a strengthening of social variety conservation, better sustainability in the manipulation of plant resources, and the growth of novel plant products. And also, application of ethno-botany can lead to rural developments by identifying and promoting useful plants resource for local use, in natural managements and conservation identified and promoting good conservation practice and in biodiversity prospecting[31].

Most researchers in Africa have recognized the importance of recording traditional knowledge for further development, such as in Ethiopia, Tanzania and South Africa [32,33and34]. The evaluation of the efficacy, safety and dosage of traditional medicines is crucial, due to the reliance of the African population on plants as sources of medicines [35]. The higher dose of root crude extract of the plant part may contain compounds that affect the propagation and pathogeneses of rabies virus in vivo than lower doses. Other previous studies showed that the leaf extract of P. dodecandra has moderate activity against Coxsackie virus in -vitro system [36].

Stephania Abyssinica, vernacular name, "ye ayit hareg" or, "Este eyesus" (Amharic) and "Hidda Hantutaa" (Afan Oromo) is a plant with in the family Menispermaceae and genus Stephania. It is found particularly distributed in tropical Africa, including Ethiopia. Its natural habitat includes grassland, abandoned fields and roadsides, at elevations up to 3500 m. The members of genus Stephania are slender, climbers with peltate and membranous leaves. The arrangements of flowers are in umbelli form cymes, which arise from axils or from old leafless stems. S. Abyssinica is a liana wood at the base, 2-3m in height. The leaves are peltate, broadly ovate, round base and membranous leaves. Flowers are cream or reddish. Fruits are yellow or pinkish green round and 5-8 mm in diameter. Its flowering seasons ranges from spring till early autumn [37,38and 39].

Traditionally, in Abeokuta south resident administration part of Ogun state in Nigeria, the leaves of *S. abyssinica* have been used for treatment of different ailments such as the menstrual disorders and child birth problems [40].

In Ethiopia, it is used for the treatment of anthrax, stomach problems miscarriage, rabies, syphilis and external tumor. *Stephanie* and Stephavanine isolated from *S. abyssinica* have been shown to exhibit antitumor activity and antineoplastic activity[41]. The roots and rhizomes of *S. abyssinica* were evaluated from body parts on in-vitro. The test showed antioxidant activity [42]. The root and leaf of *S. abyssinica* extracted by aqueous and methanol was evaluated on mice showed anti-diarrheal and antispasmodic activities [43]. The 80% methanol leaf extract of S. abyssinica showed analgesic and anti-inflammatory activity on mice [44]. The solvent fraction *S. abyssinica* Walp leaves showed antimalarial activity in in- vitro test against chloroquine sensitive and resistant laboratory adapted strains of *P. falciparum* [45].

Kalanchoe lanceolata; - vernacular name, ‟Endehuahula‟‟(Amaharic)and ‟Bosoqaa Buree‟‟(Afan Oromo) The genus name *Kalanchoe* comes from the Chinese word Kalan Chauchy," which mean falls grows "It defines the asexual reproduction in the genus, where plantlets are designed on leaf margins and fall onto the ground to produce new plants and the species name 'lanceolata' comes from Latin, describing the lance-shaped leaves of the plant.

It is succulent, yearly herb, growing up to 1.5 m height. A rosette of sessile, green or yellowish green leaves are borne on an erect, more or less, definitely 4-angled, hairy stem that grows from a fairly swollen rootstock. Leaves of *K. lanceolate* are smooth or with granular hairs; margins are usually entire on upper leaves and scalloped on lower leaves. Attractive, star-like, apricot-yellow flowers are borne in an elongate thyrse on the axil of upper leaves [46].

Kalanchoe lanceolate is cultivated worldwide in the agricultural manufacturing as a common ornamental plant. It is similarly used in several rural societies as traditional medicine. In the Batswana society. Roots of the plant are dried and ground into a powder, to treat headaches and neck pains. The powder is taken as a snuff. It is believed that when one sneezes after taking the powder, the pain goes away [47].

Ethnic communities in India use the leaf juice of the plant to cure dysentery. In Ethiopia use fresh leaves to heal wounds, by heating them moderately, and applying them to the affected area. In Namibia, the Kwanyama tribe boils the leaves of the plants to produce an infusion that is poured into a child's ear, to reduce fever [48].

Convolvulus; - is a genus of the Convolvulaceae family (bindweed or glory family), which is one of the medicinally and economically important family, including about 250 species of flowering plants, present as trees, shrubs, and herbs.). The use of the Latin and common name of *Convolvulus* plants for each species was complicated before the currently accepted names because of the widespread of this family in the world [49].

Naturally occurring chemicals from *Convolvulus* species are considered to be an organic food preservative for controlling the food spoilage. Antioxidant and antimicrobial activities of natural compounds prevent lipid oxidation and growth of food pathogens and extend their shelf life. *C. arvensis* ethanol extract has been applied on beef patties to prevent lipid oxidation[50].

Convolvulus plants have great medicinal value to humanity and society. The pharmacological potential of these plants comes from their chemical constituents with biological activities. Pharmacological activities were reported on some *Convolvulus* species. *Convolvulus* plants include a large number of important species, which have the properties of treatment of serious diseases such as fever, loss of memory, insomnia, heart disease, and hair growth[51].

Convolvulus genus was used in traditional medicine dates back to 1730s. In general, Concolvulus plants displayed profuse medicinal uses. Different parts of plant C. arvensis have been used as antispasmodic, antiinflammation, anti-swelling, treatment for painful joints, treatment against flu, antihemorrhagic, antiangiogenic, laxative, treatment for wound, diuretic, antidandruff, and treatment for parasitic infections and jaundice[52].

According to the report of Meresa A et al indicates that a total of 199 plant species to 47 families were listed as good indicators for the presence of considerable diversity of plant species prepared using fresh material, dried form and either fresh or dried form of the plant parts are used for the management of rabies in Ethiopia. However, only three medicinal plants had been scientifically evaluated on mice to evaluate the anti-rabies activity [53].

A study conducted in Ethiopia by Admasu and his collagenous indicated that traditional healers use *Phytolacca dodecandra* locally called Endod or Handoodee in Oromic; is most widely used in Ethiopia to treat rabies, the efficacy of the crude extracts of roots and leaves of P. dedecandra evaluated in mice showed anti-rabies effect [54].

A study conducted in Ethiopia, by Deressa and his collagenous reported that the evaluation of the efficacy of the crude extract of Salix Subserrata locally called Aleltu and *silene subserrata* locally named Wegert for the treatment of rabies on mice. The result indicated that chloroform and

80%methanol extracts of *S.* macroselen and chloroform and aqueous extracts of *S. subserrata* of the root of the same plant did show to increase the survival time of mice significantly [55].

The study conducted by Guyid A. et al on the leaf of *Justicia schemperiana*: locally named sensel or Dhummuugaa in Oromic; is used for treatment of rabies, malaria, syphilis, leprosy, gonorrhea and measles. His report showed that methanol extraction of the plant is preferable with appropriate probable concentration. Polyphenols, unsaturated sterols and saponins chemical compounds were present in the preparations which are supposed to have anti-microbial and anti-viral properties [56].

3. Objectives

3.1. General Objective

To evaluate the in vivo acute toxicity and anti- rabies activity of *Convolvulus kilimandschari, Kalanchoe lanceolate and Stephania abyssinica*, traditionally used for the treatment of rabies in Ticho wereda Arsi zone Oromia regional state, Ethiopia.

3.2. Specific Objectives

To evaluate in vivo acute toxicity effects of the root extracts of *Convolvulus kilimandschari, kalanchoe lanceolate and Stephania abyssinica,*

To examine the effect of plant crude extract on liver function test and renal function test on from experimental animals.

To identify the presence of phytochemical compounds in the crude extracted root of study plants.

To assess the anti-rabies activity of *Convolvulus Kilimandschari, Kalanchoe lanceolata and Stephania abyssinica,* used for the treatment of rabies.

4. Hypothesis

Null hypothesize; - medicinal plants will have toxic effect and they have not anti-rabies activity for the treatment of rabies case

5. Material and Methods

5.1. Study Area

Arsi is one of the zones of the Oromia Region in Ethiopia. It is also the name of a former province. Arsi is bounded on the south by Bale, on the southwest by the West Arsi Zone, on the northwest by East Shewa, on the north by Afar Region and on the east by West Hararghe. The highest point in Arsi is Mount Chilalo; other notable mountains in this zone include Mount Kaka and Mount Gugu. The administrative center of this zone is in Asella;

Ticho is one of the woreda in Arsi zone, Oromiya Region of Ethiopia. It is 257km far from the capital city of Addis Ababa. The altitude of this woreda ranges from 1800 to over 4100 meters above sea level; the highest point in this woreda is Mount Bada (4195 meters)[57].

5.1.1. Study Setting

The study was conducted at Ethiopia Public Health Institute (EPHI), Vaccine and diagnostics production Directorate (VDPD), Traditional and modern medicine Research directorate (TMMRD), National reference Clinical Chemistry Laboratory and Rabies laoratory, Addis Ababa Ethiopia, during the period of October 2020- June 2021.

5.1.2. Collection of medicinal plant and identification

The root of remedial plants was collected based on the information about the traditional healer from the river of Welkisa, Diminsho and Angadocho around Ticho town 257 km from the capital city of Addis Ababa October 2020 use by Principal Investigator, traditional healers, and Traditional medicinal plant experts from EPHI. They were collected from natural habitat of the plant by removing the root of the plants from the soil, then washed under running tap water and cut it in to pieces. The collected root of plants was packed separately with a plastic container and transported a day after collection to EPHI. Botanical identification and authentication were done at Ethiopian Public Health Institute by Traditional medicinal plant experts. The laboratory duties and evaluations were performed at EPHI. Each plant materials were dried in an open air without being exposed to direct sunlight and the dehydrated root of plants ingredients were separately powdered to suitable size for extraction.

5.1.3. Preparation of the root of plant extract

The roots of plants sunlight *Stephania Abyssinica, Convolvulus kilimandschari and kalanchoe lanceolate* were dried in an open air without being exposed to direct sun light for a month, forty-

five days and two months respectively. The dehydrated root of plants was grounded to various grades of distinction by means of a grinding mill (Hamburg 76 West Germany). The preparation of the extraction of the root of study plants were carried out in modern and traditional medicine research directorate in chemistry laboratory. The powdered root of study plants were balanced by digital balance and extracted using 70% hydro- ethanol maceration method [58]. 100g of each powdered root of plants were soaked in 500ml of 70%hydro ethanol in Erlenmeyer flask of one liter's capacity then mixed together. The flasks were plugged with Para film and each study plant was labeled based on the name of study plant, percentage of hydro ethanol, date and starting time of extraction at that time kept on a rotary shaker on 130rpm for 24hours. After 24hours the supernatant of the mixtures of the extracted plant materials were first filtered using gauze and again filtered with Whitman's filter paper No.1 to remove the solid particles from the solution. The residue was demarcated two times again by adding additional solvent and filtered two times the same procedure was repeated to the first one. Totally the extraction process takes place for 72 hours to increase the yield. Then the extracts were concentrated under vacuum in a rotary evaporator to remove the solvent (water and ethanol). The remaining solvent was evaporated after 7,10 and 15 days for *Convolvulus kilimandschari, kalanchoe lanceolata, and Stephania Abyssinica* respectively on water bath of +40°C to obtain dried extracted yield. Finally, yield of extracts was kept in desecrator to prevent moisture in airtight container throughout the study period [59].

5.1.4. Study design and Period

The experiment was conducted from October2020 to June 2021 and the type of study used to evaluate in-vivo acute toxicity and anti-rabies activity of medicinal plants both individually extracted medicinal traditional plant samples were used.

5.2. Preparation, Grouping and Management of Experimental Animals

A total of 84 healthy female Swiss albino mice with age of 4 weeks old that weight between 15.5-19.3g were obtained from a standard laboratory animal house of Ethiopian Public Health Institute (EPHI) from the laboratory animal unit. The animals were adapted to the laboratory environments for 7 days before conducting any experiment. The mice were kept in a littered clean metal cage at a temperature of 22^0c ± 2°C and with a 12 h light/12 h dark cycle with litter changed every two days [60]. They were given clean pellet diet, water and libitum. All experimental mice were treated under similar feeding management. And each mouse was used only for one experiment. All mice

assigned to the experiment were handled according to standard guidelines for the use and care of laboratory animals [61].

5.3. Experimental Design

5.2.1. In vivo Acute toxicity Study:

A total of 84 mice were randomly assigned in to 14 groups (one negative control group and 13 treatment groups). Each group consisting of 6 mice and each mouse was assigned a unique identification number then their cage was labeled based on dosage groups of mice and date of administered. After the mice were fasting for 4hrs. The body weight of each mouse was weighted and the dose was calculated according to the body weight. Then the root of plants crude extracts was prepared based on the dose by mice weight and each plant extract was weighted with digital balance and mixed with per ml distilled water[62].

The treatment groups of mice were classified into four sub-groups for each dose of 70%hydro-ethanol all root of plants extracts *S. Abyssinica* 175, 300, 550, and750 mg/kg body weight of mice. *K. Lanceolate and C. kilimandschari* were administered orally at the doses of 175, 300, 1000 and 2000 mg/kg body weight of mice by using intra-gastric needle in10ml/kg based on the mice body mass [63].

After administering the study plant materials, the mice were kept in their cage then followed continuously for 2 hours and 2 times every 24hours for 14 consecutive days for any sign of delayed toxicity. Observations of the clinical signs of toxicity include change in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavior pattern [64]. The mortality of each group of mice were recorded within 24 hours [65].

5.2.2. Biochemical Test for Acute Toxicity Study:

After Forten days, the experimental animals were fasted for 4 hours then their body weight were measured. They were then and sedated with diethyl ether and immediately individually mice were placed in supine position on operational board. The extremities of the animals were stretched and fixed on a dissecting board. The blood sample was withdrawn by cardiac perforation by means of sterile needle of 5ml syringe. The blood samples for biochemical assay were placed into test tubes without anticoagulant [66]. The samples were centrifuged using an electrical centrifuge at 3000

revolution per minute (RPM) for 10 minutes. Direct bilirubin and Total bilirubin, Albumin, AST, ALT, ALP and CHOL2 and HDL were used for liver function test. Urea, creatinine, Calcium, Phosphors, Glucose, Sodium, Potassium and chlorine were analyzed on Cobas 6000 (Roche Diagnostics, Mannheim, Germany) using Roche Serum Index Gen2 (SI2) at Ethiopian Public Health Institute, National References Laboratory for Clinical Chemistry, Addis Ababa, Ethiopia. Data quality was ensured through the use of standardized operating procedure for laboratory analysis quality control samples were run to assess the functionality of the instrument.

5.2.3. Histopathological test for In Vivo Acute toxicity Study:

After 14 days, all experimental mice were euthanized and their liver and kidney were collected for Physical examination. Physical examination of all collected samples was examined during collection of samples at TMMRD. The samples of liver and kidney were weighted then preserved with 10% formalin for microscopic examination. (Annex)

5.3. Phytochemical analysis

Qualitative phytochemical screening test was carried out by means of the standard method [67].

5.3.1. Test for Alkaloids

To screen the existence of alkaloids in the study plants, Mayer's test and Wagner's test were used.as follows;

a. Mayer's test

A few ml of extract samples was added in to a test tube then two drops of Mayer's reagent was added along the sides of test tube. [68]

b. **Wagner's test**

A few ml of crude plant sample extracts was added along the sides of test tube then a few drops of Wagner's reagent were added in to plant samples extract. [68].

5.3.2. Test for Coumarin

Two ml of plant extracts were added in to each test tube then 3 ml of 10% NaOH was poured in to the test tube then well mixed [69].

5.3.3. Test for Flavonoid

Three ml of crude plant samples extract were placed in to a test tube and added 1 ml of 10% NaOH solution then mixed well. [68].

5.3.4. Test for phenols

Few drops of crude extract was placed in the test tube and mixed with 2ml of 2% solution of FeCl3 [70].

5.3.5. Test for Saponins

Three mg of crude extracts were added into a test tube and3 ml of distilled water were in to plant extract and shaken well [69].

5.3.6. Test for steroid

Few crude plant extracts were placed in test tube and mixed with 2ml of chloroform, then concentrated with 2ml of H2SO4 was added sidewise [70].

5.4. In-vivo anti-rabies activity of traditional medicinal plants:

To evaluate in vivo anti-rabies activity of experimental crude root of plant extracted based on the information of traditional healer prescribed to the patient used for the treatment of rabies in the study area.Eighty Swiss albino mice were obtained from laboratory animals breeding unit at EPHI and acclimatized to the laboratory conditions for 7 days. Then randomly assigned into treatment and control groups. The treatment groups were categorized into three sub-groups for each dose root of crude extracts of Stephania Abyssinica 300and 550mg/kg and Convolvulus kilimandschari and Kalanchoe Lanceolata 1000 and 2000 mg/kg) [63]. For each part of plant crude extracts were administered. Positive and negative control group of mice were assigned.

5.4.1. Rabies Virus Strain

The PV-12 virus strain was prepared from rabies virus infected rabbit brain for NTV production stocks. First, we determined the titer of the virus by inoculating on Swiss albino mice at Vaccine and Diagnostic production directorate, nerve tissue anti-rabies vaccine production laboratory EPHI. Before the experiment was conducted, protocols for this experiment followed the guidelines on care and well-being of research animals and standard protocol observed in accordance with the Good Laboratory Practice (GLP) Regulations in rabies [71]. PV-12 virus inoculation was conducted intra-cerebrally based on the procedure by Wunderli and his colleagues [69]. 10^{-5} PV-12 virus strain (Annex2).

5.4.2. Rabies Virus Inoculation

After the mice were acclimatized to the laboratory conditions for 7 days, they were randomly assigned into treatment and control groups. Ten mice per groups were used and each mouse was assigned a unique identification number. Cages were labeled based on dosage groups of mice and date of inoculation. All groups of mice were fasted prior to inoculation and dosing for 4 hours. after fasting, each mouse was weighed with digital balance and the dose was calculated according to the body weight of each mouse. PV-12 virus was prepared from rabies infected rabbit brain and the virus was prepared in 10^{-5} suspension in phosphate-buffer saline (PBS) solution. The rabies virus inoculation was conducted intra-cranial based on the procedure by Wunderli and his colleagues [71]. All groups of mice except a negative control, positive control and treatment groups were inoculated with10^{-5} PV-12 virus strain. The dosage of inoculum used for intracranial injection of mice was 0.05ml. After injecting the desired volume of inoculum, the needle was removed gently and slowly to prevent reflux of the inoculum. Then all information (the type of mice, age of the animals, the inoculation date, the inoculum used and dose administered, the number of mice per cage) were recorded on mice history follow up recording format [72].

5.5. Extract Administration

After challenged with PV-12rabies virus, the mice were allowed to stay in their respective cages for an hour to make them calm. Then, the treatment groups were orally administered with dose of 1000 and 2000mg/kg of the root of Convolvulus kilimandschari and Kalanchoe Lanceolata extracts, 300mg/kg and 550mg/kg of the root extract of Stephania Abyssinica using an intra-gastric needle based on the animal's body weight at 10ml/kg vehicle [73].

5.6. Determination of Mean Survival time

The experimental animals were monitored daily for any sign of rabies and mortality for 21 days after inoculation with rabies virus and administered with roots of crude extracts which were recorded throughout the follow up period. The mean survival times (MST) for each group of mice was calculated by SPSS Version 26 software by Kaplan-Meier.

5.7. Determination of mortality rate

The mortality rates of rabies virus challenge mice were determined by clinical signs and direct fluorescent antibody test [64]. All mice were kept and followed for 21 consecutive days after rabies virus challenge and roots of crude extract was administered. The mice were observed two times

per day after inoculated with rabies virus for signs of rabies (Roughening fur, tremors, in-coordination, immobility and prostration) and any symptom of rabies were recorded each day on the mouse history cards [65].Confirmatory diagnosis of rabies through direct FAT was conducted by opening the skull of mice and brain samples of the mice were collected randomly from those that showed clinical sign of rabid during the experiment period of time[66].The heads were held definitely in a vice fitted on the operation table with the rostral end of the head and the tail of mice pined. A midline incision was made on the dorsal surface of the head using scalpel and blade. The skull was then exposed by dissecting away the skin, apo-neurosis and temporal muscles and reflecting them laterally. Then the brain tissues were collected by cutting upper of the skull by scalpel [67]. Any available brain tissues were taken by forceps and kept in a petridish. The brain sample consists of cerebellum, hippocampus and brain stem. The smears were made by standardized protocol for the direct FAT [68]. The brain impression smears on slide were prepared from brain tissues and air dried for 15-20 minutes at room temperature. The smears were fixed in acetone for about one hour at -20°C. Brain impression smears were stained with FITC-labeled anti-rabies conjugate and incubated for 30 minutes at 37℃. Then they were soaked in PBS to decrease any nonspecifically binding substances. Afterwards they were rinsed with PBS and air dried. Finally, after mounting media was applied, the slides were then investigated under 40X objective of fluorescence microscope to detect characteristic green fluorescence associated with rabies antigen [18].

5.8. Data analysis and interpretation

Data were analyzed using SPSS version 26 for windows software (Armonk, NY: IBM Corp.). Statistical analysis was undertaken by one-way analysis of variance (ANOVA) tests coupled with survival analysis to compare results of treatment and control groups. Mean survival time was calculated and expressed as mean ±SD for each treatment and control groups. Paired t test statistic was applied for body weight change of mice in acute toxicity determination.

5.9. Ethical clearance

Ethical approval was gained from Addis Ababa University, Department Of Medical Laboratory Science Research Committee and permission certificate No DRERC/543/20/MLS for the study. The investigator and all personnel managing experimental animals were administered with pre-exposure anti-rabies vaccine Verorab. According to WHO guidelines of anti-rabies pre-exposure

prophylaxis, three doses of vaccine injection on days 0, 7 and 21 intramuscularly at deltoid area of the arm. All the mice used in the experiment were handled with care as per the guideline aimed at the care and use of laboratory animals, eighth edition.

5.10. Operational Definitions:

Acute oral toxicity: - is the adverse effect of happening subsequent oral administration of only one dose or various doses of substance given with in 24houres.

Dose: - is the volume of crude extract administered and expressed as weight of roots of crude extract per weight of experimental mice (mg/kg).

Extraction: -is the process in which one or components are separated selectively from a liquid or solid mixture.

LD50: -is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of laboratory animals whe

n administered by the oral routine.

Inoculation: - is the process of inoculating pathogen or antigen in the living organism to stimulate the production of antibodies to produce the specific disease.

6. Results

6.1 Root of plant extract results

A 100gm of powdered plant material was extracted using 70%hydro-ethanol by maceration method. Total percentage of yields obtained from 70% hydro-ethanol extraction per 100gm powder were20,15 and30 grams of *C.Kilimandschari, K.lanceolate and S. Abyssinica* respectively.

6.2 Acute toxicity study.

6.2.1 Acute toxicity by dose of crude extracts.

The roots of crude extracts of *C. kilimandschari and K. Lanceolate* did not exhibit any significant changes and was found to be completely normal for conditions shivering, stretching, skin and fur, eyes and mucous membranes, aggressiveness, convulsion, straub tail, jumping, fear, head twitches, sedation, loss of balance, respiratory and somatomotor activity. Only a mouse was dead at dose 1000mg/kg of *C. kilimandschari* due to nonspecific death which did not show any sign and symptom of toxicity. There was no any symptom and mortality observed from the administered mice during the period of acute toxicity study for both plants crude extracts. The lethal dose (LD50) for these two plants was above 2000mg/kg. No change also was found for S. abyssinica at 175mg/kg and 300mg/kg treatment group of mice. But administrated mice with dose level of *S. abyssinica* crude extract of 550mg/kg and 750mg/Kg showed shivering and stretching, 33.33% (2/6) and 83.33% (5/6) died within an hour respectively. The LD50 of root extract of *Stephania abyssinica* was 560mg/kg as shown (in table 2 and figure 1).

Table 1. In vivo acute toxicity in mice administrated with crude extract of S. abyssinica at different dose levels.

SA	Dose(mg/kg)	Log10	Response	% of Max death response
	175	2.2	0	0
	300	2.5	0	0
	550	2.7	2	40
	750	2.9	5	100

Table 2. LD50 administered with root of crude extract of Stephania abyssinica

Karber`s Method

Dose/mg	Dose diff (a)	Died	Mean mortality (b)	Product (a*b)
175	0	0	0	0
300	125	0	0	0
550	250	2	1	250
750	200	5	3.5	700
LD50	560			

Note: This method has more accurate and precise as well as good statistical properties. Its mathematical methods: the sum of the product is divided by the number of animals in a group and the results quotient was subtracted from the least lethal dose in order to obtain the LD50 value.

6.2.2. Liver and Renal function Test for Acute Toxicity of *C. kilimandschari and K. Lanceolate and Stephania abyssinica.*

The liver and renal function tests were conducted by evaluating serum enzymes. Mice treated with the root of crude extracts of *C. kilimandschari* the result shown that level of serum enzyme ALP and CHOL2 were increased however, AST, ALT, BILD and BILD showed decrease and K. Lanceolate were conducted by evaluated serum enzymes AST, ALT, phosphorus and urea were increased (Table4).In mice treated with crude extracts of *S. abyssinica*, the level of ALP, AST, CHO and HDL were increase in treatment groups of mice compared with negative control groups observed in this study (table 4).

Table 3. Liver and renal function in mice administered with roots crude extract of *C. kilimandschari* and *K. Lanceolate* at dose level of 300, 1000 and 2000mg/kg.

	Type of test	**Negative control**	300mg/kg	1000mg/kg	2000mg/kg
LFT for CK	ALB2 (g/dL)	3.11	3.19	3.30	3.37
	ALP (U/L)	68	45.4	56.33	89.67
	ALT (U/L)	86.13	81.37	172.13	48.7

	AST (U/L)	. . 376.85	306.92	285.03	203.3
	BILD (mg/dL)	0.19	0.26	0.11	0.1076
	BILT (mg/dL)	. 0.30	0.43	0.15	0.13
	CHOL2 (mg/dL)	93.6	97.72	118.1	93.3
	HDL (mg/dL)	83.57	69.44	106.8	80.17
RFT for CK	CA (mmol/L)	2	1.81	1.89	1.85
	Cl (mmol/L)	112.33	109.78	111.17	111.7
	CREA (mg/dL)	0.10	0.10	0.11	0.21
	GLUC2(mg/dL)	144.3	115.6	95.7	125.93
	K (mmol/L)	7.45	8.64	5.59	4.95
	Na (mmoL/L)	152	149.2	154	153
	PHOS2(mmol/L)	2.99	2.67	2.87	2.05
	UREAL(mg/dL)	38.28	34.94	45.97	37.27
LFT for KL	ALB2(mg/dL)	3.11	3.11	3.65	3.67
	ALP (U/L)	68	. 98.5	. 60	63.00
	ALT (U/L)	86.13	. 96.35	354.75	597.67
	AST (U/L)	376.85	460.45	953.7	1555.70
	BILD (mg/dL)	0.19	0.17	0.15	0.33

	BILT(mg/dL)	0.30	. 0.38	0.36	0.69
	CHO2(mg/dL)	. 93.6	. 118.3	89.15	129.30
	HDL (mg/dL)	83.57	. 105	. . 67.35	77.90
RFT for KL	CA (mmol/L)	2	. 1.83	1.86	2.15
	Cl (mmol/L)	112.33	111	106.8	108.8
	CREA (mg/dL)	0.10	0.165	0.195	0.11
	GLUC (mg/dL)	144.3	83.95	95.45	98.5
	K(mmol/L)	7.45	7.02	10.54	15.545
	Na(mmoL/L)	152	154	149.5	152
	PHOS2(mmol/L)	2.98	2.73	2.99	3.85
	UREA(mg/dL)	38.28	44.4	45.95	51.43

Table 4. liver and renal function in mice administered with crude extracts of roots of *S. abyssinica* 300,550 and 750mg/kg.

	Type of Test	Negative control	SA (300mg/kg)	SA (550mg/kg)	SA(750mg/kg)
LFT	ALB2 (mg/dL)	3.11	3.33	3.24	3.33
	ALP (U/L)	68	199.67	98	87
	ALT (U/L)	86.13	43.63	61.8	66.9
	AST (U/L)	376.85	303.57	410.2	426.3
	BILD (mg/dL)	0.19	0.17	0.24	0.10
	BILT (mg/dL)	0.30	0.39	0.58	0.263
	CHO (mg/dL)	93.6	106.53	98.6	119.4
	HDL (mg/dL)	83.57	89.43	79.65	94
	CA (mmol/L)	2	1.99	1.92	1.78

RFT	Cl (mmol/L)	112.33	111.63	108.1	107.1
	CREA (mg/dL)	0.10	0.25	0.135	0.25
	GLUC (mg/dL)	144.3	75.9	134.95	79.2
	K (mmol/L)	7.45	5.67	9.71	5.88
	Na (mmol/L)	152	154.67	149	151
	PHOS (mmol/L)	2.99	2.32	3.10	2.32
	UREA (mg/dL)	38.28	52.13	33.5	52.2

The mortality of mice were observed at dose level 550 and 750mg/kg of administered with crude extract of *Stephania abyssinica* and response mice

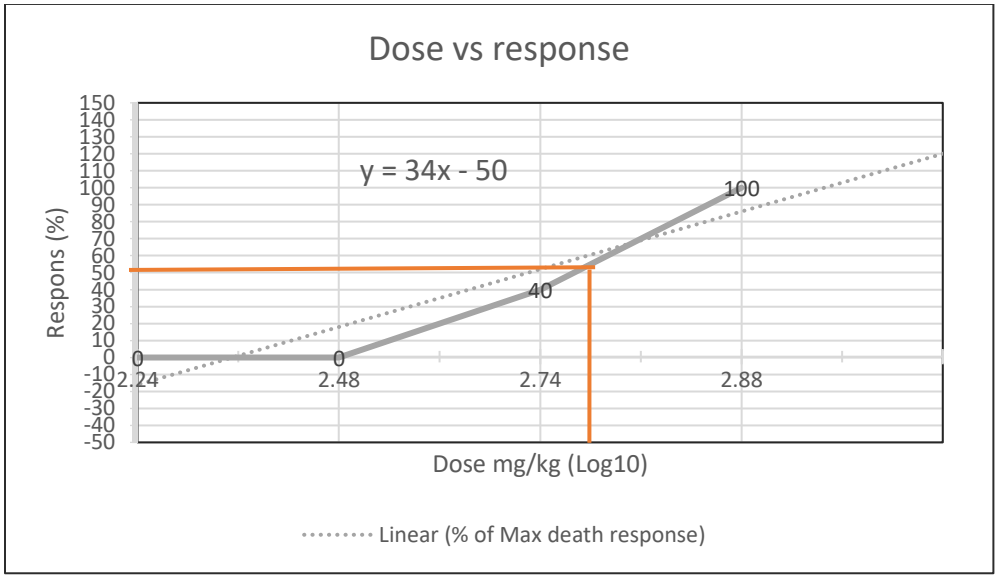

Figure 1 Mortality of mice administered with crude extract of *S.abyssinica* at dose level 550 and 750mg/kg.

6.2.3. The liver and renal function Test for acute toxicity of *C.kilimandschari*.

Liver function test; the result of root of crude extracts of *C.kilimandschari* was conducted by evaluating serum enzymes. The level of alanine aminotransferase (ALP) and CHO2 showed increased. However, AST, ALT, BILD and BILD showed decrease ; the result also showed a dose-dependent decreased in the treatment groups of mice compared with the negative control group of mice.

Liver function test of mice treated with root crude extracts of *C. kilimandschari,* the parameters serum enzyme ALP and CHO2 raised . However, the level of AST, ALT, BILD and BILD showed redused in all treated group of mice compared with control group. It is highly dose dependent and it might be due to the presence of saponin in plant crude extract.

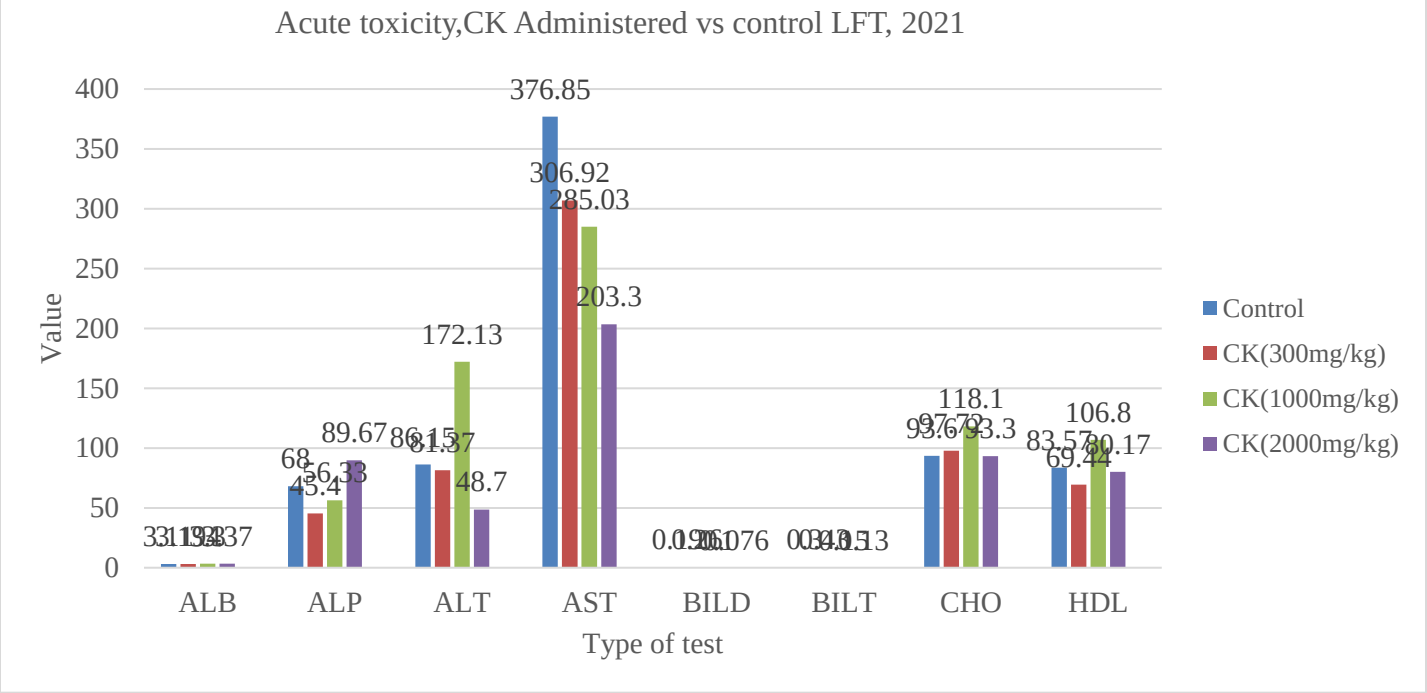

Figure 2 .Acute toxicity of liver function test of mice administered with *C. kilimandschari* at dose level of 300, 1000 and 2000mg/kg.

In the renal functional test; the result of the root of crude extracts of *C. kilimandschari*, urea was increased in the treatment groups of mice compared to with the negative control group of mice. It is considered as important markers for kidney disease

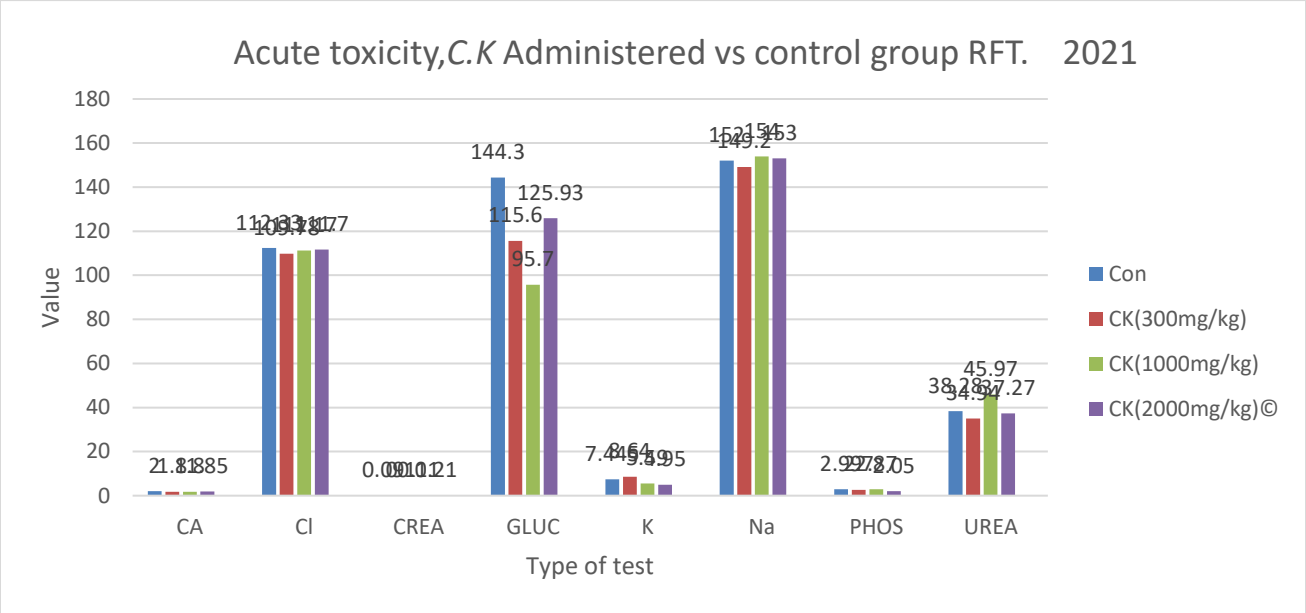

Figure 3. Renal function test mice administered with root crude extracts of *C. kilimandschari* at dose level 300, 1000and 2000mg/kg .

6.2.4. The liver and renal function Test for acute toxicity of *K. Lanceolate*.

Liver function tests of the root of crude extracts of *K. Lanceolate* was conducted by evaluating serum enzymes. The level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed extreme increase; the result also showed a dose-dependent increase in the treatment groups of mice compared with the negative control group of mice. The extremely elevated transaminase levels of enzymes indicated that it may have toxic biochemicals found in the crude extracted plant.

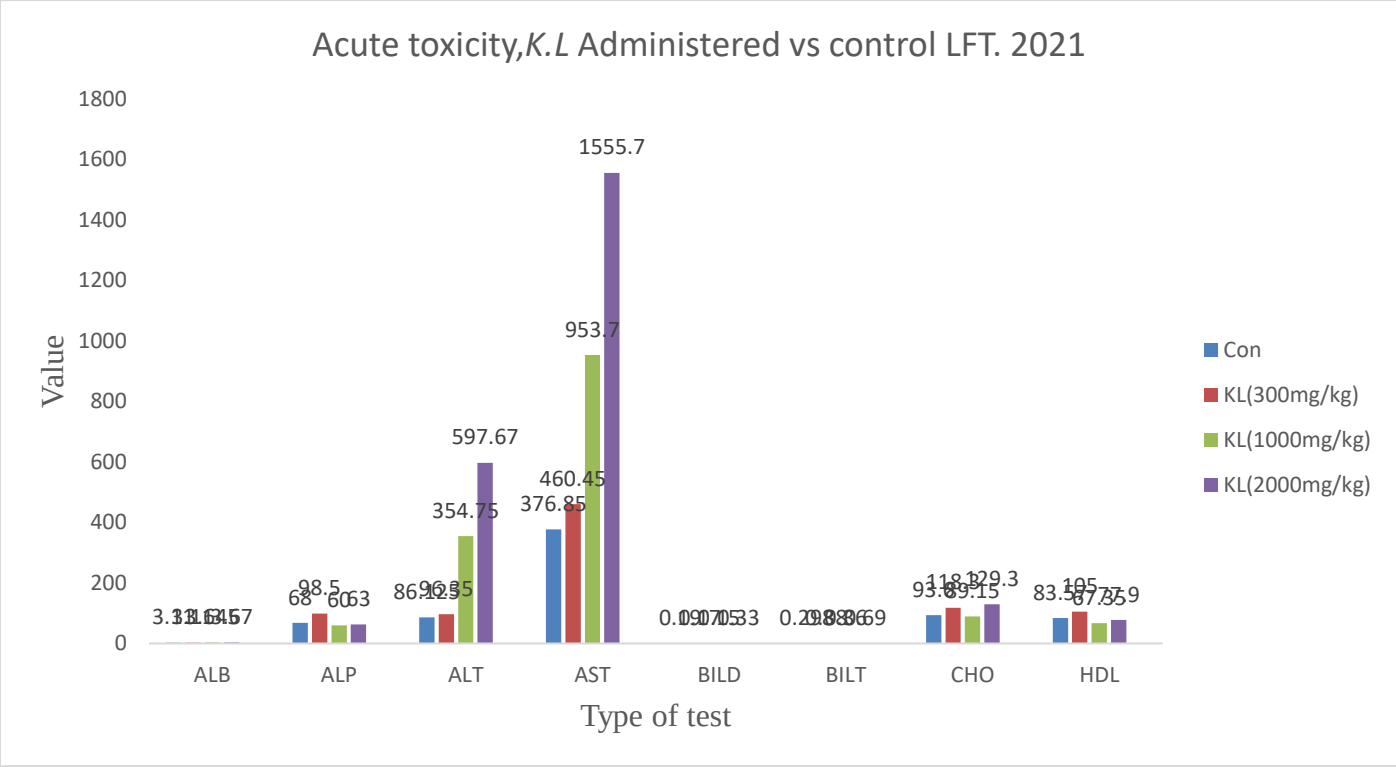

Figure 4 . Liver function test of mice administered with root crude extracts of K. Lanceolate at dose level 300, 1000and 2000mg/kg.

In the renal functional test; the result of the root of crude extracts of *K. Lanceolate*. phosphorus, potassium and urea were increased in the treatment groups of mice were compared to with the negative control group of mice. It is considered as important markers for kidney disease

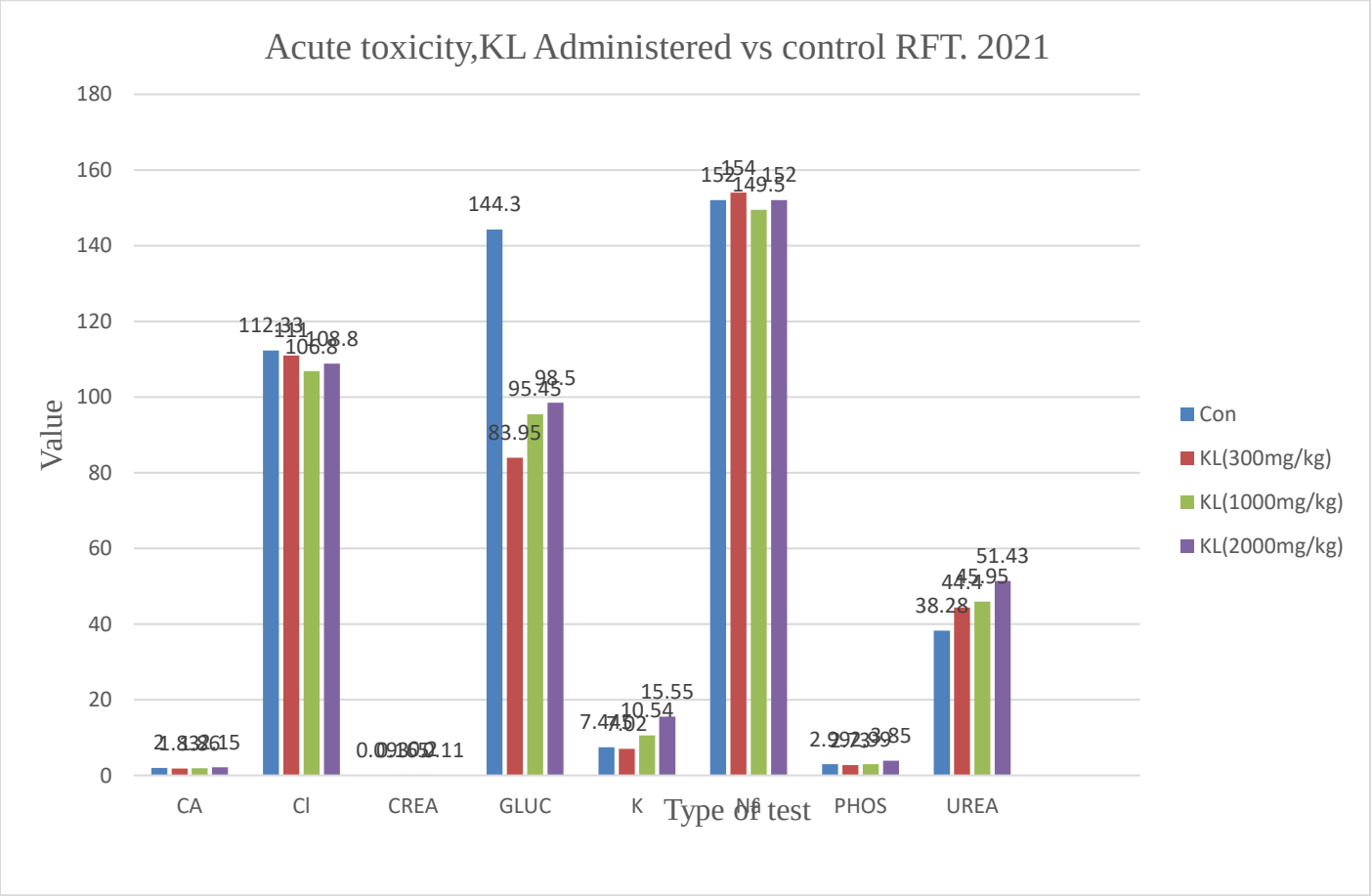

Figure 5.Rernal function test mice administerted with root crude extracts of Kalanchoe Lanceolata at doses 300, 1000 and 2000mg/kg.

6.2.5. The liver and renal function test for cute toxicity study of *Stephania Abyssinica*

Liver function tests of the root of crude extracts of *S. abyssinica* was conducted and the level of ALP, AST, BILT, CHO and HDL and showed increase in 550and 750mg/kg treatment of group of mice compared with negative control groups in this study

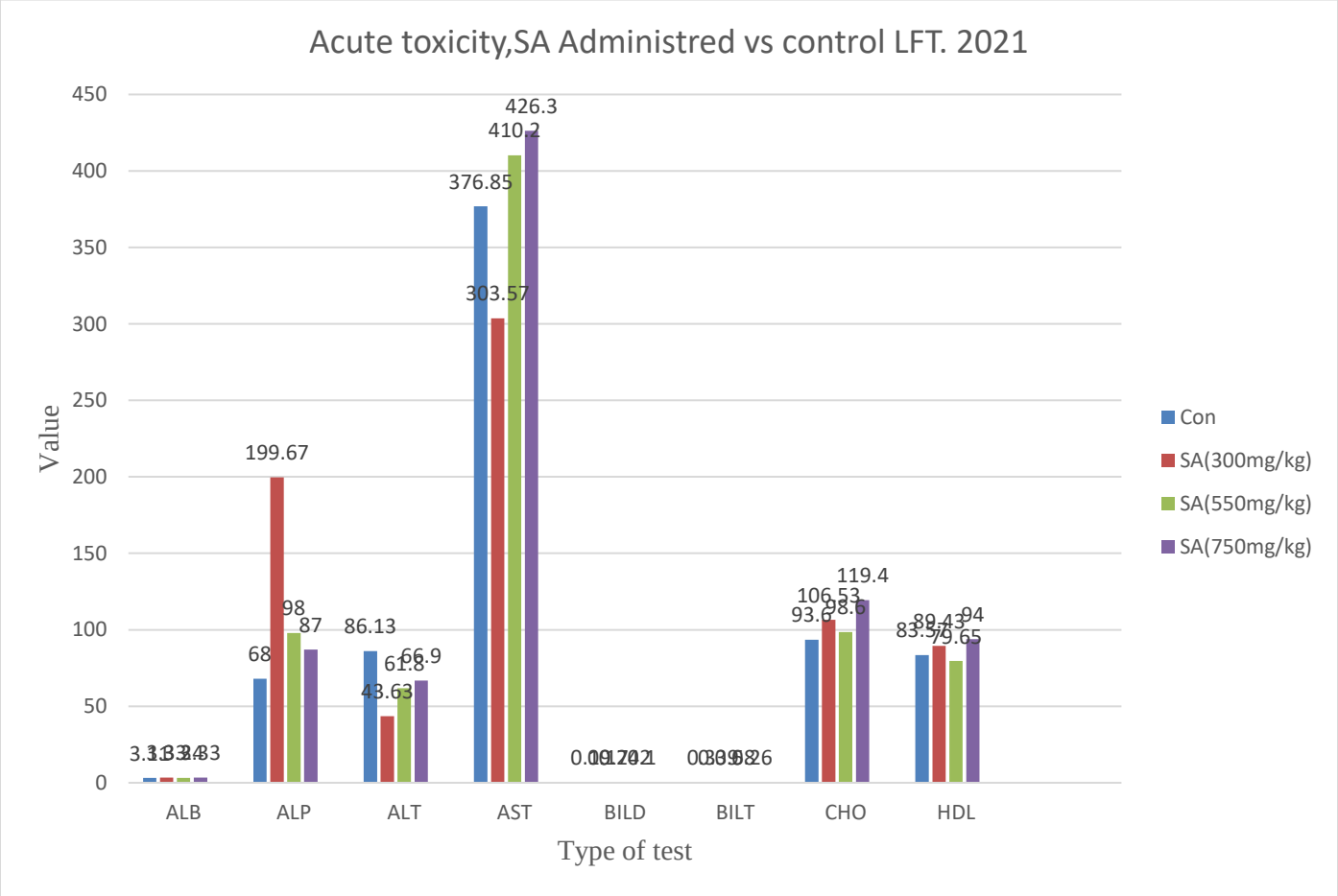

Figure 6. Liver function test of mice administered withcrude extracts from roots of Stephania Abyssinica at doses 300, 550 and 750mg/kg.

The result of acute toxicity of extracts from roots of S. a*byssinica*; the level of urea and creatinine increase, however, glucose showed decrease in the treatment groups of mice as compared with the negative control group of mice.

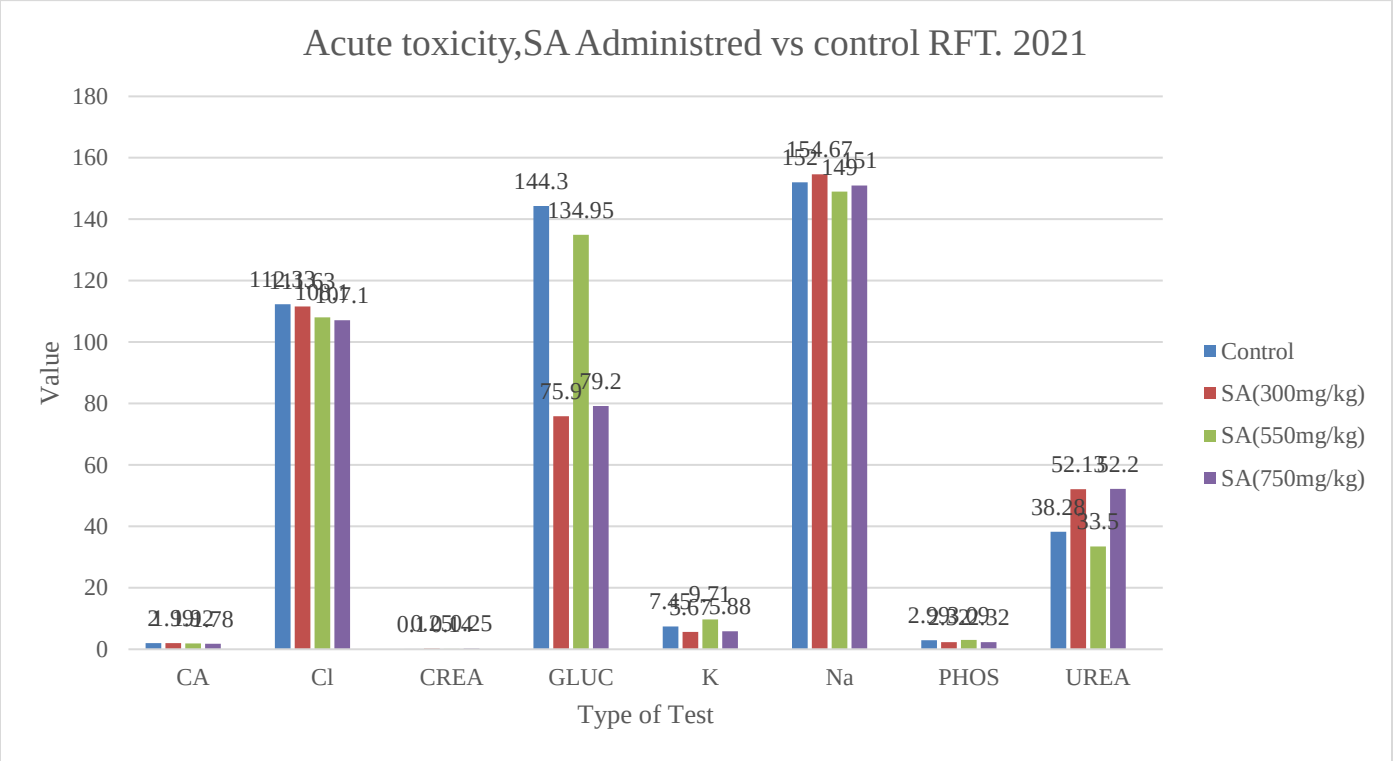

Figure 7. Liver Function Test of mice admimistered with crude extracts from roots of Stephania abyssinica at doses 300, 550 and 750mg/kg.

6.3. Histopathological test for In vivo Acute toxicity Study:

Histopathological examination for acute toxicity was done by the observation of experimental animals for 14 days their activity, behavior and indications of illness. After 14 days all experimental animals' and negative control groups of mice were euthanized and collected their liver and kidney were collected. No morphological change was observed on liver and kidney of all study animals. The result of the liver and kidney for acute toxicity evaluation of root of crude extracts of all plant extracts in the treatment and negative control groups of mice did no showed significant values at all doses extracts ($p>0.05$). However crude extracts of mice did

showed significance at dose 2000mg/kg extract of *K. lanceolate* (P<0.05) show in an annexes table in terms of liver and kidney.

6.4. Phytochemical screening of hydro-ethanolic plants crude extracts

The phytochemical screening of hydro-ethanolic plant crude extracts of the *C. kilimandschari. K.lanceolate and S. abyssinica* showed the presence Alkaloids**,** Coumarin, Flavonoid, phenols, saponin, steroids and Tannins which is shown in (table 6).

Table 5. Result of preliminary screening of 70%hydroethanol extracts from powdered roots of C. kilimandschari K.lanceolate and S. abyssinia showed the presence of secondary metabolites.

Type of tests/ Name of plant	Color Observed	C.K	k. L	S.A
Alkaloid (Mayer's test)	White creamy precipitate	+	+	+
Alkaloid (Wagner's test)	reddish- Brown precipitate	+	+	+
Cumarin	yellow colour	+++	+	+
Flavonoid (Alkaline reagent test)	yellow colour	+	+	-
Phenols	blue-green	-	++	++
Saponnin	Stable foam	+++	-	-
Steroids	. Red colour in the lower chloroform layer	-	+	+
Tannins	Blue-green	-	++	++

CK=*Convolvulus kilimandschari KL=Kalanchoe Lanceolata, S.A =Stephania Abyssinica*

+++ =strong positive, ++ = Moderate positive, + = Weak positive and - = Negative test

6.5. Percentage survival and mean survival time of mice against rabies virus

6.5.1. Anti-rabies activity of crude extracts from roots of *C. kilimandschari* at 1000 and 2000mg/kg treatment group of mice.

Percentage of survival and mean survival period of groups of mice infected with rabies virus and treated with the root of crude extract of *S. abyssinia* study plant extracts identified as finding of

the study. The control groups of mice inoculated with rabies virus from 22.22% (2/9) of mice survived and 77.77% (7/9) mice dead within 7 days. From the total of 17 mice treated with 70%hydro-ethanolic crude extract of root of *C. kilimandschari* at dose level 1000 and 2000mg/kg 52.9% (9/17) mice were dead. The mean survival time of mice treated with root of crude extract were 10days and 47.1% (8/17) of mice survived for 28 days.

6.5.2. Anti-rabies activity of crude extract roots of *K. lanceolate* at dose 1000and 2000mg/kg treatment groups of mice.

Percentage of survival and mean survival period of groups of mice inoculated with rabies virus and treated with the root of crude extract of *K. Lanceolate* was identified as finding of the study. From the total of 16 mice treated with 70%hydro-ethanolic crude extract of root of *K. Lanceolate* at dose level 1000 and 2000mg/kg. 31.2% (5/16) of mice were dead. The mean survival time of mice treated with root of crude extract were 10days. 68.8(11/16) of mice survived at the end of the experimental (28) days.

6.5.3. Anti-rabies activity of root crude extracts of *S.abyssinica* at dose 300and 550 mg/kg treatment group of mice.

From the total of 20 mice were infected with rabies virus and treated with 70%hydro-ethanolic crude extract root of *S. Abyssinica* at 300and550mg/kg dose, four mice were dead within one hour after administration with crude extract due to toxic effect rather than accident during inoculation and the effect of the virus.

6.5.4. Percentage of survival mice treated with crude extracts against rabies virus

Percentage of survival mice infected with rabies virus and treated with of crude extracts groups of mice that were identified as finding in this study. The control groups of mice challenged with rabies virus and treated with crude extracts of *C kilimandschari, K. lanceolate and S. abyssinica* is shown in (table 6).

Table 6 .The of Effect of crude extracts of the study on percentage survival of mice against challenged rabies virus.

Drug type	Total N	N of Events or death	N	Percent
CK	17	9	8	47.1%
KL	16	5	11	68.8%
PC	9	7	2	22.2%
SA	15	8	7	46.7%
Overall	57	29	28	49.1%

CK=*Convolvulus kilimandschari* KL= *Kalanchoe lanceolate*

PC=positive groups of mice SA= mice treated with *S. abyssinica*

The mortality of control groups of mice was at the fifth and seventh days after inoculation the mean survival time was 6days however, the mean survival period of micewere10days.

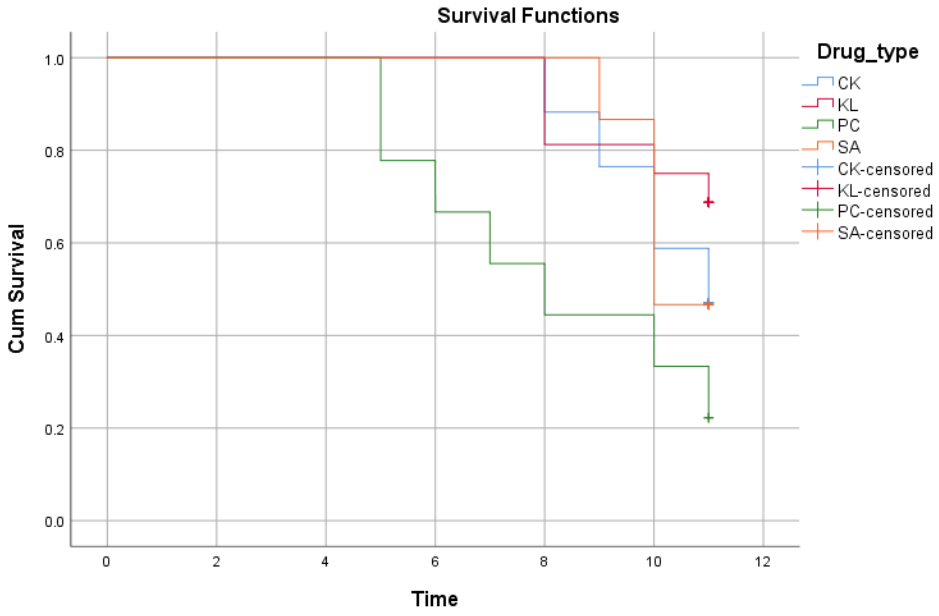

Figure 8. Effect of plant crude extracts on Percentage survival and mean survival time of mice.
CK= *Convolvulus kilimandschari* KL= *Kalanchoe lanceolate*

PC=positive groups of mice SA= *S. abyssinica*

6.5.5. Significance level of root of crude extracts used for the treatment of mice inoculated with rabies virus.

The overall plant's crude extract of *C. Kilimandschari, K Lanceolate and S. Abyssinica* were significant at all dose level of crude extract ($p<0.05$) and there was an increase in the existence time of treatment group of mice compared with the positive control group of miceTable 7).

Table 7. Significance level of root of crude extracts study plants

Test methods	Chi-Square	Df	Sig.
Log Rank (Mantel-Cox)	8.880	3	.031
Breslow (Generalized Wilcoxon)	10.278	3	.016
Tarone-Ware	9.585	3	.022

Test of equality of survival distributions for the different levels of drug type.

6.5.6. Results obtained from fluorescent antibody test

Confirmatory tests were conducted by collecting brain sample from mice that showed sign of rabies those that were treated and none treated groups of mice challenged with rabies virus. From total of 23 mice showed sign of rabies between 7 and 9 days, 10 samples were collected and tested for dFAT. All samples were positive for rabies virus antigen. After twenty-eight days of inoculation and treatment 10 and 2 samples were collected from the survived group of mice and negative control group of mice respectively. All samples didn't show any antigen against rabies virus as shown in (table 8).

Table 7. Result of direct Fluorescent Anti body test from mice brain.

Group of mice	Two samples were collected form each para meter Groups of mice.	
	Result from rabid mice Within nine Days	Result from survivors' group of mice After 28days.
CK001A	++++	–
CK 001B	++++	–
CK002A	++++	–
CK002B	++++	–
L001A	++++	–
KL002B	++++	–
SA001A	++++	–
SA001B	++++	–
SA002A	++++	–
SA002B	++++	–
PC	++++	–
NC		–

Note: CK001A and CK001B= *Convolvulus kilimandschari* 1000mg/kg
CK002A and CK002B=2000mg/kg,
KL001A and KL00B = *Kalanchoe lanceolate* 1000MG/KG, KL002B=2000mg/kg

SA001A andSA001B =*Stephania abyssinica* 300mg/kg and SAOO2A andSAOO2B=550mg/kg

PC= Positive control group of mice and NC = Negative control group mice (-) show no antigen detected and (++++) show high concentration of Antigen detected.

7. Discussion

The study was designed to evaluate in vivo acute toxicity effect and anti-rabies activity of *C kilimandschari, K. lanceolate and S. abyssinica.* Medical plants are widely used as source of medication for treatment and prevention for different kinds of disease for the reason that of the phytochemical elements naturally occurring in different parts of medicinal plants [18].

Ivivo acute toxicity test is essential for the initial phase of drug development for the reason that several of them can be toxic and it helps to determine the dose to be used [26]. Mice observed for acute toxicity determination of root crude extracts of *C. kilimandschari and K. lanceolata* in the present study did not exhibit any mortility and sign of toxicity at all dose during the 14 days of experment.The lethal dose (LD50) of these plants could be geater than limit dose 2000 mg/kg. This encourages the safety use of plant extracts for anti-rabies activity evaluation in mice model. The roots crude extracted of *S. abyssinica* no mortality and signs of toxicity were observed after administration at 175mg/kg and 300mg/kg. At dose 550mg/kg and 750mg/kg administered groups of micewere showed sign of toxicity on two and five mice respectively. The result of the root crude extract acute toxicity of *S. abyssinica* was dose dependent. Which might be due to availability of toxic substance found in the plant. The lethal dose (LD50) is 560mg/kg.

The study of biochemical parameters are indicators of toxicity, raising the effectiveness or the installation of a toxicity on the vital organs[78]. In this study,mice treated with root crude extracts of *C. kilimandschari*, the parameters serum enzyme ALP and CHO2 raised . However, the level of AST, ALT, BILD and BILD showed redused in all treated group of mice compared with control group. It is highly dose dependent and it might be due to the presence of saponin in plant crude extract. Because the saponin compounds in the plant is exaggerated by the herbal species, genomic derivation, the portion of plant examined, the environment, storage and during processing the compound found in saponin modified their activities, the property and concentration of extracting solvent[79]. The level of serum enzyme AST, ALT, phosphorus and urea were raiesd mice treated with root of extracts of *K. lanceolate* at dose 2000mg/kg. This might be due to existence of different type and concentration of phytochemical constitutes found in the plant cause liver or heart injury. Mice treated with root of extracts of *S.abyssinica*, the level of ALP, AST, BILT, CHO,

HDL and Urea showed increase in the treated groups of mice compared with the negative control group. It indicated that hepatocellular damage, hepatobiliary, kidney and heart biomarkers.

All plant root crude extracts showed that anti-rabies activity and increased the mean survival time($P<0,05$)treatment group of mice compared to the positive control group. The presence of phytochemical compounds found in the plant crude extract were similar evaluated in vivo and invitro anti-rabies activity of other traditional medicinal plants used for the treatment of bacterial, fungal and viral disease in our society. Alkaloids, Cumarin, flavonoid, phenol, sterols and saponin have antiviral activity as reported [45,68,69].

There were other reports of plants that showed antirabies/antiviral activity tested in vitro and/or in vivo systems. Admasu and his colleagues [54] reported that hydroethanolic extract of leaves of *P. dodecandra* showed significant increased survival period of mice compared to positive control group of mice.Similarly, Demeke and his colleagues [80]reported that hydro-ethanolic extracts of leaves of *J. schemperiana*, roots of *P. dodecandra*, root bark of *C. macrostachyus* and their combination treated mice increases the survival time as compared to positive control group of mice. In addition,Deressa and his colleagues [55] reported that crude hydromethanolic and chloroform extracts of roots *S. macroselen* and chloroform and aqueous extracts of leaves of *S. subserrata* showed significant improvement on the survival period of experimental mice compared to negative control group of mice. On other hand, Muller and his colleagues [81] investigated methanolic extract of leaves and flowers of *Alamanda schottii* with some antirabies activity tested in vitro system. Similarly, Abad and his colleagues [82] reported aqueous extract of leaves *Nepeta nepetella* also has antiviral activity.

Generally, in the current most of plant crude extracts evaluated for anti-rabies activity showed a modrate antirabies activity of mice challenged with rabies virus at dose dependent. This might be that the dose decreased the active biochemical ingridiane present in the plant part would be reduced to the level of not inhibiting the activitiy of the viruses.root crude extracts in this study showed anti rabies activity which might be these plant extracts hold active compounds that have ability to inhibit the viral protein synthesis or affect the propagation and pathogeneses of rabies viruse.

8.Strength and Limitation

8.1. Strength

Strength:- Covid-19 was pandemic during our research activities and challenged to our laboratory activities but with in this challenge multi professional's participated in this research and share their knowledge.

8.2.Limitation

Hematological test was not include in our research activity for analysis of hematological profile on each plant extract .

9.Conclusion and Recommendation

9.1. Conclusion

Based on our results, distinct and common phytochemical mixtures were found in three plant extracts. The root of crude of extracts of *S. Abyssinica* in vivo on mice at dose level of 550mg/kg show dose dependent toxic effect. This is may be associated with the presence of different biochemicals found in this plant. This ensures the rejection of the predetermined alternative hypothesis. All plant crude extracts had demonstrated significant anti-rabies virus survival rate as compared to positive control groups. It may be the presence of bio-active biochemical constituents such as Alkaloid, flavonoids, cumurine, phenol, steroids, saponin and others that are able to inhibit the propagation and activities of rabies virus at all doses of administered root crude extracts of experimental group of mice as compared to positive control group of mice. This ensures that the rejection of the predetermined null hypothesis. However, there is an essential of large scale and detailed studies.

9.2. Recommendation

Based on the above concluding remarks, the following recommendations will be forwarded:

- Future for need isolation and evaluation of each bio-active chemicals constituents of plant crude extracts that exhibits anti-rabies activity using improved techniques are recommended.
- The standardization of the method of extraction and an in-vitro testing is recommended for anti-viral activity.
- In the study setting many traditional healers use different types of plants for the medication of different types of disease and for the treatment of rabies. This needs further bio-active chemicals identification to prevent the antagonistic effect of crude extract of medicinal plants.
- Sub-acute and chronic toxicity tested on the crude extract of the study plants should be examined to setup to starting dose for anti-rabies activity.

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Annexes :

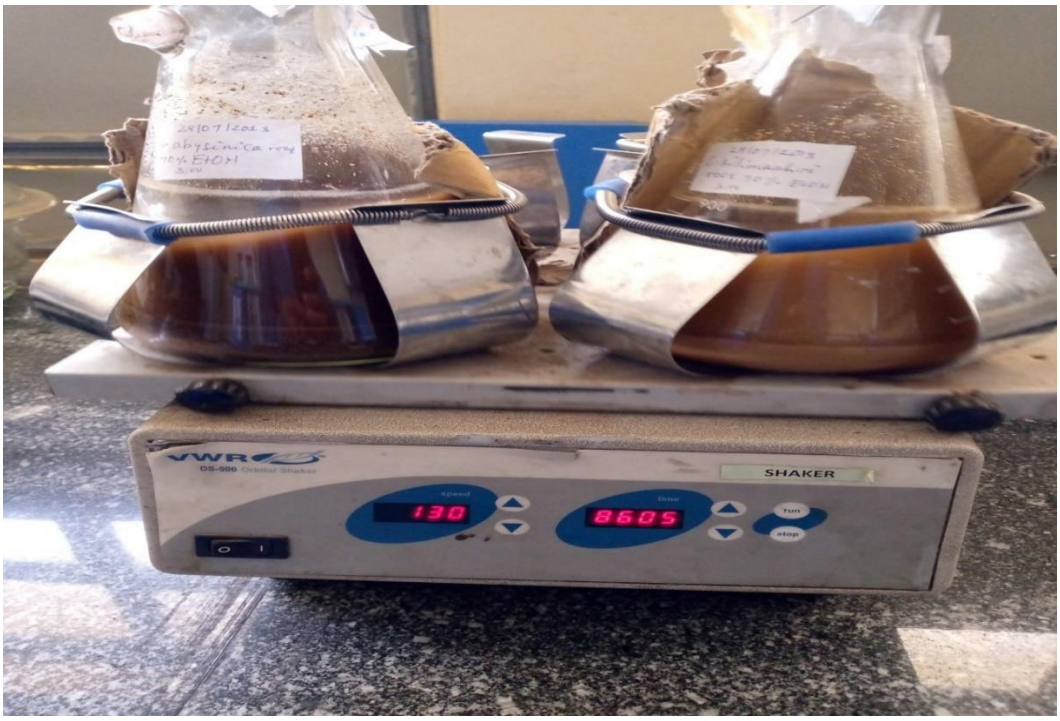
Annex 1.Processing and 70% hydroethanolic extraction of medicinal plants.

1. Washing and preparation of roots of medicinal plants

2. powder of medical plants

a.*K. Lanceolate* b. *C. kilimandschari* c.*S. Abyssinica* from left to right.

3. prepared samples for extraction



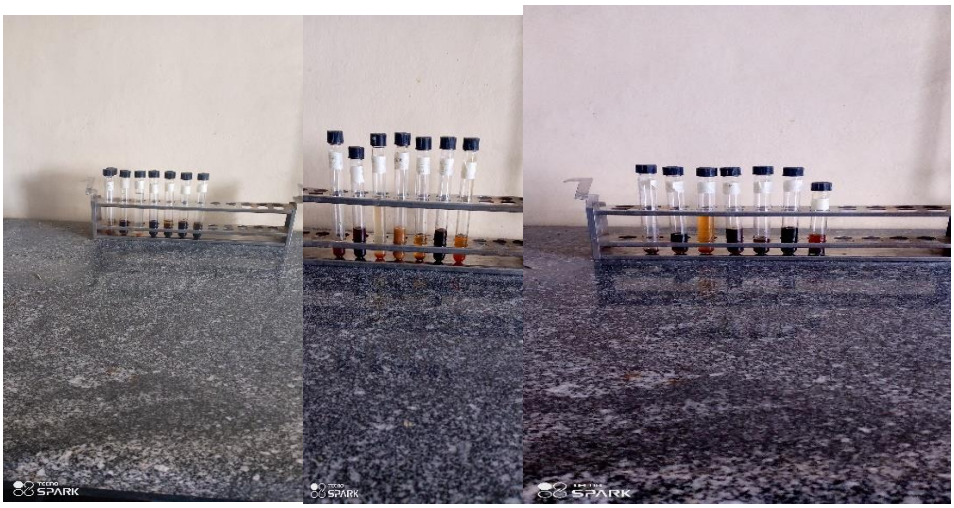
4. Rotary shaker adjusted at 130rpm

5. Filtration of the root of S. Abyssinica and C kilimandschari.

6. Adjusted Rota evaporator for concentration process.

5. Buchi rota vapor (on condensation process)

Annex 2**.** Preparation, Grouping and Management of Experimental Animals



Annex 3. phyto chemical screening of medicinal plantFigure

Annex 4. 1.Preparation of mice brain for fluorescent antibody test.

2.prepared slides for fluorescent antibody test.

Table 1. Result of histopathological Test for Acute Toxicity Study of Extracts from roots *C. kilimandschari* 300, 1000 and 2000mg/kg weight of liver and kidney samples of mice per gram

			F	Sig.
CK300mg/kgL-* C_L	Between Groups	(Combined)	8.467	0.246
		Linearity	24.669	0.12
		Deviation from Linearity	0.365	0.760
	Within Groups			
	Total			
CK00mg/kgL_ * C_L	Between Groups	(Combined)	2.837	0.405
		Linearity	2.179	0.379
		Deviation from Linearity	3.166	0.369
	Within Groups			
	Total			
CK2000mg/kgL_ * C_L	Between Groups	(Combined)	0.867	0.639
		Linearity	0.723	0.551
		Deviation from Linearity	0.939	0.590
	Within Groups			
	Total			

CK = *C.kilimandschari* L= liver of mice tereated C-L =liver of control groups of mice

Table 2. Result of histopathological Test for Acute Toxicity Study of mice administered with root crude extracts frofom roots *Kalanchoe Lanceolate* 300, 1000 and 2000mg/kg weight of liver samples of mice per gram.

			F	Sig.
KL300mg/kg L * C_L	Between Groups	(Combined)	8.200	0.250
		Linearity	1.783	0.409
		Deviationfrom Linearity	11.408	0.205
	Within Groups			
	Total			
KL1000mg/kg L * C_L	Between Groups	(Combined)	1.283	0.558
		Linearity	2.485	0.360
		Deviation from Linearity	0.682	0.650
	Within Groups			
	Total			
KL2000mg/kg _L * C_L	Between Groups	(Combined)	204638713.092	0.000
		Linearity	249872139.772	0.000
		Deviation from Linearity	182021999.753	0.000
	Within Groups			
	Total			

KL = *K.lanceolate* *L*= liver of mice tereated C-L =liver of control groups of mice

Table 3. Result of histopathological Test for Acute Toxicity Study of mice administered with root of crude extracts with *S,abyssinica* at dose 300 and 550mg/kg weight of liver samples of mice per gram.

Plant Type			F	Sig.
SA300mg/kg-L * C_L	Between Groups	(Combined)	3.133	0.388
		Linearity	4.590	0.278
		Deviation from Linearity	2.405	0.415
	Within Groups			
	Total			
SA550mg/kg_L_ * C_L	Between Groups	(Combined)	2.505	0.428
		Linearity	4.626	0.277
		Deviation from Linearity	1.445	0.507
	Within Groups			
	Total			

SA = S,abyssinica L= liver of mice tereated C-L =liver of control groups of mice

Table 4. Effect of plant crude extracts on Percentage survival and mean survival time(days) of mice.

	Mean[a]				Median	
			95% Confidence Interval			
Drug type	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error
CK	10.235	.272	9.701	10.769	11.000	.
KL	10.375	.326	9.736	11.014	.	.
PC	8.222	.878	6.501	9.944	8.000	1.491
SA	10.333	.181	9.979	10.687	10.000	.
Overall	9.982	.207	9.576	10.389	11.000	.

Declaration

I, the undersigned, declare that this research thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the research project have been duly acknowledged.

M.Sc. candidate: **Anberber Alemu (BSc.)**

Signature: _____

Date of submission: _____

This research proposal has been submitted with our approval as advisors.

Advisor: Dr Wondatier Nigatu (PhD, Associate professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Kassu Deseta (PhD candidate, Associate professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

Advisor: Regasa Deriba (BSc, MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

Advisor: Brihanu Hurisa (BSc, MSc,PhD Candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Firehiwot Teka (**BSc**, MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

Advisor: Eshetu Lemmas(MSc.MA, PhD Candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia