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Phytochemical analysis and safety evaluation of ethanol roots extract of *Erythrina sacleuxii* hua in Wistar albino rats

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***Erythrina sacleuxii*, one of five *Erythrina* species found in Kenya, is a stiffly multi-branched deciduous tree reported to have antiplasmodial, antifungal, anti-bacterial and anti-cancer activities. This study aimed at evaluating acute and sub-acute oral toxicity of *E. sacleuxii* roots ethanolic extract. Phytochemical analysis was conducted on Fourier transform infrared spectroscopy and Gas Chromatography-Mass Spectrometer. For acute and sub-acute toxicity studies 16 male rats n=4/group were used. The control group received 2% DMSO while treatment groups received 400, 1200 and 2400 mg/kg single dose (acute toxicity assay) and repeated for 14 days in subacute toxicity assay. Histopathological, serum biochemistry and hematological tests were analyzed. The results of FT-IR analysis recorded 12 dominant peaks, confirming availability of N-H, C-N, -C=C-, C-H, S=O, C-O and C-C functional groups. GC-MS analysis revealed presence of 17 bioactive phytochemicals mainly terpenoids and phenols. No toxic effect, organ toxicity or death was recorded in acute toxicity assay. In the subacute toxicity investigation, rats' behavior, gross pathology, and hematological parameters showed no significant changes. Biochemical analysis indicated an increase in alkaline phosphatase from 147 U/L at 400 mg/kg to 164 U/L at 2400 mg/kg however this was not statistically significant and no mortality was recorded. In comparison to the negative control, treatment groups' gross and histopathology of livers and kidneys had no significant difference. These results demonstrate that 80% ethanolic extract of E. sacleuxii roots has no severe effects in both acute and subacute study. However, sub-chronic and chronic toxicity studies are necessary for further validation.**

Key words: *Erythrina sacleuxii*, acute and sub-acute studies, phytochemical analysis.

INTRODUCTION

Erythrina sacleuxii Hua, one of approximately 130 plant species of *Erythrina* is a stiffly multibranched deciduous tree that can reach a height of 9-24 m and has an open rounded spreading crown. It is represented by about 30 species in continental Africa and 6 species occurring in Madagascar. In Kenya, five species of *Erythrina* that is *E. abysinnica*, *E. burttii, E. excelsa, E. malancantha*, and *E. sacleuxii* are found in the wild. Traditional African healers

have used the stem bark of *E. sacleuxii* in the management of trachoma, malaria, and elephantiasis, and the roots for the management of syphilis (Mitscher et al., 1987). Decoctions of *E. sacleuxii* leaves are used in East Africa to treat peptic ulcers, diarrhea, and as an antimicrobial agent (De la Parra, 2014). Isoflav-3-enes, isoflavones, pterocarpans, isoflavanones, and flavones have been isolated previously from this plant in past studies. Various researches have been conducted on different parts of *E. sacleuxii* to determine its efficacy. Biochanin A, 5′-(3-methylbut-2-enyl) pratensein and 3′-(3-methylbut-2-enyl) biochanin A isolated from the leaves demonstrated weak to moderate antifungal activity (Ombito et al., 2018), isoflavones from the twigs showed weak human cancer cell lines (HeLa-S3) cytotoxicity (Ombito et al., 2020), isoflavonoids from the bark of stem and roots of *E. sacleuxii* demonstrated antiplasmodial activities against two plasmodium strains: the chloroquine-sensitive (D6) and chloroquine-resistant (W2) (Andayi et al., 2006), 3'-prenylbiochanin A, genistein, daidzein parvisoflavone, erythrabyssin II and shinpterocarpin isolated from root wood, root bark, and stem bark extracts exhibited antimicrobial activity against *Staphylococcus aureus*, *Shigella* species, and *Candida albicans* (George et al., 2013). Although efficacy studies on various components of the *E. sacleuxii* has been conducted, limited information on the safety of this medicinal plant is available. As a result, this study investigated the acute and subacute toxicological safety of *E. sacleuxii* via oral administration.

MATERIALS AND METHODS

Sample collection and extraction

E. sacleuxii roots and leaves were collected from Kwale County, Kenya on July 2021. The leaves were used for plant identification and authentication by a taxonomist at Jomo Kenyatta University Agriculture and Technology (JKUAT, GoK Laboratories) and for future reference a voucher number MOM-JKUATBH 001A-2021 was kept in the herbarium. The plant roots were washed off dirt in distilled water, dried under the shade away from direct sunlight, pulverized using a plant mill into a powder. Maceration process was then conducted for 72 h using 80% ethanol in water for the powdered plant roots under 300 rpm agitation. The extract was decanted; the supernatant was filtered using Whatman Grade 1 filter paper, thereafter the filtrates were pulled together and concentrated using rotary evaporator. Obtained concentrate was then freeze-dried to obtain dry powder. From the dry plant powder mass, the resulting plant extract yield was calculated.

Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a non-destructive characterization technique that employs infrared radiation to irradiate the sample, and the absorbed radiation produces a distinct spectrum depending on the chemical composition of the sample. FTIR spectrometer coupled with a TGS (Tri-glycine sulphate) detector, 4 cm^{-1} resolutions in the 4000–400 cm^{-1} mid-IR region was used to detect the chemical composition of *E. sacleuxii* roots ethanolic extract (ESRE). Briefly, in the preparation of 3 mm dia salt-discs, potassium bromide (KBr), 100 mg was mixed and flattened with 1 mg of dried sample. The resulting discs were then analyzed on Alpha Bruker, D8, (Fourier transform infrared spectrophotometer) Germany model, equipped with OPUS 6.5 software with ATR sampling unit.

Gas chromatography-mass spectrometer

Further investigation of ESRE was qualitatively performed on GC-MS (Shimadzu QP2010SE, Japan) machine following the method described by Wamalwa et al. (2015). The sample was introduced via an all-glass injector working in the split mode with a split ratio of 10. The peaks were identified on the basis of computer matching of fragmentation spectral mass, peak area percentage and retention indices pattern with those stored in the database of the National Institute of Standards and Technology (NIST 08 and NIST 08s) library and by comparing with data already published by other authors. The structure, molecular weight, name, formula, and bioactivities were thereafter ascertained.

Grouping and acclimatization of experimental animals

Thirty-two healthy, 6 to 8 weeks and weighing 120 g – 140 g male Wistar albino rats, were procured from Kenyatta University's Biochemistry Department. For 5 days, the animals were acclimatized to laboratory surroundings. They were kept under standard living conditions in normal bedding covered plastic cages (OECD, 2008b). Each day the rats were given fresh water and standard diet.

Following the acclimatization period, the animals were randomly assigned into 4 groups each containing four animals. The groups were: one control group and three experimental groups. Picric acid was used to scientifically mark each animal with a unique identifying number. For the sub-acute toxicity investigation, 16 male rats were utilized, four animals per group. Similarly, 16 male Wistar albino rats, four animals per group were used in the acute toxicity study.

Study of acute toxicity

Acute toxicity testing evaluates the adverse effects that occur within a short period of time after a single dose of a test substance is administered. In accordance with OECD guidelines 423 (OCED 423, paragraph 23), healthy Wistar albino male rats were used in the acute toxicity study (Jonsson et al., 2013). To begin experiment, the animals which were procured from Kenyatta University were grouped into four groups (n=4). After grouping the animals were placed into their respective cages for five days before being dosed to allow them to acclimatize to the laboratory environment setting. Following acclimatization, all groups lacked access to food for 16 h but had a free supply of water prior to administration. All animals were weighed after the fasting period, and using their body weights, the respective doses to be administered were calculated. Two (2) %

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DMSO was used to reconstitute the plant extract. Group 1 serving as the negative control received 2% DMSO while groups 2-4 received ESRE at 400, 1200 and 2400 mg/kg/bwt respectively via oral gavage. After the plant extract at the respective doses were administered, all groups of rats were monitored periodically after 1 h after ESRE administration, then 4 h later. This was routinely done for 7 days (Nana et al., 2011). Behavioral changes, any clinical sign of toxicity and mortality, were monitored during this study period. The body weight of the animals was taken at the beginning of the experiment and on the last day of the experiment. On day seven, the animals were starved overnight and euthanized on the eighth day. For anesthesia, the rats were subjected to diethyl ether followed by cervical dislocation. Liver, pancreas, heart, kidneys, and lung were excised out, and gross pathology and weights noted.

Sub-acute toxicity study

Sub-acute toxicity study lasted 14 days and it examined the extract's toxicity on full blood count as well as adverse effect on major organs (liver and kidneys) following OECD 407 guidelines (OECD, 2008b). A total of 16 healthy male Wistar albino rats were randomly grouped into four groups (n=4). They were then fasted for 16 h (food suppressed, but not water) prior to administration. All animals were weighed and treated after the fasting period as follows: Groups I serving as the negative control was orally administered with 2% DMSO, while groups 2-4 were orally administered with ESRE at 400, 1200, and 2400 mg/kg/bwt dosage respectively, for 14 days via oral gavage. Clinical signs, general behavior and hazardous symptoms (tremors, lethargy, salivation, diarrhea, convulsions, sleep, and coma), and mortality were monitored after 1, 4 and 24 h, and once a day for 14 days. The amount of feed and water consumed were noted each day while their general body weights were assessed once a week for two weeks. The rats' ultimate weights were assessed on the 15th day, after which they were subjected to anesthesia under diethyl ether. Using the cardiac puncture method, 4 ml of blood was drawn from each rat.

Amount of water and feed consumed

The respective quantity of water (ml) and feed (g) each group consumed was determined daily by subtracting the amount remaining after 24 h from the total amount of feed and water supplied the previous day.

Change in the weight of body

The change in body weight is an important metric for determining toxicity (Vahalia et al., 2011). To determine the health status of the rats in this study, body weights of rats were weighed on a weekly basis. Each rat's body weight in each group was taken at the beginning of the experiment prior to dose administration. The weights were then taken once a week for the entire experiment period. After each weight measurement, the dosages of the extracts to be administered in each group were adjusted to ensure consistent dosage/ml.

Hematology and serum biochemistry

On the last day of experiment, the rats were euthanized by dipping their heads into diethyl ether followed by cervical dislocation, and using the cardiac puncture method, 2 ml/tube blood were collected into heparin and EDTA (ethylene diamine tetra acetic acid) tubes for measurement of serum biochemistry and hematological parameters, respectively. The blood in EDTA tubes was immediately assessed for hematological indices such as lymphocyte count (LYM), erythrocytes count (RBC), monocyte count (MON), white blood cell count (WBC), mean corpuscular hemoglobin (MCH), hemoglobin (HGB), hematocrit (HCT), red blood cell distribution width (RDW), mean corpuscular volume (MCV), thrombocytocrit (PCT), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and mean platelet volume (MPV) using an automated chemistry analyzer.

Centrifugation of blood in heparin tubes was carried out for 10 min at 4°C at a speed of 4,000 rpm. The separated plasma was used to measure the quantity of biochemical parameters which included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin (BIL), creatinine (Cr), total cholesterol (TCH), Urea and triglyceride (TG) using an automated dry chemistry analyzer (Roche-Refltron).

Relative organ weight

According to Lu (1985), a significant difference in relative organ weight between treated and untreated animals is an indicator of toxicity because organ weight is affected by body weight suppression. Major organs such as the liver, kidney, pancreas, heart, and lungs were excised, observed macroscopically and their weights noted. The following formula was used to compute each organ's relative weight (ROW):

Relative Organ Weight = Absolute organ weight (g) / Body weight (g) of rats on the final day × 100%.

Histopathology study on the liver and kidneys

Following euthanization of the animals, liver and kidney were removed and placed in 10% formalin for fixation overnight at room temperature. The following day, sections were taken randomly from the fixed organs. To remove excess formalin, the sections were rinsed in distilled water for 6 h, then increasing alcohol concentrations of 70% for 2 h, 90% for 2 h and absolute alcohol overnight. This was followed by clearing in xylene-for 4 h. The sections were then infiltrated in three setups of paraffin wax, the first one staying for 1.5 h, followed by changing into the next set for 2.5 h, and lastly in the third set overnight. Finally, tissue blocks were made embedding in paraffin wax on square metal plates, which were then labeled and kept at room temperature for sectioning. With a microtome blade, the tissue blocks were sliced into 4 μm thick ribbons. At every fifth section, the section ribbons were gathered and placed on a warm water bath set at 40°C followed by mounting onto pre-cleaned frosted end slides. The paraffin wax-coated slides were then fixed for 20 min in an oven set at 40°C. The fixed slides were then cooled for 30 min at room temperature before staining for 6 min in haematoxylin and then 20 s in eosin. Following routine H and E staining, the sections were first placed in a jar to remove paraffin and hydration and then into another jar for dehydration and clearing. To enable complete removal of paraffin, the tissues were placed in two set of xylene, one set for 5 min and then placed in a second fresh xylene for 2 min. The tissue sections were then hydrated in absolute ethanol for 2 min followed by decreasing concentrations of alcohol as follows: 95%: 2 min, 70%: 3 min, and 50%: 5 min. This was followed by rinsing the tissue sections in tap water for 5 min, and then staining for 6 min in haematoxylin before washing again for another 5 min in running tap water. The slides were then submerged in acidic alcohol for 1 s, followed by bluing in sodium bicarbonate. Counter staining in eosin of the fixed slides was then done for 20 s, followed by washing for two min in tap water, following bluing. Dehydration of the sections followed in alcohol at 50%: 2 min, 70%: 2 min, 95%: 2 min and lastly two times in absolute for two min. Two setups of

Table 1. FTIR spectra analysis of 80% *Erythrina sacleuxii* roots ethanolic extract.

No.	Wavenumber (cm^{-1}) (test sample)	Wavenumber (cm^{-1}) (reference table)	Functional group assignment	Phytocompound identified
1.	3375.25	3400-3250 (m)	N-H stretch	Amine/Amide
2.	3296.35	3330-3270 (n, s)	C-H	Alkyne
3.	1639.18	1690-1640 (m)	-C=C-/ C-N	Alkene/imine
4.	1597.58	1600-1585 (m)	C-C stretch (in–ring)	Aromatic
5.	1409.04	1410-1380 (s)	S=O stretch	Sulfate/sulfonyl chloride
6.	1258.24	1310-1250 (s)	C-O stretch	Ester/ether
7.	1123.07	1124-1087(s)	C-O Stretch	Secondary Alcohol
8.	1073.53	1085-1050 (s)	C-O Stretch	Primary Alcohol
9.	744.74	750 ± 20	C-H	Aromatic monosubstituted

xylene each at three min were used to clear dehydrated sections before they were mounted with DPX and cover slips on microscopic slides permanently. The sections were examined for any histological changes between the groups treated and the control group under a light microscope. Following the examination, photomicrograph for sections of kidney and liver were taken for all groups using PixelPro software with a general purpose laptop at a magnification of x20.

Ethical consideration

The toxicity tests, both acute and subacute were carried out with approval obtained from Ethical and Scientific Committee, Department of Anatomy, Kenyatta University, Kenya. This was done in accordance to the guidelines stipulated in biomedical research ensuring the animals were used in a compassionate and humane manner. In this investigation, the animals were not subjected to any undue suffering or frightening situations (OECD, 2008b). A well-trained individual carried out all the surgical procedures in these studies after the animals had be anaesthetized with diethyl ether to keep pain and suffering to a minimum. By placing the animals in an appropriate environment and having their beddings changed every three days, they were protected from pathogens. The numbers of animals used in this study was the minimal possible number that allowed the investigators to achieve the study's specific scientific objectives.

Statistical analysis

Numerical data in the current study were subjected to one-way ANOVA analysis using R-program. Comparisons of the mean were conducted using Tukey HD multiple-comparison test, with p-values ($p \leq 0.05$) used to specify the confidence interval. Graphical representation was conducted using Origin Pro software and all the data are expressed as mean ± standard error mean (SEM).

RESULTS

Analysis of ESRE using Fourier Transform Infrared Spectroscopy (FTIR)

The active components analyzed in the extract based on the peak values in the infrared radiation region showed dominant peaks at 3769.04, 3375.25, 3262.45, 3296.35, 1639.18, 1597.58, 1409.04, 1258.24, 1123.07, 1073.53, 744.74 and 408.3 wave number cm^{-1} (Table 1). The peak at 3375.25 cm^{-1} is attributed to by O-H stretch vibrations in alcohol. Polyphenols in plant extract show absorbance at this peak. Absorbance peaks at 3262.45 cm^{-1} and 3296.35 cm^{-1} are attributed to by C-H stretch in alkyne while carbonyl stretch in ketones show absorbance peak at 1639.18 cm^{-1}. The absorbance peak at 1597.58 cm^{-1} is due to C-C stretch in aromatic rings and the peak at 1409.04 cm^{-1} is attributed to C=O stretch in sulfate. The peak at 1258.24 cm^{-1} is due to C-O stretch in ester/ether, peaks at 1123.07 cm^{-1} and 1073.53 cm^{-1} are attributed to C-N stretch vibrations in aliphatic amines indicating presence of alkaloids in the sample. The absorbance peak at 744.74 cm^{-1} is attributed to by C-H stretches in aromatic mono- substituted compounds (Figure 1).

GC-MS analysis on 80% ESRE

GC-MS analysis of ESRE identified a total of 17 peaks (Figure 2). These correlate to bioactive chemicals identified through comparison of their spectral mass fragmentation patterns, peak area (%), and retention time to the standards stored in National Institute of Standards and Technology (NIST) Library. The presence of; Caryophyllene oxide, 6-epi-shyobunol, 1,2-Benzenedicarboxylic acid butyl 2-methylpropyl ester, 3H-Cyclodeca[b]furan-2-one 4,9-dihydroxy-6, Diisooctyl phthalate, Glycerin, Octadecanoic acid 2,3-dihydroxypropyl ester, D-Allose, D-Arabinitol, Sorbitol, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, Dimethyl(bis[(2Z)-pent-2-en-1-yloxy])silane, 2-(4-Methyl-1H-1,2,3-triazol-1-yl)ethan-1-amine, 1-Butanol, 3-methyl-, acetate, But-3-enyl (E)-2-methylbut-2-enoate, Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexene and Cyclohexanone was discovered using GC-MS analysis (Table 2).

Acute toxicity of 80% ESRE

At the various doses, the acute toxicity investigation revealed no adverse signs or symptoms. During the

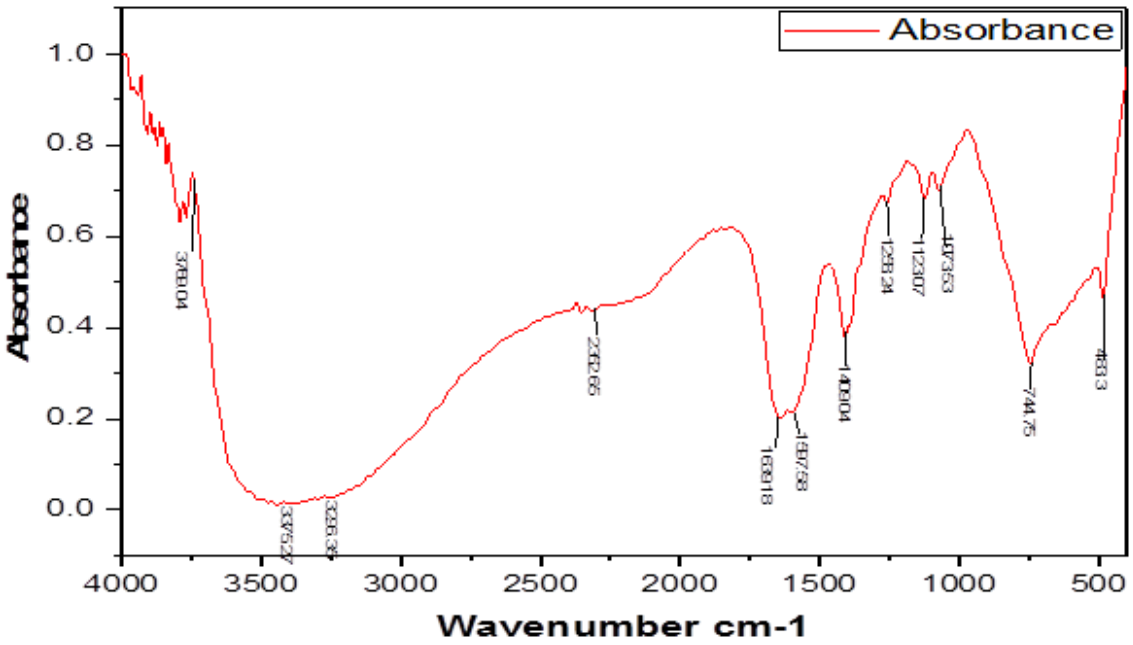

Figure 1. *E. sacleuxii* roots ethanolic extract FT-IR spectrum.

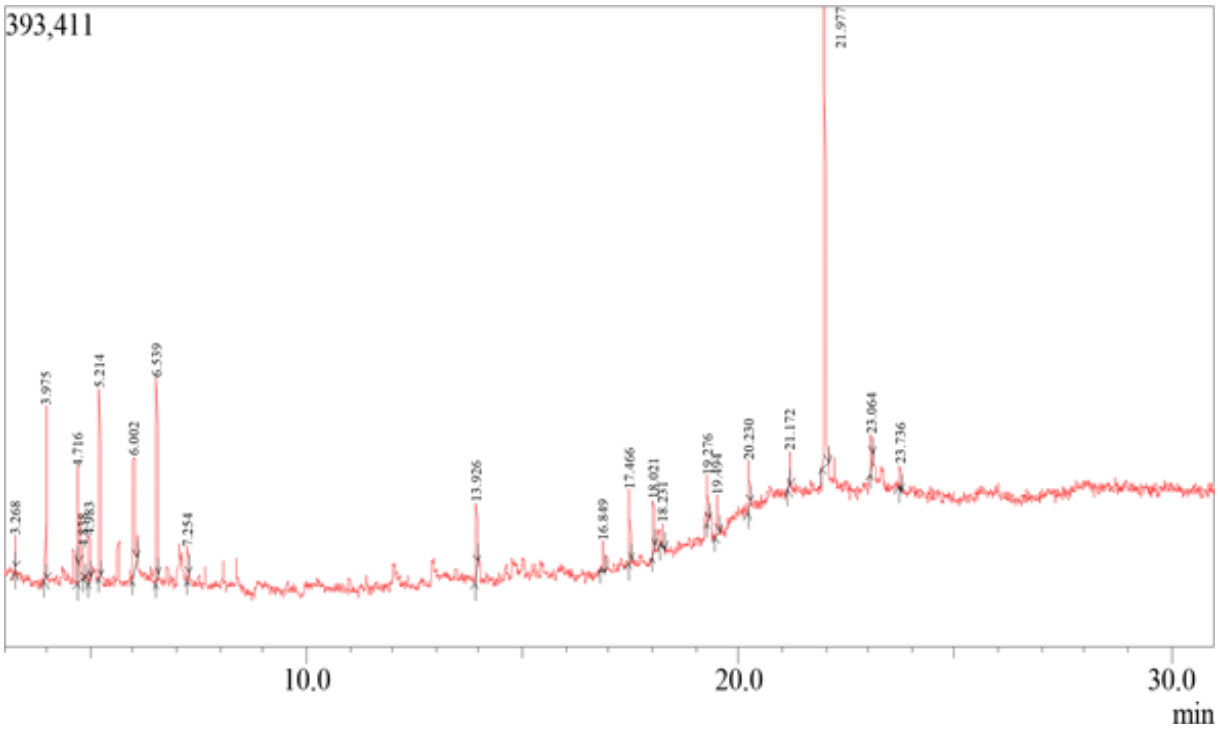

Figure 2. GC-MS chromatogram of 80% *E. sacleuxi roots* ethanolic extract.

acute toxicity assays, there were no recorded deaths in all groups. Due to this report, the extract's LD_{50} can be estimated to be higher than 2400 mg/kg/bwt. Gross macroscopic investigation of the kidneys and liver of study groups revealed no significant aberrant alterations in color, texture, shape and size compared to the control

Table 2. The phytochemical compounds identified in 80% *E. sacleuxii* roots ethanolic extract.

RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Compound nature	Reported pharmacological action
13.926	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	3.74	Sesquiterpenoid oxide	Antifungal (Yang et al., 1999)
16.849	6-epi-shyobunol	$C_{15}H_{26}O$	222	1.48	Elemane sesquiterpenoids	Anti-inflammatory, Antioxidant (Shareef et al., 2016)
18.021	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	278.3435	2.86	Phthalate ester	Antimicrobial Antifouling (Sudha and Masilamani, 2012)
23.064	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6	$C_{15}H_{20}O_4$	264.32	1.64	Terpene lactones	Unknown
23.736	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.6	1.69	Phthalate ester	Anti-inflammatory, Antitumor (Sudha and Masilamani, 2012)
4.992	Glycerin	$C_3H_8O_3$	92.09382	6.62	Polyol	Anti-inflammatory (Szél et al., 2015)
12.914	D-Allose	$C_6H_{12}O_6$	180.16	2.75	Enantiomer	Antioxidant (Ishihara et al., 2011)
13.856	D-Arabinitol	$C_5H_{12}O_5$	152.15	1.95	Sugar alcohol	Protective function against ROS (Christensson et al., 1999)
17.558	Sorbitol	$C_6H_{14}O_6$	182.17	1.24	Sugar alcohol, or polyol	Diuretic, laxative and cathartic property (Parthipan et al., 2015)
23.554	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5026	6.58	Ester	Antioxidant (Singh et al., 2008)
26.644	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{25}H_{46}O_6$	442.6	1.63	Ester	Emulsifier in food
12.129	Dimethyl(bis[(2Z)-pent-2-en-1-yloxy])silane	$C_{12}H_{24}O_2Si$	228.40	3.07	Silane	Unknown
8.314	2-(4-Methyl-1H-1,2,3-triazol-1-yl)ethan-1-amine	$C_5H_{10}N_4$	126.16	0.84	Amine	Unknown
7.016	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130.1849	1.42		Unknown
5.435	But-3-enyl (E)-2-methylbut-2-enoate	$C_9H_{14}O_2$	154.2063	1.32		Unknown
18.231	Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohex	$C_{15}H_{20}O_2$	232.32	1.04		Unknown
3.975	Cyclohexanone	$(CH2)_5CO$	98.14	6.85	Ketone	Human xenobiotic metabolite (Abba et al., 2018)

(Figure 3). Relative organ weight of the kidneys and liver of the treatment groups recorded no significant change when compared to the control group (Table 3). The body weight of all the test groups as well as the control group showed a normal gradual increase in body weight. For the control group, an initial 110±14.72 g increased to 145±17.61 g mean body weight on the 8th day, recording a gain of 35 g mean body weight. On the first day of experiment, rats in 400, 1200 and 2400 mg/kg treatment groups showed mean body weights of 151.75±11.32, 148.5±3.87 and 153.5±5.32 g, respectively. On the 8th day of experiment, the mean body weight of rats in 400, 1200 and 2400 mg/kg treatment groups had increased to 186.25±11, 186.5±6.76 and 184±11.46 g, respectively. This showed a gain of 34.5, 38 and 30.5 g, mean body weight, respectively (Figure 4)

Sub-acute assay of 80% ESRE

ESRE effect on gross pathology and behavior

During the 14-day study, no mortality was recorded. There were no observable changes in general behavior of animals; however, less feeding accompanied with loose stool was recorded in the group treated with 2400 mg/kg dose. Also, there were no changes in the general appearance of all the animals and their movements were also normal after administration of the extract.

ESRE effect on water intake

There was a gradual increase in water intake in the low-dose (400 mg/kg) group, similarly to the control group. Treatment groups 1200 and 2400 mg/kg demonstrated a decrease in water intake though not statistically significant (Figure 5).

ESRE effect on amount of feed consumed

The amount of feed consumed in all the treatment groups (400, 1200 and 2400 mg/kg b.w.t) as well

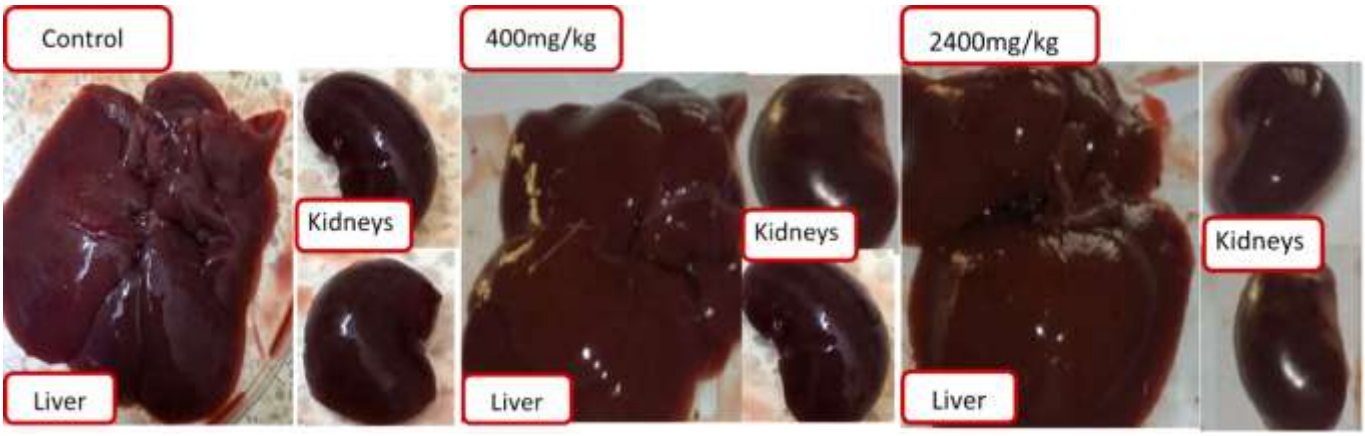

Figure 3. Livers and kidneys of the rats subjected to 80% *E. sacleuxii* roots ethanolic extract at different dosages.

Table 3. Effect of *E. sacleuxii* roots ethanolic extract on relative organ weight during sub-acute assay.

Group	Liver	P-Value	Kidneys	P-value
Control	9.27±0.67		0.81±0.1	
2400 mg/kg	7.22±0.38	0.148	0.67±0.04	0.211
1200 mg/kg	8.21±0.23		0.81±0.08	
400 mg/kg	8.39±0.1		0.92±0.08	

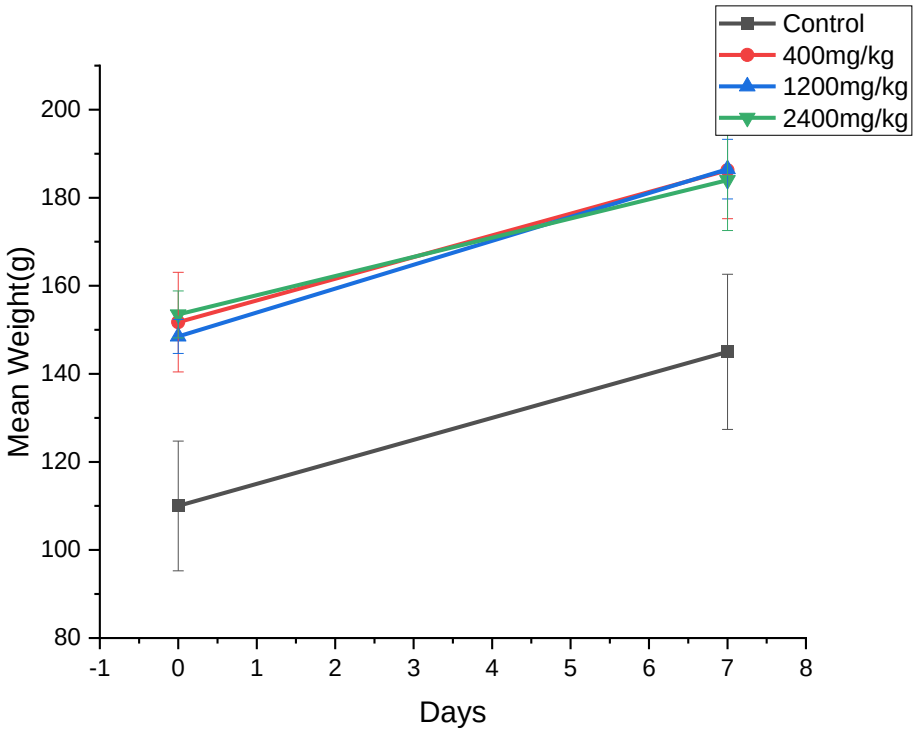

Figure 4. Acute effect of 80% *Erythrina sacleuxii* roots ethanolic extract on mean body weight in rats. Data presented as means with standard error of the mean represented as error bars (n=4/group and p>0.05).

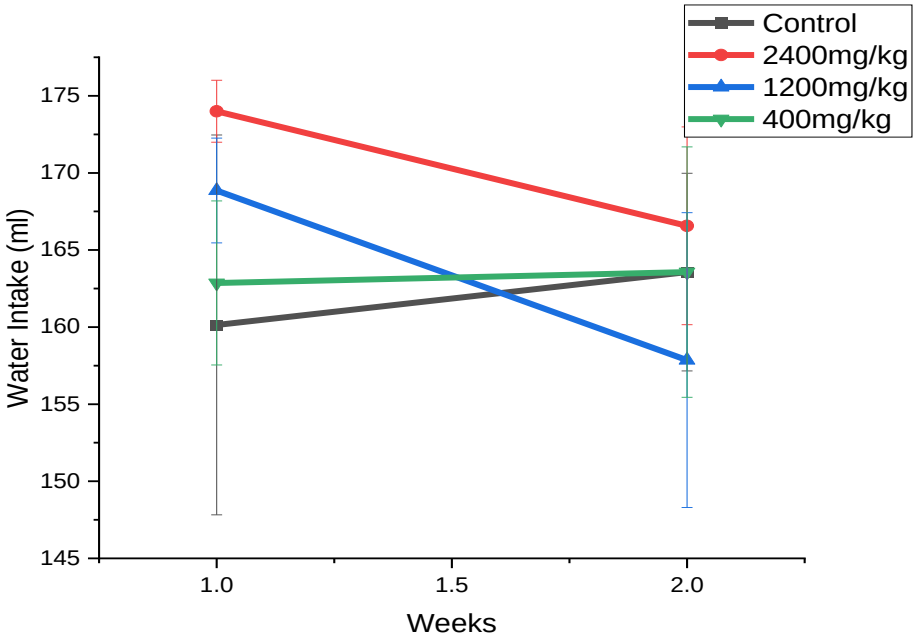

Figure 5. Effect of 80% *Erythrina sacleuxii* root ethanolic extract on mean water intake. Data are presented as means with S.E.M represented as error bars (n=4/group and p>0.05).

as the control group are presented in Figure 6. All treatment groups recorded a statistical significant (P≤0.05) increase in their food consumption when compared to the control group throughout the study period.

Effect of ESRE oral administration on body weight

Throughout the 14-day study period, the control group as well as all the test groups recorded no statistically significant progressive body weight gain (Table 4).

Effect of ESRE oral administration on relative organ weights

During the sub-acute toxicity experimental period, there were no significant alterations in the relative weight of all major organs except for pancreas which recorded a statistically significant ($p < 0.05$) decrease in its relative weight (Table 5).

Effect of ESRE oral administration on hematological parameters

The majority of the measured hematological indices of all as well as the control group were normal with reference to the acceptable standard ranges for rats. A rise from the normal reference ranges in WBC, LYM and NEU was observed, however, the increase was not significant compared to the control. All the parameters of hematology measured for the treated groups compared to the control group demonstrated no significant difference (Table 6).

Effects of ESRE oral administration on serum biochemistry

Parameters of serum biochemistry in the sub-acute toxicity study for all the test groups and the reference group were normal (except bilirubin) with reference to the standard ranges for rats (Table 7).

The level of ALT recorded at 400, 1200 and 2400 mg/kg doses were 37.85, 38.1 and 46.4 U/L respectively. The increase in ALT levels in the treatment groups was not significant ($p > 0.05$) when compared to the levels recorded in the control group (37.85 U/L). Similarly, the levels of AST in the treatment group 400, 1200 and 2400 mg/kg were 103, 136 and 142 U/L respectively showing a decrease from the control group (137 U/L) in treated groups 400 and 1200 mg/kg though not significant. Generally, the level of ALP in the treated groups increased and there was a significant increase ($p < 0.05$) at dose 2400 mg/kg when compared to dose 400 mg/kg. Increase in ALP levels of the treatment groups to that of the control were not statistically different. Following analysis, the mean amount of creatinine increased slightly in all treated groups, that is, the levels at 400, 1200 and 2400 mg/kg were 0.37, 0.42 and 0.47 mg/dl respectively

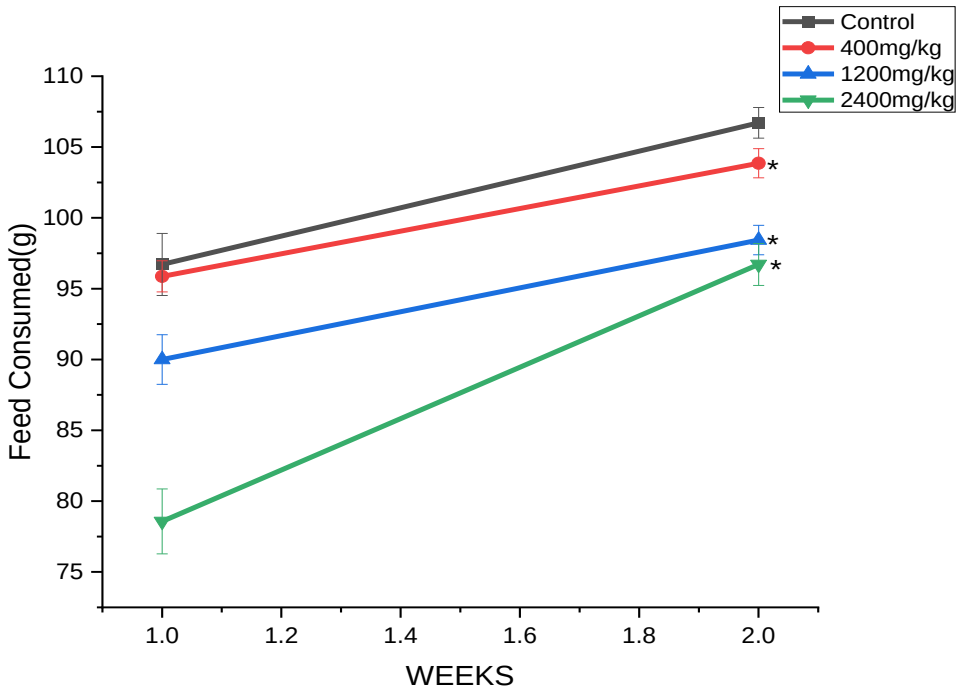

Figure 6. Effect of 80% *Erythrina sacleuxii* root ethanolic extract on mean water intake. Data are presented as means with S.E.M represented as error bars (n=4/group and p>0.05). Significant differences are represented by*.

Table 4. 80% *Erythrina sacleuxii* roots ethanolic extract effect on body weights of rats.

Group	0	P-value	Week 1	P-value	Week 2
Control	108.5±3.58	0.916	122.5±2.96	0.214	157.5±1.04
2400 mg/kg	143.25±7.61		161.75±7.6		220.25±6.76
1200 mg/kg	160.25±17		180.5±16.83		231±12.47
400 mg/kg	145.5±8.3		162.5±10.67		207±18.49

n=4/group; Mean ± S.E.M (Standard Error of Mean). P-Value represents % body weight change.

Table 5. Effect of 80% *E. sacleuxii* roots ethanolic extract on absolute organ weight of treated and control groups during subacute toxicity study.

Organs	Control	400 mg/kg	1200 mg/kg	2400 mg/kg	P-value
Pancreas	0.83±0.1	0.62±0.07	0.51±0.07	0.48±0.07*	0.0291
Liver	6.93±0.76	5.2±0.15	4.81±0.15	5.04±0.8	0.0735
Heart	0.4±0.03	0.43±0.02	0.35±0.03	0.32±0.05	0.161
Kidneys	0.72±0.06	0.76±0.05	0.65±0.03	0.62±0.07	0.268
Lungs	0.87±0.02	0.72±0.02	0.78±0.05	0.76±0.08	0.211

n=4/group; Values are presented as Mean ± S.E.M (Standard Error of Mean), * = significant at p>0.05.

but when compared to the control group (0.36 mg/dl) the increase was not statistically significantly. Serum bilirubin levels decreased in all treated groups, that is, the levels at 400, 1200 and 2400 mg/kg were 0.4, 0.45 and 1.08 mg/dl respectively and the decrease when compared with the control (1.2 mg/dl) was significant ($p < 0.05$) at dosage 400 mg/kg. Following analysis, the mean amount triglycerides and total cholesterol levels were not

Table 6. Effect of 80% *Erythrina sacleuxii* roots ethanolic extract on indices of hematological during the 14-day sub-acute toxicity study in Wistar rats.

Parameter	Unit	Control	400 mg/kg	1200 mg/kg	2400 mg/kg	Reference ranges	P-value
WBC	10^9/L	18.35 ±7.03	13.67±4.38	12.6±5.65	13.3±2.62	4.5-11	0.856
RBC	10^12/L	4.98±1.4	6.43±0.57	6.47±0.85	7.29±0.2	6.76-9.75	0.343
HGB	g/L	98.25±25.17	137.25±5.12	128.25±18.96	128.5±16.9	137-176	0.473
HCT		0.3±0.08	0.39±0.03	0.39±0.05	0.4±0.04		0.491
MCV	fl	63.9±2.66	61±1.3	59.3±1.27	61.28±2.71	29.41-123.07	0.51
MCH	pg	21.35±1	21.73±1.4	19.75±0.7	19.58±0.57	18.37-36.98	0.328
MCHC	g/L	333.5±3.23	333.75±2.78	335.5±4.97	355.5±19.19	254.1-805.5	0.385
PLT	10^9/L	302.5±143.89	508±162.39	584.75±248.62	656.75±127.29	638-1177	0.546
LYM	10^9/L	15.47±5.67	11.27±3.78	10.92±5	12.26±2.75	4.78-9.12	0.882
MON	10^9/L	0.025±0.02	0.083±0.05	0.005±0.003	0.005±0.003	0.03-0.18	0.122
NEU	10^9/L	2.84±1.46	2.3±0.66	1.68±0.72	1.77±0.43	0.22-1.57	0.787
RDW-CV		0.15±0.01	0.17±0.03	0.15±0.006	0.14±0.003		0.539
MPV	fl	7.025±0.15	7.58±0.13	7.33±0.17	7.45±0.18	6.2-9.4	0.145
PCT	ml/L	2.12±0.99	3.88±1.25	4.21±1.81	4.79±0.74		0.503

Values are presented as mean ± S.E.M, n= 4 rats/group. WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelets, LYM: lymphocytes, MON: monocytes, NEU: neutrophils, RDW-CV: MPV: mean platelets volume.

Table 7. Effect of 80% *Erythrina sacleuxii* root ethanolic extract on parameters of serum biochemistry during the 14-day sub-acute toxicity study in Wistar rats.

Parameter	Unit	Control	400 mg/kg	1200 mg/kg	2400 mg/kg	P-Value	Reference range
ALT	U/L	37.85±6.16	37.7±8.62	38.1±10.09	46.4±5.02	0.185	18-45
AST	U/L	137±14.03	103±12.26	136±9.2	142±13.2	0.754	75-143
ALP	U/L	157±20.55	139.75±5.39	148.5±22.86	164.75±18*	0.0336	62-230
CRE	mg/dl	0.36±0.06	0.37±0.06	0.42±0.03	0.47±0.03	0.407	0.2-0.5
BIL	mg/dl	1.2±0.31	0.4±0.04*	0.45±0.07	1.08±0.17	0.0161	0.2-0.55
CHO	mg/dl	85±5.57	87.5±5.38	91±4.74	98.5±0.96	0.229	40-130
TRI	mg/dl	73.85±3.85	73.98±3.17	74.1±2.78	75.05±3.42	0.994	20-114
UREA	mg/dl	20.05±5.22	24.78±1.74	24.33±0.63	25±1.9	0.401	12.3-24.6

Values are presented as n=5; Mean ± S.E.M (Standard Error of Mean); *= Significant different at p>0.05. ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, CRE: Creatinine, BIL: Bilirubin, CHO: Cholesterol, TRI: Triglycerides.

significant (p>0.05) for all the treatment groups when compared with the control group. Analysis of urea levels demonstrated an increase in all the treatment groups, however not significant (p > 0.05).

Effects of oral administration of ESRE on the liver's histology

The observed parameters in the histopathological study of the liver included: endothelial cells (EC), hepatic sinusoids(S), central vein (CV), interlobular bile duct, hepatic artery and hepatic portal vein. All these parameters had a normal appearance in all the rats treated with 2400 mg/kg (Figure 8a), 1200 mg/kg (Figure 8b) and 400 mg/kg (Figure 8c) ESRE as well as the control group (Figures 7a and b).

Effects of oral administration of ESRE on the kidney's histology

There were no significant changes on the kidneys histopathological of all the treatment groups 2400 mg/kg(Figure 10a), 1200 mg/kg (Figure 10b) and 400 mg/kg (Figure 10c) compared with the controls (Figures 9a and b. The observed indicators in the histopathology study of the kidney included: Proximal convoluted tubules (PCTs), squamous cells (SC), urinary space (US), macula densa (MD), glomerulus (G) and distal convoluted tubules (DCTs).

DISCUSSION

Herbal plants have been used since time immemorial by

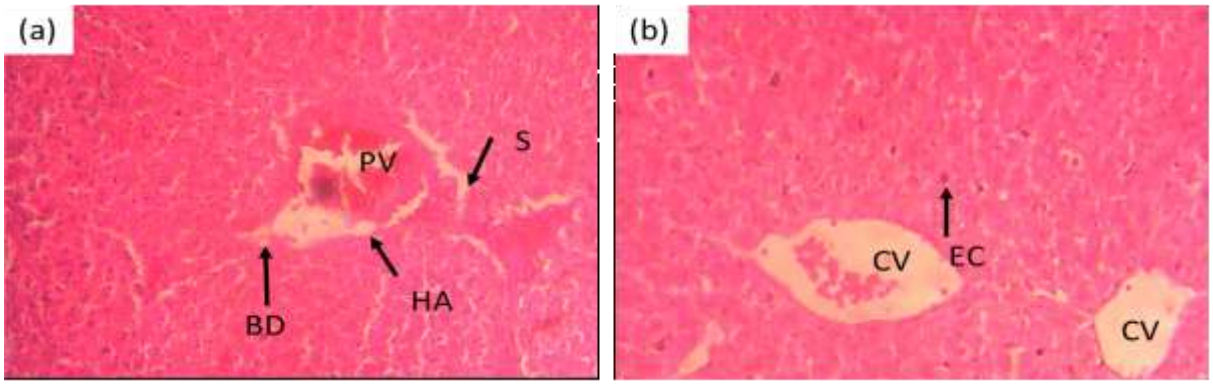

Figure 7. Images representing the liver section of control rats (2% DMSO) showing no visible lesions. PV= Portal vein, CV= Central vein, HA= Hepatic artery, BD= Bile duct, EC= Endothelial cells, S= sinusoids.

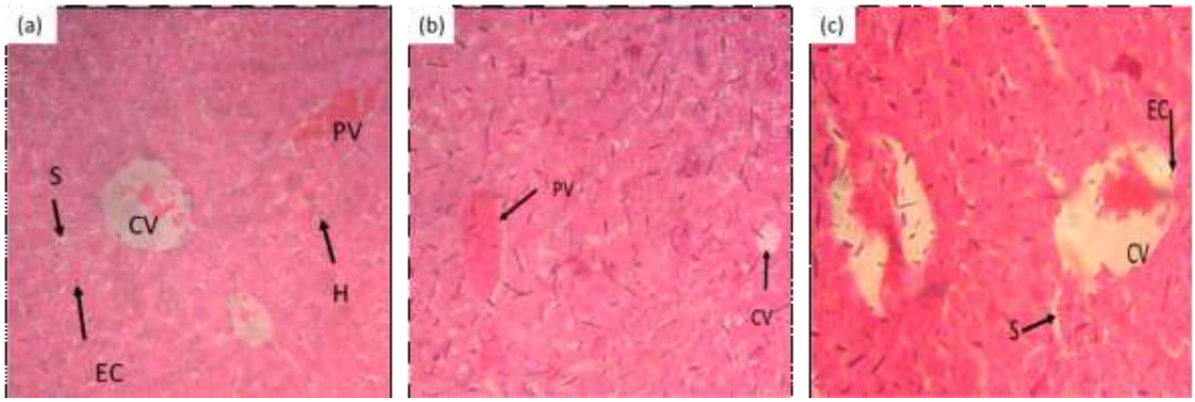

Figure 8. Photomicrograph of liver section showing the effect of 80% *Erythrina sacleuxii* roots ethanolic extract in treatment groups. a: 2400 mg/kg, b: 1200 mg/kg, c: 400 mg/kg. Indicators: HA= Hepatic artery, CV= Central vein, BD= Bile duct, PV= Portal vein, EC= Endothelial cells, S= sinusoids.

local communities as remedies for various ailments all over the world. Even today, medicinal plants are still in use all over the world especially in developing countries as a primary and easiest healthcare mode (Pe, 2015). Therefore, establishing the safety of medicinal plants is important in protecting humans and animals against plant based products toxicity. This study evaluated the phytochemical and safety profiles of *E. sacleuxii* roots ethanol extract in Wistar albino rats. The first step in determining the relevance of any medicinal plant species is to screen for its phytochemicals, as this provides a wealth of information about the compounds present. The FTIR spectrum results from this study demonstrated the presence of carboxylic acids, hydroxyl, methyl and aldehyde groups, alkenes, alkynes, amines, sulfates, ester/ether and aromatics functional groups which contribute to medicinal activities of various plants. The results from FTIR can be confirmed by the presence of the compounds elucidated in the GC-MS analysis results. Presence of hydroxyl group could be due to the presence of glycerin, D-arabinitol and sorbitol which are polyol compounds. Indicated peak for carbonyl groups show presence of ketones e.g. cyclohexanone identified in the extract. Identified peak for ester could be due to hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester. Results from GC-MS analysis indicated the existence of important bioactive compounds including glycerin which had the highest percentage peak area. Glycerin is reported to have anti-inflammatory effect (Szél et al., 2015). The other bioactive compounds present in *E. sacleuxii* roots ethanolic extracts included; D-Allose reported to have anti-oxidant activity (Ishihara et al., 2011; Murata et al., 2003), diisooctyl phthalate which is linked to increase in the secretion of TNF-alpha in monocytes/macrophages, caryophyllene oxide that has antifungal activity while 6-epi-shyobunol has anti-inflammatory and antioxidant activity (Shareef et al., 2016). On the other hand, 1,2-benzenedicarboxylic has been reported to have antimicrobial and antifouling activity while D-arabinitol has been suggested to have

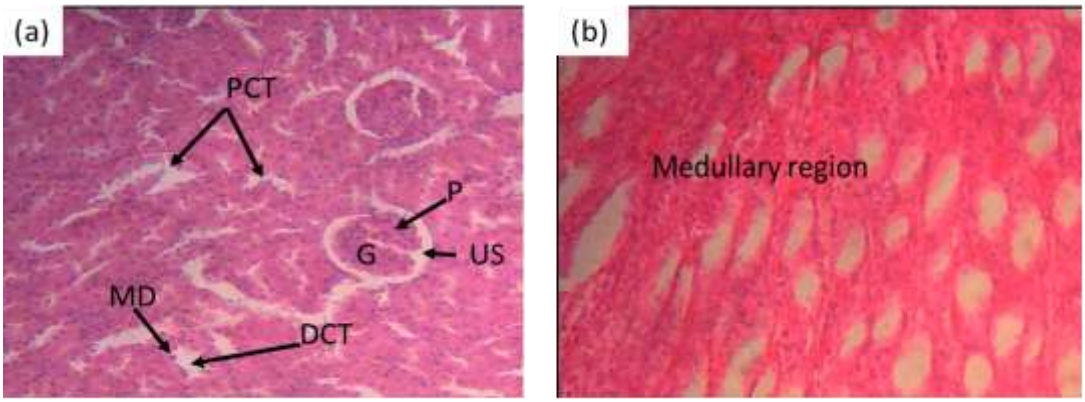

Figure 9. Images representing the kidney sections of control rats (2% DMSO) showing no aberrant change. G= glomerulus, PCT= proximal convoluted tubule, US= urinary space, DCT= distal convoluted tubule, P= podocyte and MD= macula densa.

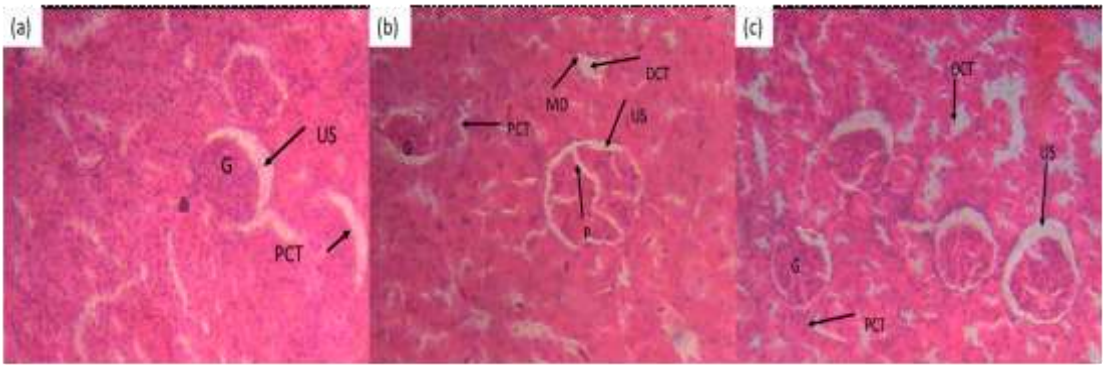

Figure 10. Photomicrograph of kidney section showing the effect of 80% *Erythrina sacleuxii* roots ethanolic extract in treatment groups. a: 2400 mg/kg, b: 1200 mg/kg, c: 400 mg/kg. Indicators: G= glomerulus, PCT= proximal convoluted tubule, US= urinary space, DCT= distal convoluted tubule, P= podocyte and MD= macula densa.

anti-oxidant activities (Christensson et al., 1999). Sorbitol has been reported to have diuretic, laxative and cathartic properties, with hexadecanoic acid having hemolytic, pesticide, flavor and antioxidant activity (Parthipan et al., 2015). Therefore, the bioactive compounds present in *E. sacleuxii* root extract support the therapeutic application of the plant. The discovery of these substances in the plant is the first step in identifying the plant's potential health advantages, as well as its future biotic and phytopharmaceutical significance.

The acute toxicity study of ESRE showed no severe side effects even at the highest dose, as such the LD_{50} can be estimated to be more than 2400 mg/kg for this plant extract. The change in body weight is used as a metric to determine toxicity (Vahalia et al., 2011).

Therefore, with all groups showing an increase in weight during the treatment period, ESRE could be considered none toxic to Wistar albino rats.

Many researchers use rat liver and kidneys to evaluate the toxicity or safety of medicines or plant materials (Satyapal et al., 2008). With the macroscopic examination of kidneys and liver histology revealing no significant differences in this study, then ESRE have no organ toxicity effects. Further safety validation was shown when no death or obvious behavioral changes occurred during the subacute toxicity assays. In subacute toxicity, organ weight determination serves as an important parameter of chemical alteration to major organs (Michael et al., 2007). It also specifies which organs will be targeted in the event of serious toxicity or buildup of the test material (OECD, 2008a). In the present experiment, the weight change of lungs, heart, kidneys and liver observed in all treatment groups was not significantly different when compared to control group. Decrease in pancreas weight had no significant effect on pancreatic functions since triglyceride and cholesterol levels were within normal ranges. The changes in body weight are an important measure of

animals' health in general (Hilaly et al., 2004). From this study, an increment in body weight was recorded indicating a positive health status of the animals (Yakubu et al., 2008). Hematological factors are linked to harmful results in sub-acute study because any modification in the system of hematology is a sensitive index for any toxicity to be seen in humans when animal studies data is translated (Olson et al., 2000). The hematological indices (RBC, PCT, WBC, HCT, PLT, HGB, MON, MCV, MCHC and MCH) in the current sub-acute toxicity investigation for rats were within the reference range. The RBC tally, as well as its parameters showed a no significant increase in the treatment groups which could be due to an increase in the absolute quantity of erythrocytes. The increase observed for platelet count in this study was not significant and it could be as a result of increased platelet aggregation.

As a result of how they react to clinical indications and symptoms caused by toxic substances, biochemical parameters serves as important markers for toxicological evaluation. The evaluation of hepatic and renal function is critical for determining the toxic characteristics of extracts and drugs *(*Rahman et al., 2001). For many years, kidney functions have been assessed by measuring plasma urea (Féres et al., 2006). In renal disorders, both acute and chronic, plasma urea levels are typically elevated. As the kidneys begin to fail, urea clearance decreases, as a result, in damaged kidneys which are not able to eliminate these chemicals at a normal constant rate, urea begins to build; also the level of urea in the blood will rise as a result of this (Féres et al., 2006). The normal serum urea level is 15-45 mg/dl in adult rat. In this study, the mean urea levels showed slight elevation in all test groups, though this was not statistically significant.

Endogenous creatinine is produced and released at a steady rate into bodily fluids with glomerular filtration primarily maintaining its concentration in plasma. Plasma concentration of creatinine as well as its normal clearance from the kidneys has been used as indicators of rate of glomerular filtration. In adult rat, creatinine reference levels are 0.2–0.8 mg/dL. In the current study, creatinine levels showed slight elevation in all the test groups; however, this increase was not significant when compared to control group and also the values were within the normal ranges. The liver is the primary metabolic organ. The ability to measure liver function through the activity of serum marker enzymes such as ALT, AST, and ALP, as well as the level of serum total bilirubin, has shown to be a valuable tool (Yakubu et al., 2008). ALT and AST, indicators of hepatocellular damage, take part in gluconeogenesis (Lala et al., 2021). Although AST is found in cytosolic and mitochondrial isoenzymes as well as other organs, it is less sensitive and specific for the liver, and an elevation in AST might occur due to non-hepatic reasons (Lala et al., 2021). The release of these enzymes into the circulation is triggered by hepatocellular damage, not necessarily cell death. The rise of these enzymes in circulation indicates that parenchymal hepatic tissue/cells are being destroyed (Anderson and Borlak, 2008). In this study, even at the highest dose (2400 mg/kg bwt) of ESRE compared to the control group, serum AST and ALT levels did not show any significant difference. Similarly, alkaline phosphatase showed an increase in the treatment groups; this rise, however, was not statistically significant in comparison to a control group.

The increase in ALT, AST, ALP and the decrease in total bilirubin levels reported with reference to the histology of the liver tissues may not be an indication of liver damage but due to anti-inflammatory and antioxidant properties of the plant extract (Ekam and Ebong, 2007). Total bilirubin, a product of hemoglobin catabolism, is said to be a marker for determining liver function and cholestasis (Satyapal et al., 2008). Decrease in total bilirubin is common in anemia caused by chronic glomerulonephritis (Aba et al., 2018) or aplastic anemia. A significant decrease in bilirubin was recorded; however, this could not be a result of anemia since the red blood cells levels were normal and thus it could be due to anti-inflammatory properties of plant extract. Results presented for the treated rats microscopic histology of their livers demonstrates no change. The structural architecture of hepatic sinusoids, central veins, portal triads and hepatocytes as well as the general structural appearance of the liver were normal as compared to the control group. The result was accompanied by the extract having no negative effects on any of the biochemical markers (such as ALT and AST), which in comparing them to the control group showed no statistically significant changes.

The kidney's histopathological results in this study revealed a normal general structure of the kidney and appearance of glomeruli and tubules, as well as intact macula densa, proximal convoluted tubules and distal convoluted tubules. The values of biochemical indicators in the blood corroborated these findings, indicating that the *E. sacleuxii* roots extract was relatively safe in Wistar albino rats.

Conclusion

From this study, the acute toxicity investigation of ESRE had no negative impacts on the rats' behavior or gross pathology. As a result, the oral LD_{50} of the extract was larger than 2400 mg/kg. The body weight, feed and water consumed, relative organ weight as well as hematological, and biochemical parameters of tested doses were also not negatively affected in the sub-acute toxicity assay. Tissue sections obtained for the kidney and liver of treated rats showed no signs of toxicity. Therefore, the study concludes that ESRE taken orally can be considered non-toxic, especially at 400 mg/kg. However, sub-chronic and chronic toxicity studies should be

conducted before developing extract-based healthcare products to validate their effectiveness and long-term toxicological safety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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