

ZIBELINE INTERNATIONAL™
PUBLISHING

ISSN: 2521-0815 (Print)

ISSN: 2521-0432 (Online)

CODEN: MSPAFY

Matrix Science Pharma (MSP)

DOI: <http://doi.org/10.26480/msp.03.2026.57.65>

RESEARCH ARTICLE

EVALUATION OF ACUTE AND SUBACUTE TOXICITY INDUCED BY ETHYL ACETATE EXTRACT OF *O. LATIFOLIA* KUNTH AND *O. TETRAPHYLLA* CAV. LEAVES IN SWISS ALBINO MICE AND WISTAR ALBINO RATSVishnu Kumar, S^a, Pugalenthi, M^b., Divya Bharathi G^c.^aSasi Bioprospecting Laboratory, Coimbatore - 641 045^bDepartment of Botany, Government Arts and Science College, Coimbatore, Tamil Nadu, India - 624 101.^cDepartment of Botany, Sri Sarada College for Women, Salem, Tamil Nadu, India - 636016*Corresponding Author Email: vishnu.sasi55@gmail.com

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ARTICLE DETAILS

Article History:

Received 12 January 2025

Revised 17 January 2025

Accepted 08 February 2025

Available online 04 March 2025

ABSTRACT

Objective: The evaluate acute and subacute toxicity of ethyl acetate extract of *Oxalis latifolia* and *O. tetraphylla* leaves (family: Oxalidaceae) in Swiss albino mice and Wistar albino rats. **Methods:** Swiss albino mice were used in the 500, 1000, and 2000 mg/kg acute toxicity investigations. Systematic measurements were taken at 1, 2, 3, and 24 hours post-dose. For the subacute toxicity, the animals were grouped into six groups of 6 rats where animals were fed with food and distilled water and the groups were control, 200 and 400 mg/kg of extracts respectively every 24 h orally for 28 days. **Results:** In acute toxicity studies, the extract did not induce death after dose up to 2000 mg/kg, the leaf ethyl acetate extracts from *O. latifolia* and *O. tetraphylla* did not result in any mortalities and were shown to be non-toxic to animals. After 28 days of treatment, there was no discernible difference in the body and organ weights between the treated group and the control group. Analysis of the extract's hemological composition and biochemical characteristics showed no harmful effects. Histopathological alterations were not present, nor were there any obvious abnormalities. In 28 days, no deaths were noted. **Conclusions:** The lack of toxicity at larger doses in rats suggests it may be worth exploring further for potential medical and therapeutic applications.

KEYWORDS

Oxalis latifolia and O. tetraphylla, acute and subacute toxicity

1. INTRODUCTION

Herbal medicines Directly or indirectly, contributed to the advancement of conventional medicine, which benefits the health of millions of people around the world (Yuan et al., 2016). Literature reveals that more than 80% of the population in developing nations rely only on traditional medicine practises and herbal medicine for their primary health care requirements, while 60% of the global population relies on traditional medicine (Chikezie Ojiako, 2015). The herbal remedies are increasing as a result of their accessibility, effectivity and social acceptability (Parveen Upadhyay et al., 2007; Ankur et al., 2010). Natural medicinal are used in treatment of various conditions which includes obesity, cardiovascular disease, arthritis, osteoporosis, diabetes, renal and liver problems (Bashar et al., 2011). Herbal medicines are believed to have less negative side effects since they have derived naturally (Latha et al., 2010; Kim et al., 2017; Dharma et al., 2018; El-Far et al., 2018; Ambika et al., 2019). Herbal remedies are complex mixes of various bioactive phytochemicals that may work through a variety of methods (Bello et al., 2016; Roy et al., 2016). Medicinal plants are necessary to learn more about their qualities, safety, and effectiveness. Negative effects on reproduction in addition to acute, subacute, chronic, carcinogenic, and long-term effects (Zahi et al., 2015). The cumulative toxicity of a chemical in organs, the metabolism of a molecule, and the detrimental consequences of single or sustained dose exposure are all factors can be learned via sub-acute toxicity testing and used to advise dose selection (Gandhare et al., 2013).

The genus *Oxalis* (Family: Oxalidaceae) includes more than 570 species

and is found all over the continent except cold places. The many species of *Oxalis* are employed for their various ethnomedicinal characteristics (Patel et al., 2004). Different parts of *Oxalis* spp have been used for the treatment of Antiseptic (Sharmila et al., 2016), Digestive problem (Savithamma et al., 2014), Vomiting (Jane et al., 2011), Anemia (Sharmila et al., 2016), Piles (Padal et al., 2016) and Wound healing (Harsh et al., 2012) Various studies indicated that *Oxalis* species are widely used in the management of various types of diseases like anaemia, dyspepsia, cancer, dementia, convulsion, piles, bleeding from wounds, diarrhea, headache and jaundice (Ameenah et al., 1993; Mohammad et al., 2000; Hebbar et al., 2004; Sumei et al., 2006; Madhavachetty et al., 2008). However, to my knowledge, there is no previous research work has been carried out in then selected sample *O. tetraphylla* and only a limited work has been reported in *Oxalis latifolia*.

Both *Oxalis latifolia* and *O. tetraphylla* stemless herbs with a worldwide range, can be found growing in abundance in cultivated fields, gardens, and lawns. The leaves of *O. latifolia* and *O. tetraphylla* are usually eaten raw and sometimes cooked (Sasi et al., 2011). The plant is reported to be a treasure of medicinal properties and usage. These plants are used as first aid remedies to cure anemia, inflammation and wound healing (Savithamma et al., 2014). Eventhough the plant has higher nutritional values and several medicinal uses, these plants have not yet explored to make its own position in the herbal drug research. Therefore, in the present study, is focused on both acute and subacute toxicity studies.

2. MATERIALS AND METHODS

Quick Response Code



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Website:
www.matrixscpharma.comDOI:
[10.26480/msp.03.2026.57.65](https://doi.org/10.26480/msp.03.2026.57.65)

2.1 Laboratory Animals

Wistar albino male rats weighed between (100 - 250 g) while Swiss albino male mice weighed between 25 and 30 grammes. These animals were obtained from the Small Animal Breeding Station (SABS), located at the College of Veterinary and Animal Sciences in Mannuthy, Thrissur, Kerala. The animals had access to clean, dry, well-ventilated cages with the appropriate amount of food and water as is typical in a laboratory setting. They were housed in ideal laboratory settings (22 ± 2°C, 30-70% relative humidity, and 12 hours of light and darkness per day). Prior to the start of the experiments, the animals were given two weeks to become acclimated to the laboratory. The permission to conduct the experiment was given by (Reg. No. NCP/IAEC 2018-19/03) as per IAEC (The Institutional Animal Ethics Committee) guidelines.

2.2 Acute Toxicity

An acute oral toxicity test was conducted and its results analysed in accordance with OECD recommendation 423. Swiss albino mice weighing 20–30 g was used in the acute toxicity experiments, and an oral single dosage was used. A total of 36 mice were randomised into 6 different groups to test for acute toxicity via oral administration. Six mice (three males and three females) were divided into three groups and given a single oral dose of 500, 1000, or 2000 mg/kg of body weight of an extract from the *Oxalis* species, with a control group receiving distilled water. After administering the drug orally, researchers monitored the mice's behaviour and looked for symptoms of toxicity continuously for the first hour, every four hours for the next twelve hours, and every twenty-four hours thereafter (Twaij et al., 1983).

2.3 Subacute Toxicity

According to the OECD's 407 standard, a subacute oral toxicity research was conducted utilising the 28-day Repeated Dose Toxicity Method (OECD, 2008). Three males and three females of the Wistar albino strain were randomly assigned to receive either 10 millilitres per kilogramme of body weight (mL/kg) of distilled water, 0.1% carboxymethylcellulose (CMC), or 200 or 400 milligrammes per kilogramme of body weight (mg/kg) of an ethyl acetate extract of the plant species *Oxalis*. Subacute toxicity test doses were set with the LD₅₀ in mind. Body weight changes were recorded on days 0 (days before treatment began), 7, 14, 21, and 28 of the 28-day experiment, while the animals' overall health and clinical symptoms of toxicity were monitored daily. All animals were fasted for a full night at the end of the study period so that blood could be collected. Under ether anaesthesia, blood was drawn from the Sinus puncture and placed in two different tubes: one containing EDTA and the other without any additions. Hematological parameters were promptly evaluated from the EDTA-anticoagulated blood sample. The serum for the biochemical examination was obtained by centrifuging the second tube at 3000 rpm at 4° C for 10 minutes. Liver and kidneys were also removed and weighed; wet slices were then inspected histopathologically.

2.4 Haematological Parameters

An automatic haematological analyzer was used for the haematological analysis (Model ABX-Micro-S-60). White blood cells (WBC), red blood cells (RBC), and other haematological markers were tallied (OECD, 2008).

2.5 Biochemical Analysis

The retro-orbital plexus was punctured while the animals was sedated with isoflurane to get blood. Ethylene Diamine Tetra-Acetic Acid (EDTA), an anticoagulant, was used to prevent clotting when collecting whole blood for haematograms, but not when collecting blood samples for biochemical analysis. Serum was separated from whole blood by centrifuging it twice for ten minutes at 3000 rpm. The serum's biochemistry was analysed using a biochemical autoanalyzer (Type 7170, Hitachi). Blood plasma was examined for bilirubin, Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT), and Serum Glutamic Pyruvic Transaminase (SGPT) concentrations in order to assess liver function. By evaluating the concentrations of urea and creatinine, biochemical parameters for kidney function were examined (OECD, 2008).

2.6 Histopathological Examination

The tissues of the kidney and liver. Soon after the sacrifice, important organs were examined under a microscope. Surgery was used to remove the kidneys and liver, which were then immediately sent for histological processing after being weighed (absolute organ weight) on an analytical balance. The relative organ weight (ROW) of each animal was then determined using the formula: relative organ weight (%) = (organ weight/body weight) 100 (Pieme et al., 2006). Hematoxylin and eosin was used to stain the liver and kidney fragments after they had been embedded in paraffin wax and preserved in a 10% buffered formalin solution. The

histology slices were then seen under optical microscopy (Optika Microscopes, Italy) and photographed using an Infinity 1 camera microscope with ×40 magnification magnification. The goal of histopathological investigation is to determine the health of the tissue by looking for symptoms of toxicity such as leukocyte infiltration, degeneration, necrosis, and apoptosis.

2.7 Preparation Of Plasma And Homogenate Tissue

Animals were given anaesthesia before being slaughtered by cervical dislocation at the conclusion of the experiment so that blood could be collected in heparin tubes. After 10 minutes of centrifugation at 3000 rpm, plasma was collected. Homogenized livers and kidneys were prepared using ice-cold KCl (1.15%). The upper lipid layer was carefully removed to acquire the supernatant, which was then centrifuged at 5000 rpm for 10 min. at 4°C to extract the supernatant. The enzymes catalase, glutathione peroxidase, reduced glutathione (GSH), lipid peroxidation, and superoxide dismutase (SOD) were identified from this supernatant.

2.8 Estimation of Superoxide Dismutase

The SOD assay was carried out were by (Xin et al., 1991). 5 mg of adrenaline was dissolved in 10 ml of distilled water to create the adrenaline solution. Following that, 0.10 ml of serum was stirred in potassium buffer with a pH of 7.8. In a cuvette, buffer and 0.3 ml of an adrenaline solution were combined with 0.2 ml of the extract, stirred, and measured at 450 nm.

2.9 Estimation of Catalase

The method developed by was used to calculate catalase activity (Aebi et al., 1983). This is based on how catalase gradually breaks down hydrogen peroxide (H₂O₂) by absorbing UV light. Absorbance is easily measured at 240 nm.

2.10 Estimation of Glutathione Peroxidase

The absorbance of the yellow-colored complex was determined at 412 nm after being incubated for 10 minutes at 37°C versus distilled water. An unhomogenized sample was processed the same way and kept as the blank. One unit of enzyme activity was defined as the decrease in log GSH by 0.001 min/mg protein after deducting the decrease in log GSH per minute for the non-enzymatic process (Hafeman et al., 1974).

2.11 Estimation of Lipid Peroxidation

The method of was employed in lipid peroxidation. The reaction mixture for basal peroxidation contained 2 mL of tissue extract, 0.03 M tris HCl buffer (pH 7.4), and 0.2 mM sodium pyrophosphate. The tubes were continuously shaken at 37°C during the 20-min. incubation period. The reaction was stopped by the addition of 1 mL of 10% TCA. The tubes were shaken well and 1.5 mL of TBA was added and was heated in boiling water bath for 20 min. After centrifuging the tubes, the colour development was measured at 532 nm (Hogberg et al., 1974).

2.12 Estimation Of Reduced Glutathione

GSH determination used the method developed its foundation is the idea that in an alkaline media, GSH reacts with alloxan and oxygen at a wavelength (320 nm) (Xifan et al., 2015).

2.13 Statistical Analysis

The data were expressed using the Standard Error of the Mean (SEM). After one-way analysis of variance, Dunnett's test was used to determine whether there were any differences between the groups in the subacute toxicity test (ANOVA). At P > 0.05, the level of significance was chosen.

3. RESULTS

3.1 In Vivo Studies In Animal Model

3.1.1 Acute Oral Toxicity

The ethyl acetate leaf extract of *O. latifolia* and *O. tetraphylla* were subjected to acute toxicity study in Swiss albino mice. After extracts were given at doses of 500, 1000, and 2000 mg/kg, the animals were watched for 24 hours. The findings after taking into account the characteristics of alertness, grooming, touch and pain reaction, tremors, convulsions, righting reflex, grip strength, etc. Up to 2000 mg/kg, the leaf ethyl acetate extracts from *O. latifolia* and *O. tetraphylla* did not result in any mortalities and were shown to be non-toxic to animals.

3.1.2 Sub-Acute Toxicity Study

The acute toxicity study revealed that *Oxalis* leaf extracts of both plant samples of the present study did not cause any mortality upto 2000 mg/kg

Further, sub-acute studies were carried out based on OECD Guidelines, of which, ethyl acetate leaf extract of *O. latifolia* and *O. tetraphylla* were administered in a dose of 200 and 400 mg/kg orally for 28 days. No death has occurred in tested animals.

3.1.3 Body Weight

There were declined significant difference in body weight of the control

and drug treated rats and the data are presented in Table 1 and 2 and Plate 1. In comparison to the control group, the weight gain declined markedly differently. Following 28 days of treatment with *O. latifolia* and *O. tetraphylla* leaf extracts, the body weight of the rat gradually increased at doses of 200 and 400 mg/kg, which may be a sign that the animals' nutritional status had improved.

Table 1: Body weight of rat fed with *O. latifolia* and *O. tetraphylla* ethyl acetate leaf extracts

Groups and Doses	Body weight (g/rat)				
	0 th day	7 th day	14 th day	21 th day	28 th day
Group I (Untreated)	110.47 ± 0.6	122.23 ± 1.09	139.99 ± 1.02	156.60 ± 0.94	168.67 ± 1.23
Group I (Control 0.1% CMC)	125.34 ± 0.97	135.13 ± 0.8	144.16 ± 1.17	155.94 ± 1.74	171.05 ± 0.95
Group III (<i>O. latifolia</i> 200mg/kg)	210.56 ± 1.41	220.78 ± 1.20	239.74 ± 0.77	247.49 ± 10.5	259.06 ± 1.08
Group IV (<i>O. latifolia</i> 400mg/kg)	150.40 ± 0.38	163.21 ± 0.64	175.63 ± 0.54	191.71 ± 0.42	207.37 ± 0.26
Group V (<i>O. tetraphylla</i> 200mg/kg)	100.08 ± 0.90	120.65 ± 0.43	149.20 ± 0.18	160.89 ± 0.77	168.50 ± 0.50
Group VI (<i>O. tetraphylla</i> 400mg/kg)	175.27 ± 0.13	190.87 ± 0.24	207.44 ± 0.22	213.81 ± 0.28	225.42 ± 0.12

CMC: Carboxy Methyl Cellulose; There is no significant difference at $p < 0.05$ when compared to normality in the data, which represent the mean and SEM (n = 6). (untreated).



Grouping of Animals for drug administration



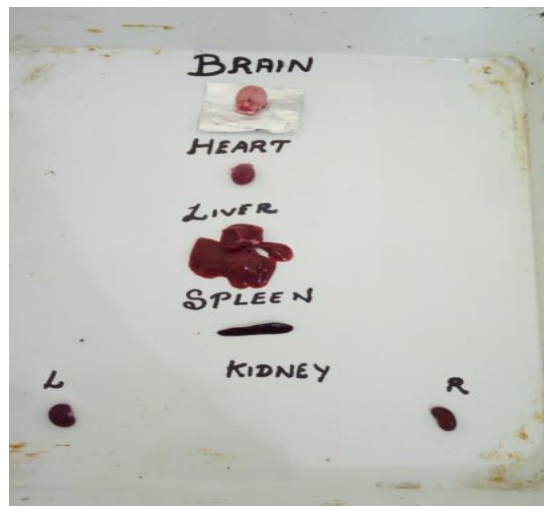
Drug preparation



Drug administration



Collection of blood through Retro-orbital puncture



Dissected organs from sacrificed animal

PLATE 1: In vivo Toxicity Studies in Animal Model Organ weight

In general, there were no statistically significant differences in the relative weights of the rats' liver, spleen, heart, kidney, brain, and testes when compared to the control group (Table 3). However, the relative with 200

and 400 mg/kg of the rat during 28 days of drug administration of *O. latifolia* and *O. tetraphylla* leaf extracts.

Table 2: Effect of ethyl acetate extract of *O. latifolia* and *O. tetraphylla* leaves on organ weight

Groups and Doses	Organ weight (g)				
	Liver	Spleen	Heart	Kidney	Brain
Group I (Untreated)	5.40 ± 0.65	0.47 ± 0.08	0.53 ± 0.05	1.19 ± 0.17	1.54 ± 0.14
Group I (Control 0.1% CMC)	5.60 ± 0.12	0.46 ± 0.08	0.56 ± 0.46	1.21 ± 0.35	1.57 ± 0.098
Group III (<i>O. latifolia</i> 200mg/kg)	5.06 ± 1.00	0.46 ± 0.12	0.54 ± 0.10	1.17 ± 0.23	1.56 ± 0.8
Group IV (<i>O. latifolia</i> 400mg/kg)	5.32 ± 0.69	0.48 ± 0.29	0.57 ± 0.49	1.2 ± 0.14	1.59 ± 0.14
Group V (<i>O. tetraphylla</i> 200mg/kg)	5.14 ± 0.64	0.45 ± 0.07	0.57 ± 0.75	1.18 ± 0.13	1.57 ± 0.32
Group VI (<i>O. tetraphylla</i> 400mg/kg)	5.66 ± 0.51	0.49 ± 0.06	0.59 ± 0.08	1.21 ± 0.09	1.61 ± 0.050

CMC: Carboxy Methyl Cellulose; There is no significant difference at $p < 0.05$ when compared to normality in the data, which represent the mean and SEM (n = 6). (untreated).

3.1.4 Haematological Parameters

Table 3 displays the impact of ethyl acetate leaf extracts from *O. latifolia* and *O. tetraphylla* on the haematological parameters of experimental and

control rats. Throughout the course of the treatment, the evaluated haematological variables, such as WBC and HB, were within the physiological range (28 days).

Table 3: Haematological values of rat fed with ethyl acetate extract of *O. latifolia* and *O. tetraphylla* leaves after 28 days

Groups and Doses	Haematological parameter	
	WBC (cells/cmm)	HB count (g/dL)
Group I (Untreated)	7600 ± 4.1	14.9 ± 0.24
Group I (Control 0.1% CMC)	10056 ± 4.1	13.17 ± 0.36
Group III (<i>O. latifolia</i> 200mg/kg)	8641 ± 3.8	13.4 ± 0.44
Group IV (<i>O. latifolia</i> 400mg/kg)	9742 ± 3.4	13.9 ± 0.61
Group V (<i>O. tetraphylla</i> 200mg/kg)	9785 ± 5.1	13.01 ± 0.78
Group VI (<i>O. tetraphylla</i> 400mg/kg)	10080 ± 2.18	13.92 ± 0.63

CMC: Carboxy Methyl Cellulose; Values are mean ± SEM (n = 6); cmm: Cubic Millimeter.

3.1.5 Biochemical parameters

The information for the biochemical parameters in the treated and untreated rats is presented in Table 4. Sub-acute oral administration of *O. latifolia* and *O. tetraphylla* extracts (daily for 28 days) did not result in any

appreciable alterations in the liver's biochemical parameters (Serum Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and kidney's biochemical parameters) (Creatinine & urea).

Table 4: Biochemical parameters of rat fed with ethyl acetate extract of *O. latifolia* and *O. tetraphylla* leaves after 28 days

Groups and Doses	Liver function test				Kidney function test	
	ALP KA units	SGOT (IU/L)	SGPT (IU/L)	Bilirubin (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Group I (Untreated)	200 ± 1.18	486 ± 3.25	130 ± 1.85	1.05 ± 0.47	1.17 ± 0.07	48.9 ± 2.12
Group I (Control 0.1% CMC)	174 ± 1.72	367 ± 2.23	105 ± 1.23	1.02 ± 0.59	0.56 ± 0.04	50.7 ± 2.78
Group III (<i>O. latifolia</i> 200mg/kg)	134 ± 2.48	333 ± 1.78	95 ± 1.80	0.97 ± 0.41	0.83 ± 0.49	48.21 ± 1.71
Group III (<i>O. latifolia</i> 400mg/kg)	191 ± 1.78	360 ± 3.29	102 ± 3.29	1.02 ± 0.67	0.95 ± 0.14	51.30 ± 0.79
Group III (<i>O. tetraphylla</i> 200mg/kg)	169 ± 2.12	380 ± 1.40	105 ± 1.40	0.98 ± 0.41	0.69 ± 0.51	51.76 ± 0.63
Group III (<i>O. tetraphylla</i> 400mg/kg)	223 ± 1.58	455 ± 1.85	110 ± 1.85	1.04 ± 0.72	1.13 ± 0.71	52.82 ± 1.02

The data represent the mean ± SEM (n = 6).

3.1.6 Histopathological Studies

Microscopic representations of the liver, spleen, heart, kidney, and brain were shown in Plates 2 - 6. All groups' histological analyses of the liver, spleen, heart, kidney, and brain sections were normal. There were no discernible abnormalities, according to pathological analyses of the tissues. When the internal organs were examined under a microscope, no changes were discovered, and neither group's cellular characteristics stood out.

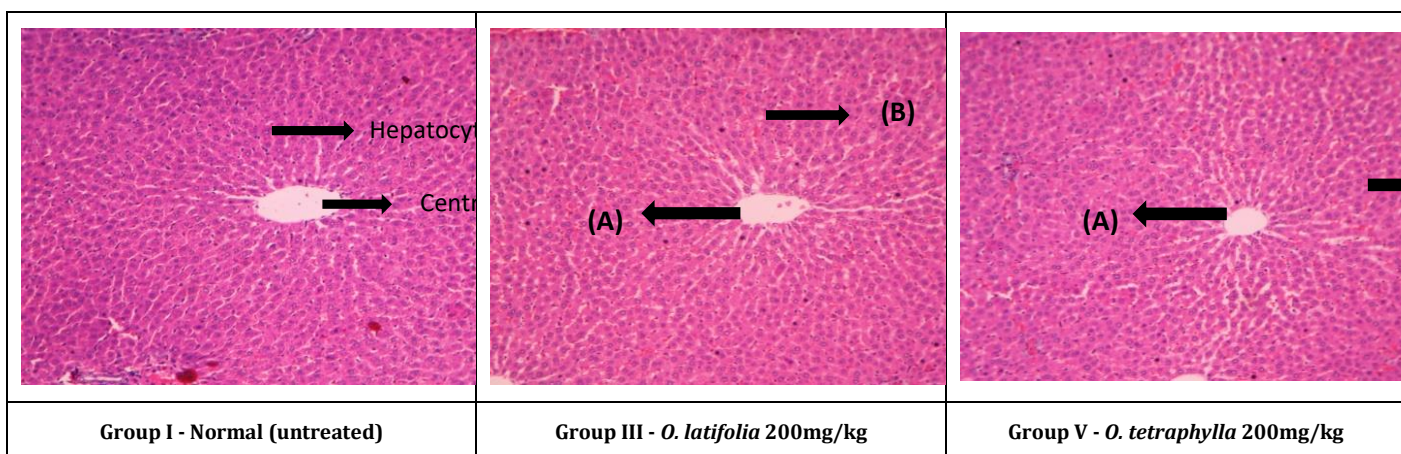
3.2 In Vivo Antioxidant Activities

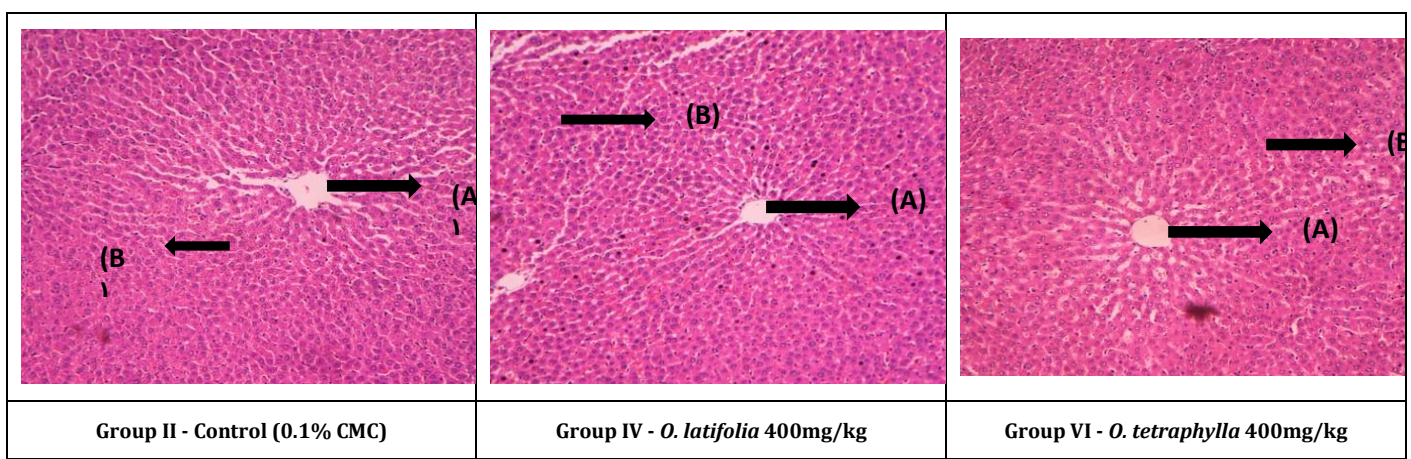
In vivo antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Lipid Peroxidation (LPO), and non-antioxidant Reduced Glutathione (GSH) are affected by *O. latifolia* and *O. tetraphylla* leaf ethyl acetate extract in the liver of rats after 28 days. These effects are shown in Tables 5. Animals given 200 and 400 mg/kg of leaf ethyl acetate extracts of *O. latifolia* and *O. tetraphylla* showed significantly higher blood levels of CAT, SOD, and GSH. On the other hand, animals given 400 mg/kg of the drug were the only ones in which raised GPx levels could be seen. Significant in vivo antioxidant activity was seen with the higher *O. tetraphylla* dose.

Table 5: In vivo antioxidant activity of *O. latifolia* and *O. tetraphylla* extracts

Groups and Doses (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	LPO (µM of MDA/mg protein)	GSH (nM/mg protein)
Group I (Untreated)	3.00 ± 0.11	8.67 ± 1.83	11.72 ± 1.82	7.42 ± 0.96	16.96 ± 0.96
Group II (Control 0.1% CMC)	2.86 ± 0.19	8.16 ± 1.39	10.82 ± 0.98	7.18 ± 1.24	15.2 ± 0.62
Group III (<i>O. latifolia</i> 200mg/kg)	2.74 ± 0.12	7.61 ± 1.78	10.62 ± 2.12	7.28 ± 1.48	15.92 ± 1.02
Group IV (<i>O. latifolia</i> 400mg/kg)	3.02 ± 0.14	8.42 ± 0.16	11.15 ± 1.78	7.58 ± 0.09	16.12 ± 0.19
Group V (<i>O. tetraphylla</i> 200mg/kg)	2.65 ± 0.09	8.43 ± 1.82	11.79 ± 1.76	7.38 ± 0.78	16.02 ± 0.90
Group VI (<i>O. tetraphylla</i> 400mg/kg)	3.06 ± 0.09	8.78 ± 0.89	11.95 ± 1.69	7.55 ± 0.89	16.86 ± 0.92

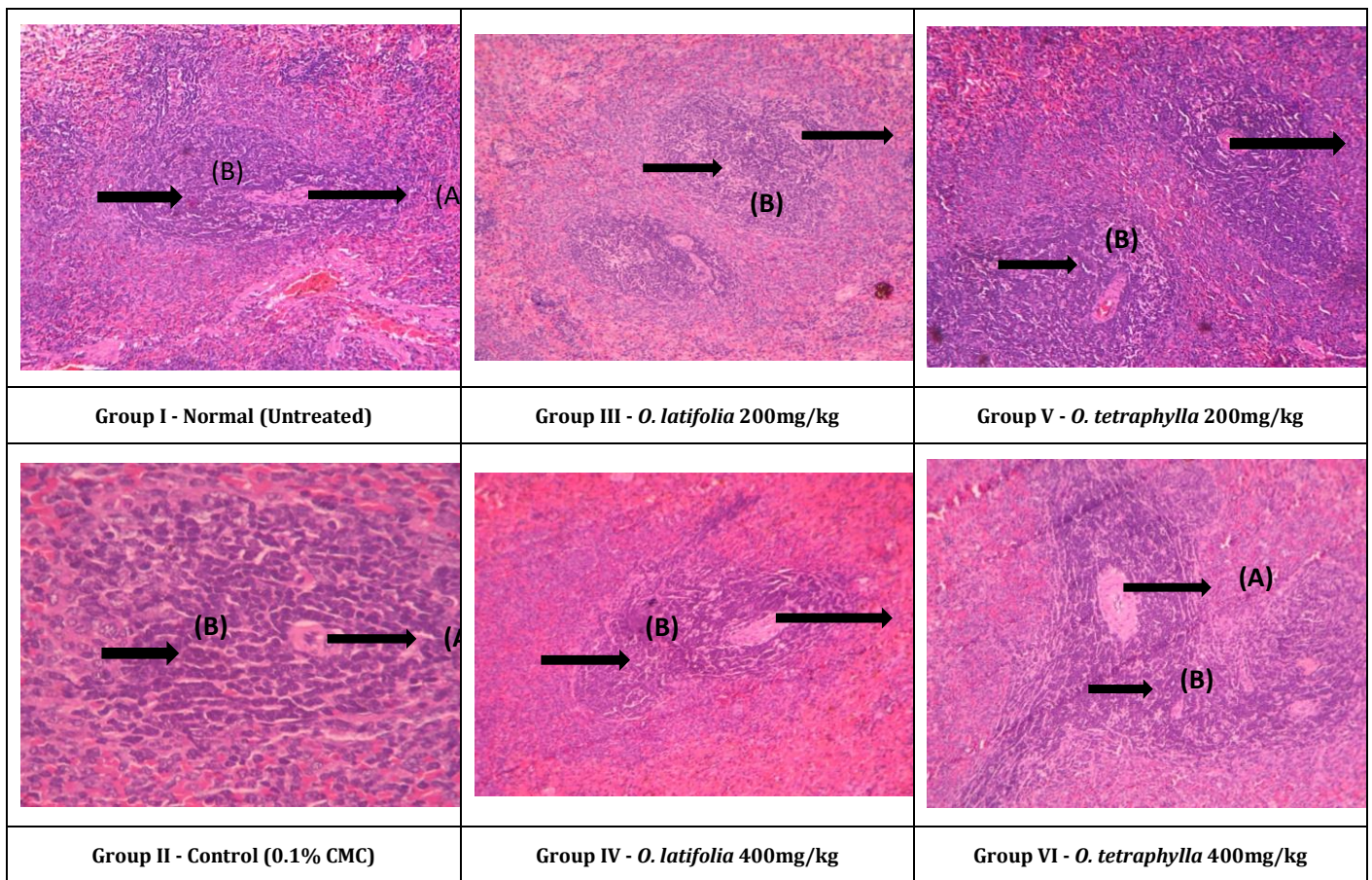
There is a significant difference at $p < 0.05$ compared to normal (untreated).





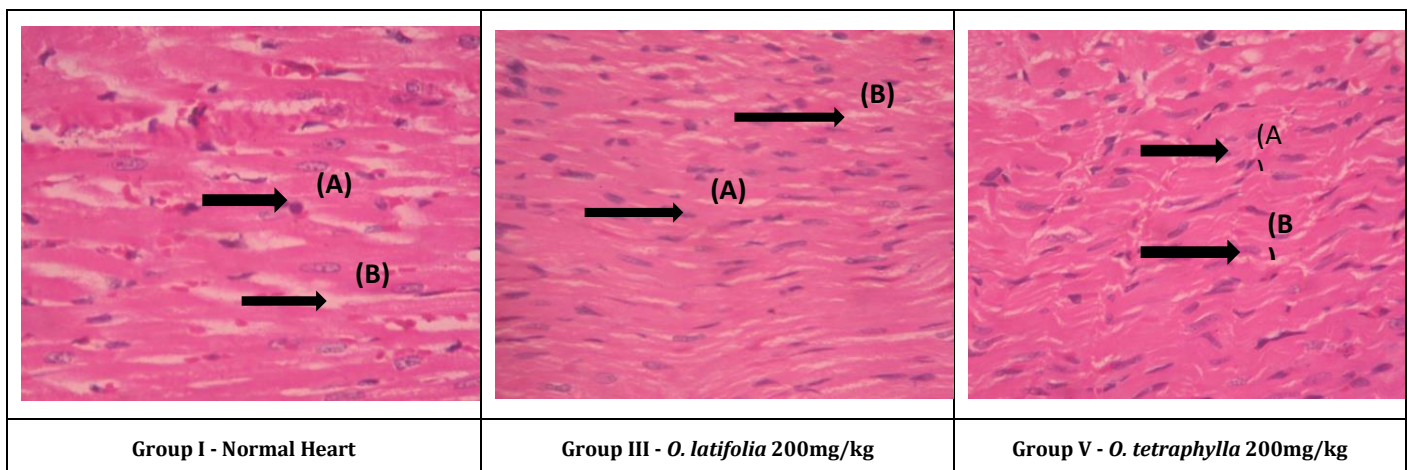
Histopathological features examined: (A) Central Vein: (B) Hepatocytes

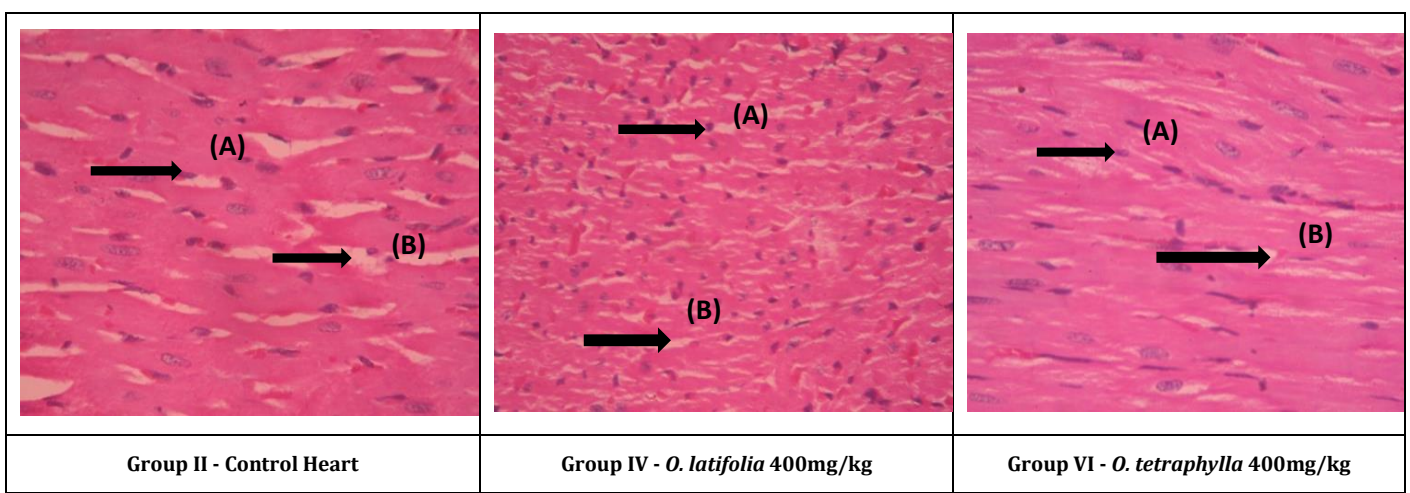
PLATE 2: Histopathological examination of liver of Wistar Albino Rats administered with ethyl acetate Leaf extracts of *O. latifolia* and *O. tetraphylla* leaves



Histopathological features examined: (A) White Pulp: (B) Red Pulp

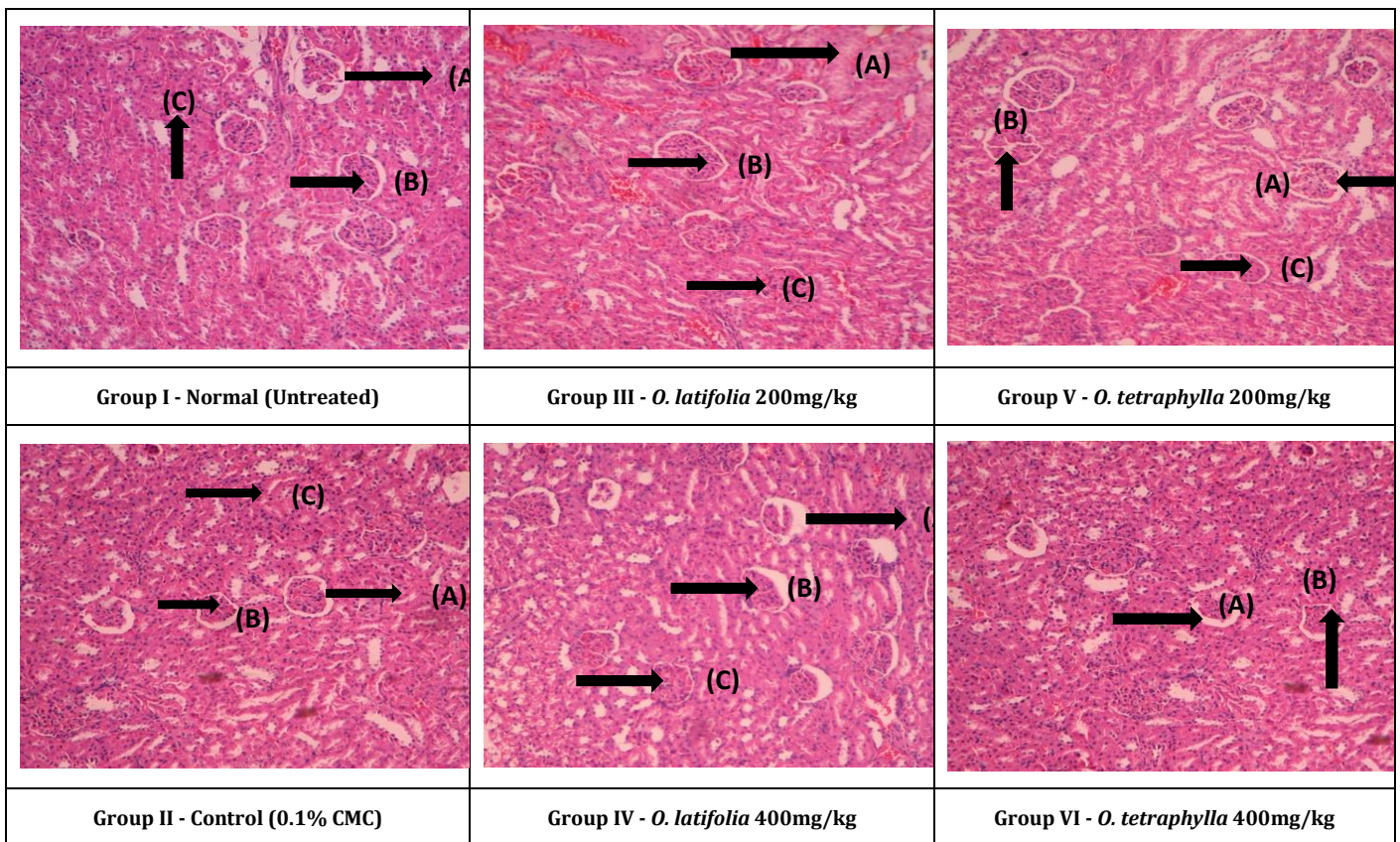
PLATE 3: Histopathological examination of spleen of Wistar Albino Rats administered with ethyl acetate Leaf extracts of *O. latifolia* and *O. tetraphylla* leaves





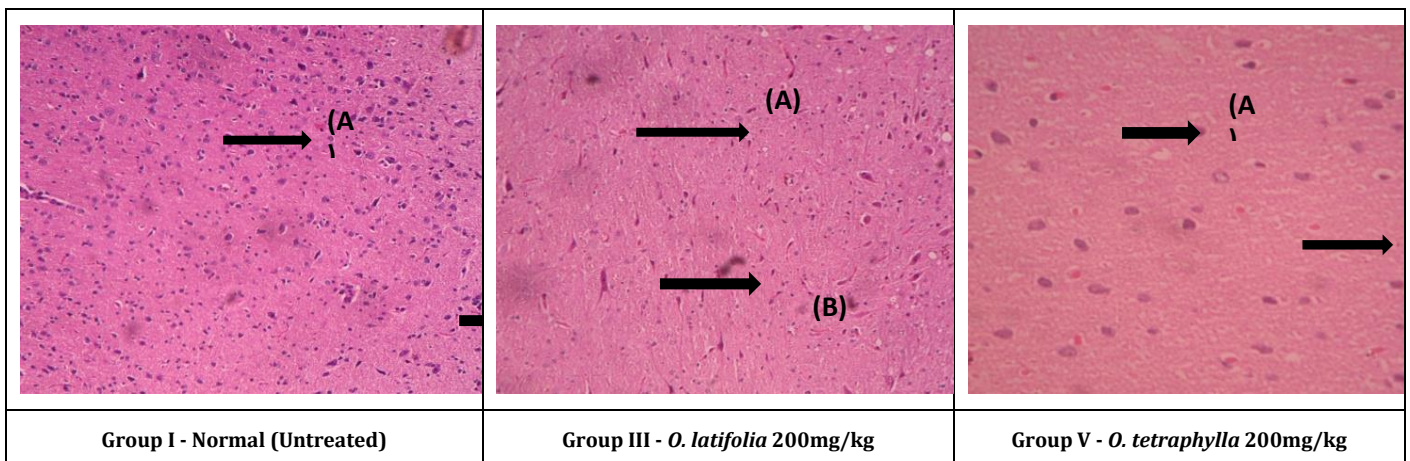
Histopathological features examined: (A) Cardiac Muscle cells: (B) Intercalary disc

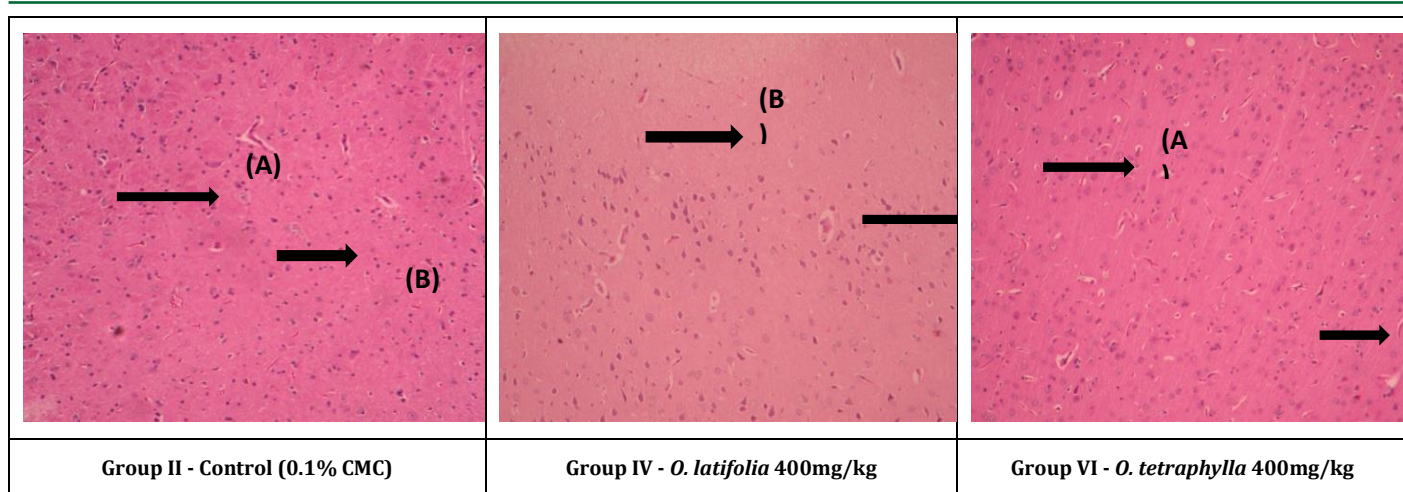
PLATE 4: Histopathological examination of Heart of Wistar Albino Rats administered with ethyl acetate Leaf extracts of *O. latifolia* and *O. tetraphylla*



Histopathological features examined: (A) Renal tubules: (B) Glomeruli: (C) Bowman's capsules

PLATE 5: Histopathological examination of Kidney of Wistar Albino Rats administered with ethyl acetate Leaf extracts of *O. latifolia* and *O. tetraphylla* leaves





Histopathological features examined: (A) Pyramidal neuron: (B) Cerebral cortex

PLATE 6 : Histopathological examination of Brain of Wistar Albino Rats administered with ethyl acetate Leaf extracts of *O. latifolia* and *O. tetraphylla* leaves

4. DISCUSSION

The daily intake and the final end point observation of treating particular diseases with repeated dose hold on with toxicity study. The collected plant samples are to be scientifically validated to identify the positive effects of the drug, which can be support the work of traditional healers and pharmacological industries which values the safety profile for phytomedicine (Aniagu et al., 2005). Herbal medicines are an alternative remedy for gaining popularity in the world. Herbal preparations have been used unselectively and the formulation has significant risk and adverse effects. The prepared medicinally formulation are usually given for a long period of time without proper dose and these are monitored by traditional healers (Eran et al., 2016). To determine the dose that might be employed later, scientific knowledge of oral toxicity is crucial. Despite the pharmacological advantages of *O. latifolia* and *O. tetraphylla*, comprehensive information about this medicinal plant's acute and subacute toxicity is limited. Hence, the current study was undertaken to evaluate and focus on the acute and subacute toxicity of *O. latifolia* and *O. tetraphylla* leaves in an animal model (mice and rat) (Meriama et al., 2020). Has done similar work to identify the toxicity of the *Oxalis cernua* in rat and mice model for a period of 14 days and has reported that the plant to be non-toxic. As a result of their genetic, biochemical, and behavioural similarities to humans, rats and mice are preferred for toxicology studies since they can reproduce any human symptom (Tan et al., 2008). It was determined that the lethal dose was larger than 2000 mg/kg body weight. Typically, it is deemed secure following a full day of monitoring (Sandipan et al., 2014). Has worked on acute and sub-acute toxicity and has identified that *Averrhoa carambola* has no side effects in both sexes of mice upto the dose 2000 mg/kg. Drug doses identified from animal studies are translated to humans after several trails (Reagan-Shaw et al., 2008). In the present work, acute toxicity study was carried out in ethyl acetate extract of *Oxalis latifolia* and *O. tetraphylla* as it did not cause any mortality up to 2000 mg/kg. Hence 1/10th and 1/5th of the maximum dose (200 and 400 mg/kg) were fixed for animal experiments (Sowndharajan et al., 2010). The selected extracts showed low toxicity when administered orally. Has reported that any substance with LD₅₀ of 1000 mg/kg body weight/oral route is been identified to be safe (Clarke et al., 1977). The high LD₅₀ value obtained indicates that the extract could be administered with a high degree of safety and the absorption of these drugs is incomplete due to inherent factors that are absorbed along the gastrointestinal tract (Dennis, 1984). Acute toxicity is achieved by adhering to certain clinical restrictions, where cumulative toxic effects occur even at very low dosages. This is one reason why repeated dose studies are generally helpful in assessing the safety profile of plant extracts. Accordingly, a sub-acute toxicity study was carried out using doses of 200 and 400 mg/kg of chosen *Oxalis* extracts (Ekar et al., 2018). Have reported that there is a long-term history of traditional and folklore medicine which promotes a false feeling of their safety, an assumption of minimal side effects and their usefulness in prevention and control of diseases. To better understand *Oxalis latifolia* and *O. tetraphylla* leaf ethyl acetate extract properties, safety and efficiency on normal metabolic condition, Swiss albino mice were used for acute toxicity study. On confirmatory result of acute toxicity study, further analysis was performed for a period of 28 successive days to observe the sub-acute toxicity and *in vivo* antioxidant activity in Wistar Albino Rats. Two doses 200 and 400 mg/kg body weight was fixed and the drugs were administered on daily basis. The effects were monitored by examining the parameters such as body weight, weight of vital organs, biochemical parameter and histological parameters (liver, kidney, heart, spleen and

brain). At the end of sub-acute study there was no behaviour changes, no loss in body weight and no death or toxicity associated symptoms. Changes in organ weight are trademark of toxicity in the tested animals (Unuofin et al., 2018). Subacute studies are done to identify the adverse effect of life span, organ damage, hematological changes and histological variation in experimental animals (Hilaly et al., 2004). Has worked on evaluating rats at different doses such as 250, 500 and 1000 mg/kg for 28 days as the body weight, feed consumption and water consumption served as a sensitive indicator of health status of animals (Debora et al., 2013). Has reported that subacute study of *A. carambola* was done using two doses of 200 and 500 mg/kg body weight for 28 days, the continuous oral administration of drug did not cause any change in the animal behavior bodyweight or in organs relative weight in drug treated animals and control group. The result concluded that after 28 days of drug administration there was a normal increment in body weight as of the selected sample *O. latifolia* and *O. tetraphylla*. Biochemical parameters investigation was done to identify the major toxic effect on specific tissues specifically kidneys and liver which may provide information about toxicity of the therapeutic agents (Yamthe et al., 2012). The evaluations of hepatic functions have been examined by Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) activities. The AST are present in different tissues such as heart, skeletal muscle, kidney, brain and liver ect., and ALT is primarily present in liver. The elevation of these transaminase is been one of the identifying factors of liver damage as it predicts toxicity (Rahman et al., 2001; Crook, 2006). Biochemical analysis of serum is done to identify the alteration of renal and hepatic function of extracts. The alteration of ALT, AST, ALP, creatinine and uric acid is an indicator of liver and kidney function. The total proteins such as albumin, globulin and total bilirubin also affects the liver's secretory functions (Debora et al., 2013; Farah et al., 2014). Has reported that the blood serum of animals tested with *A. carambola* did not show any difference in the parameters tested (Mazumdar et al., 2014). has worked on the biochemical parameter of *A. carambola* and has reported that there were no biochemical changes in plasma, urea, creatinine and uric acid levels which was fixed as kidney function test and the assessment of Hepatocellular damages are made by estimation of transaminase activities such as ALT/ SGPT, SGOT, ALP, bilirubin and Total protein. In the present study ALP, SGOT, SGPT, Bilirubin, creatinine and urea was carried out in the *O. latifolia* and *O. tetraphylla* ethyl acetate leaf extract which revealed the same activity as reported earlier and it was proved that the presently studied plant samples are to be a safe drug. The hemopoietic system is one of the most vulnerable areas for hazardous substances, which are crucial indicators of the physiological and pathological conditions in both humans and animals. Hematological parameters are an important basic source in identifying such foreign compounds including plant extracts. The results of the plant extract show no discernible alteration in the RBC, suggesting that the plant has no impact on erythropoiesis, shape, or osmotic fragility of Red Blood Cells. The first line of cellular defence against infectious agents, tissue damage, and inflammation is made up of white blood cells (WBC). Further there were no significant changes observed in neutrophils, lymphocytes and monocytes of rat administered with the selected plant sample suggesting that the extract might not have any effect on the immune system of the animals. *A. carambola* did not have any alteration in biomolecules, metabolic products and hematology functioning of which did not have any significant difference in the study which correlates with the result of the selected sample (*O. latifolia* and *O. tetraphylla*) (Debora et al., 2013; Farah et al., 2014). Animals after treatment of drug (*O. latifolia* and *O. tetraphylla*) for a period of 28 days was sacrificed and histological evaluation are done to characterize the biological response of the internal

organs (Heart, brain, spleen, liver and kidney) and also to analyze the architecture of the drug treated animals and control group. Any toxic effect of the drug can be easily identified by doing hematological and histological evaluation (Jaganathan et al., 2013). In hepatocytes cells the cytoplasm and vacuole swelling indicates the acute and subacute liver injury caused by the drug. The alteration is noted as per the size of the vacuole where the smaller ones are highly effected (Abdelhalim et al., 2011). Histological evaluation was done in *A. bilimbi* and the results revealed that the organs had intact cells, well organized tissue architecture and there was no pathological lesion in the selected organs and control group (Farah et al., 2014). Thus, the toxicity study revealed that the ethyl acetate leaf extract of *O. latifolia* and *O. tetraphylla* possess the lowest/nil toxicity effect. Against oxidative injury, antioxidant enzymes such as CAT, SOD and GPx play a major role. Through the Nrf2 transcription factor, these antioxidant enzymes get stimulated and play a major role in reducing ROS-induced damage which mainly works during the process of inflammation. In the cytosol Keap1 protein is retained by Nrf2 transcription factor. When these get exposed to free radicals, Nrf2 may dissociate from Keap1 protein and translocate into the nucleus where it regulates and activates the antioxidant genes (Hybertson et al., 2011). The homogenated liver was analysed for Catalase, Super oxide Dismutase, Glutathione Peroxidase, Lipid peroxidation activity. CAT has the highest capability of converting toxic compounds into less toxic and more stable molecules in plants, it also increases the cell wall resistance and acts as a signaling molecule for the induction of defensive genes (Caverzan et al., 2016). GST uses glutathione (GSH), thioredoxin (TRX) or glutaredoxin to reduce H₂O₂ through ascorbate-independent thiol-mediated pathways (Meyer et al., 2012; Meriama et al., 2020). Has done *in vivo* antioxidant analysis in Liver and Kidney of *O. cernua* and the result concluded to have significant value as of the control group has taken special efforts in identifying the antioxidant parameters of *Averrhoa bilimbi* in paracetamol intoxicated Wistar Albino Rats where Superoxide Dismutase, Glutathione peroxidases and Glutathione analysis were done in Liver, Kidney and Heart (Thamizhselvam et al., 2015). The efficacy of the extract was found to be significant when compared with *A. bilimbi* and the selected samples *O. tetraphylla* and *O. latifolia* showed minor significant difference. Toxicity study of *O. latifolia* and *O. tetraphylla* was conducted with two doses (200 and 400mg/kg body weight) for 28 days. The results revealed that the repeated oral administration of drug did not produce any significant changes in animal behavior in the treatment period Bodyweight and organs relative weight of the treated animals were compared to the control group, as evidenced by the absence of toxic symptoms. The extracts formulation is reportedly non-toxic because there was no mortality noted during the treatment time and no alterations were seen. This conclusion serves as scientific support for both short- and long-term safe usage.

5. CONCLUSION

O. latifolia and *O. tetraphylla* leaf extracts taken orally are safe, especially at concentrations of 200 and 400 mg/kg, as rats used in acute and subacute toxicity trials did not perish from exposure to the extract. The results of this study support the use of *O. latifolia* and *O. tetraphylla* in traditional folk medicine. To confirm its efficacy and long-term toxicological safety, additional pre-clinical studies should be conducted.

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