

## In Vivo Safety of Aqueous Leaf Extract of *Lippia javanica* in Mice Models

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### Abstract

Rural dwellers in Kenya often resort to herbal remedy and dietary control in the treatment of several diseases including diabetes mellitus (DM), hypertension, cancer and cardiac diseases. The therapeutic applications of such plants has largely rested upon their long-term clinical experience, however, their safety profiles has not been well evaluated. The present study aimed at determining the *in vivo* toxic effects of orally and intraperitoneally administering *Lippia javanica* leaf extract at dosage levels of 450 mg/kgbw, 670 mg/kgbw and 1000 mg/kgbw daily for 28 days on the body and organ weights, hematological indices and biochemical parameters in normal male swiss white albino mice. During this period, the mice were allowed free access to mice pellets and water *ad libitum* and observed for signs of general illness, change in behavior and mortality. Phytochemical composition was assessed using standard procedures. The oral and intraperitoneal administration of 450 mg/kgbw, 670 mg/kgbw and 1000 mg/kg body weight of the extract decreased the body weight gain and altered the organ to body weight percentage of the brain, kidney, liver, heart, testes and lungs. Oral and intraperitoneal administration of the same doses caused a change in levels of RBC, WBC, Hb, PCV, PLT, MPV, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophils, basophils, monocytes and biochemical parameters: AST, ALP, ALT, GGT, CK,  $\alpha$ -AMYL, LDH, T-BIL, D-BIL, I-BIL, TG, TC, LDL-C, HDL-C, BUN, UA, Urea and Creatinine. The extracts contained alkaloids, sterols, terpenoids, flavonoids, tannins and saponins.

**Keywords:** Diabetes mellitus; Hypertension; Cancer; Cardiac diseases; *Lippia javanica*; Toxic; *In vivo*; Mg/kgbw; *Ad libitum*

### Introduction

Toxicity studies are fundamental in evaluation of safety of extracts or drugs used in clinical medicine [1]. The short or long-term administration of a chemical compound may bring about significant changes in the function, metabolic transformation, structure and concentration of biomolecules, enzymes and even metabolic pathways [2]. These alterations might be rapid or slow and may lead to different biochemical mechanism of the drug producing similar pathological, clinical and laboratory findings [3]. Therefore, the measurable endpoint of toxicity may be a pharmacological, biochemical, or a pathological change, which shows percentage or proportional change [4].

The current pharmacological treatment of various diseases is based on oral and injectable agents which have so many side effects, coupled with their high costs which are not affordable in poor economic communities [5]. Consequently, in rural parts of worldwide societies, traditional remedies from plant sources with minimal side effect are frequently employed to manage such diseases and disorders [5].

*Lippia javanica* (the lemon bush) (Figure 1) is an erect, multi-branched, woody shrub that grows 1-2 m tall. The 3-4 cm long leaves are hairy on sides and have dentate, lightly toothed, margins, and are rough to the feel with deeply sunken veins from above [6]. Leaves are opposite, often in whorls of up to four and have a characteristic lemon scent when crushed. Small creamy white flowers clustered together in dense, round spikes about 1 cm in diameter are produced between February and May (but can be found throughout the year). Seeds are small brown nutlets [6].

The plant has been exploited since pre-historic time by traditional herbalists for the treatment of various ailments including diabetes, coughs, asthma, colds, 'flu, chest complaints, scabies and scalp infections and malaria [6]. Clinical studies using human volunteers have also shown that Lemon Bush extract is a more potent malaria vector mosquito repellent than most available commercial formulation



**Figure 1:** *Lippia javanica* (photograph taken in July 2013, at Kijauri Nyamira County).

[6]. Shikanga et al. [7] reported the presence of substantial levels of alkaloids, flavonoids, terpenes, saponins, phenolics, anthraquinones, phlobatannins, cardiac glycosides and tannins in the leaf. One unique characteristic of the leaf is that it has bitter lemon taste. For instance, some leafy vegetables with bitter taste have been implicated in enhancing insulin production in experimental diabetic rats and have potentials for diabetic control and management [8].

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The aim of this study was to determine the *in vivo* safety effects of aqueous leaf extracts of this plant in male swiss white albino mice. The safety of the aqueous leaf extract of *Lippia javanica* was studied in normal mice that were orally and intraperitoneally administered with different doses of 450 mg/kgbw, 670 mg/kgbw and 1000 mg/kgbw daily for 28 days by recording changes in body and organ weights, hematological indices and biochemical parameters.

## Materials and Method

### Study site

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University from July 2013 to February 2015. Kenyatta University is 23 km from Nairobi off Thika Road.

### Collection and preparation of the plant materials

The plant used in this study was collected from its native habitat on the basis of ethno-botanical information. It was collected with bio-conservation aspects in mind from Kijauri village Nyamira county Kenya. Information on the identity of the plant to collect, the precise locality where it grows, what part to collect, when curative potency is at maximum and the mode of preparation was provided by a traditional medical practitioner. For this study, the part of the plant collected was the leaves. A botanical identity of the plant was authenticated by an acknowledged authority in taxonomy and a voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi.

Leaves were collected while green and dried at room temperature away from direct sunlight. The dried leaves were ground into fine powder by use of an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed, dry plastic air tight bags ready for extraction.

### Preparation of the aqueous extracts

Each one hundred grams of the powdered plant material was extracted in 1 liter distilled water at 60°C for 6 hour. The mixture was left to cool at room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers under vacuum pump. The filtrate was then freeze-dried for 72 hours. The freeze-dried powder was then weighed and stored in airtight container at -20°C until used for bioassay.

### Experimental animals

The study used male Swiss White Albino mice (3-4 weeks old) that weighed 21-25 g with a mean weight of 23 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12 hours/12 hours darkness photoperiod and fed on rodent pellets and water *ad libitum*. The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

### *In vivo* toxicity tests

The mice were randomly divided into four different groups of five mice each. Group I and II consisted of untreated control mice intraperitoneally and orally, respectively, administered daily for 28 days with 0.1 ml physiological saline. Group III and IV consisted of normal mice intraperitoneally and orally administered daily for 28 days with the extract at 1 g/kg body weight in 0.1 ml physiological saline. The same

experimental design was adapted for toxicity evaluation of aqueous leaf extract of *Lippia javanica* at 450 mg/kg body weight and 670 mg/kg body weight doses. During this period, mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behaviour and mortality. At the end of 28 days, the mice were sacrificed.

### Determination of body and organ weight

The body weight of each mouse was assessed after every seven days during the dosing period up to and including the 28<sup>th</sup> day and the day of sacrifice (day zero, 7, 14, 21, 28). On the day of sacrifice, all the animals were euthanized using chloroform as an inhalant anaesthesia and blood samples were drawn from the heart of each sacrificed mouse. The blood samples were collected in plastic test tubes and divided into two portions. One portion was used for determination of hematological parameters. The other portion was allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at -20°C for metabolite and enzyme assays. The liver, kidney, heart, lungs, spleen, intestine, brain and testis were carefully dissected out, weighed and preserved in 10% neutral buffered formalin.

### Determination of hematological parameters

Blood parameters and indices were determined using standard protocols [9]. Red blood cells count, white blood cells count, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume were determined in whole blood with EDTA anticoagulant using the Coulter Counter System (Beckman Coulter, ThermoFisher, UK).

Differential white blood cell count for neutrophils, lymphocytes, eosinophils, basophils and monocytes were determined from giemsa stained blood films using a hemocytometer [9]. Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphology, respectively.

### Determination of biochemical parameters

The biochemical parameters determined on the sera specimen using the Olympus 640 Chemistry AutoAnalyser were Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH), Creatine Kinase (CK),  $\alpha$ -Amylase ( $\alpha$ -AMYL), Total bilirubin (T-BIL), Direct bilirubin (D-BIL), Indirect bilirubin (I-BIL), Total cholesterol (TC), Blood urea nitrogen (BUN), Triacylglycerols (TG), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Glucose (GLU), Uric acid, Urea and Creatinine.

All reagents for the machine were commercially prepared to fit the required volumes and concentrations. The reagents were in specific containers referred to as reagent cartridges. The reagent cartridges were bar coded for the identification by the machine. The machine was programmed for the selected tests for each sample. The sample sectors were then placed into the autoloader assembly. A number of events that occurred simultaneously were performed automatically under the direct control of the instrument microprocessor. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Department of Laboratory Medicine, Kenyatta National Hospital.

## Qualitative phytochemical screening

A phytochemical screening of alkaloids, flavonoids, saponins, tannins, terpenoids, sterols, and free and bound anthraquinones present in *Lippia javanica* extracts was performed using standard methods [10,11].

## Data management and statistical analysis

The data collected was entered into Ms Excel spread sheets where it was organized for statistical analysis. Analysis of data was done using SAS statistical software version 9.1.3. The results were expressed as mean  $\pm$  standard deviation (SD). Evaluation of the safety was compared by testing the statistical significant difference among groups of control mice and those treated with the plant extracts at different doses using ANOVA and followed by Tukey's post hoc tests to separate the means and obtain the specific significant differences across the four treatments. The values of  $P \leq 0.05$  were considered to be significant.

## Results

### Effect of oral and intraperitoneal administration of aqueous

### leaf extracts of *Lippia javanica* on body weights, organ weights and absolute organ body weight in mice

The results in Tables 1 and 2 show that during the four week period, intraperitoneal and oral administration of *Lippia javanica* to mice for 28 days led to a significant lower rate of weekly weight gain as the dosage increased across treatments with a dose level of 1000 mg/kg body weight recording the lowest rate of weekly weight gain. In spite of this, the plant extracts generally showed a steady weight gain throughout the four week period in both routes. The intraperitoneal administration of aqueous leaf extract showed a significant lower weekly weight gain for animals compared to the oral administration. However, during the third and the fourth week the extract showed no significant difference in the rate of weekly weight gain of laboratory mice in both routes at  $p \leq 0.05$ .

Tables 3 and 4 show that across the four treatments, the intraperitoneal and oral administration of *Lippia javanica* to mice for 28 days did not significantly change the organ weights of brain, liver, kidney, spleen, lungs and heart at  $p \leq 0.05$ . However, the administration of aqueous leaf extracts

Treatment (mg/kgbw)	Weekly weight of mice (g)					$\Delta$ Weight/Week (g/Week)
	0	1	2	3	4	
Control	20.80 $\pm$ 0.84 <sup>b</sup>	21.52 $\pm$ 0.79 <sup>b</sup>	22.04 $\pm$ 0.74 <sup>b</sup>	23.12 $\pm$ 0.53 <sup>a</sup>	23.64 $\pm$ 0.40 <sup>a</sup>	0.71 $\pm$ 0.12 <sup>a</sup>
450	22.40 $\pm$ 1.14 <sup>a</sup>	22.96 $\pm$ 1.74 <sup>a</sup>	22.52 $\pm$ 1.77 <sup>a</sup>	23.92 $\pm$ 1.76 <sup>a</sup>	24.62 $\pm$ 0.84 <sup>a</sup>	0.56 $\pm$ 0.15 <sup>a</sup>
670	22.41 $\pm$ 1.82 <sup>a</sup>	23.00 $\pm$ 1.92 <sup>a</sup>	23.92 $\pm$ 1.62 <sup>a</sup>	24.06 $\pm$ 1.62 <sup>a</sup>	24.62 $\pm$ 1.79 <sup>a</sup>	0.55 $\pm$ 0.76 <sup>a</sup>
1000	21.80 $\pm$ 1.79 <sup>a</sup>	22.42 $\pm$ 1.10 <sup>a</sup>	23.44 $\pm$ 0.98 <sup>a</sup>	23.40 $\pm$ 0.93 <sup>a</sup>	23.92 $\pm$ 1.75 <sup>a</sup>	0.53 $\pm$ 0.16 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analyzed by ANOVA followed by Tukey's post hoc test. Key-  $\Delta$ : represents change

**Table 1:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on body weight of laboratory mice.

Treatment (mg/kgbw)	Weekly weight of mice (g)					$\Delta$ Weight/Week (g/Week)
	0	1	2	3	4	
Control	20.60 $\pm$ 0.89 <sup>b</sup>	22.70 $\pm$ 0.62 <sup>a</sup>	23.74 $\pm$ 0.65 <sup>a</sup>	24.58 $\pm$ 0.77 <sup>a</sup>	25.32 $\pm$ 0.76 <sup>a</sup>	1.18 $\pm$ 0.20 <sup>a</sup>
450	22.20 $\pm$ 1.92 <sup>a</sup>	22.84 $\pm$ 1.83 <sup>a</sup>	23.74 $\pm$ 1.62 <sup>a</sup>	24.06 $\pm$ 1.62 <sup>a</sup>	25.62 $\pm$ 1.79 <sup>a</sup>	0.86 $\pm$ 0.42 <sup>ba</sup>
670	22.00 $\pm$ 1.22 <sup>a</sup>	22.62 $\pm$ 1.19 <sup>a</sup>	23.52 $\pm$ 1.13 <sup>a</sup>	24.58 $\pm$ 0.77 <sup>a</sup>	24.30 $\pm$ 1.19 <sup>a</sup>	0.58 $\pm$ 0.06 <sup>b</sup>
1000	21.40 $\pm$ 1.67 <sup>a</sup>	22.50 $\pm$ 1.54 <sup>a</sup>	23.14 $\pm$ 0.65 <sup>a</sup>	23.72 $\pm$ 1.13 <sup>a</sup>	23.64 $\pm$ 1.45 <sup>a</sup>	0.56 $\pm$ 0.08 <sup>b</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analyzed by ANOVA followed by Tukey's post hoc test. Key-  $\Delta$ : represents change

**Table 2:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on body weight of laboratory mice.

Treatment (mg/kgbw)	Organ weights of laboratory mice						
	Brain	Liver	Kidney	Spleen	Lungs	Testes	Heart
Control	0.51 $\pm$ 0.03 <sup>a</sup>	2.21 $\pm$ 0.30 <sup>a</sup>	0.40 $\pm$ 0.11 <sup>a</sup>	0.23 $\pm$ 0.05 <sup>b</sup>	0.34 $\pm$ 0.10 <sup>a</sup>	0.07 $\pm$ 0.02 <sup>c</sup>	0.12 $\pm$ 0.01 <sup>a</sup>
450	0.53 $\pm$ 0.05 <sup>a</sup>	2.27 $\pm$ 0.28 <sup>a</sup>	0.44 $\pm$ 0.07 <sup>a</sup>	0.31 $\pm$ 0.06 <sup>a</sup>	0.40 $\pm$ 0.08 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>cb</sup>	0.12 $\pm$ 0.04 <sup>a</sup>
670	0.54 $\pm$ 0.04 <sup>a</sup>	2.33 $\pm$ 0.22 <sup>a</sup>	0.45 $\pm$ 0.08 <sup>a</sup>	0.32 $\pm$ 0.04 <sup>a</sup>	0.45 $\pm$ 0.05 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.09 <sup>a</sup>
1000	0.54 $\pm$ 0.03 <sup>a</sup>	1.12 $\pm$ 0.20 <sup>b</sup>	0.46 $\pm$ 0.08 <sup>a</sup>	0.33 $\pm$ 0.02 <sup>a</sup>	0.46 $\pm$ 0.08 <sup>a</sup>	0.24 $\pm$ 0.02 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analyzed by ANOVA followed by Tukey's post hoc test.

**Table 3:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on organ weights of laboratory mice.

Treatment (mg/kgbw)	Organ weights of laboratory mice						
	Brain	Liver	Kidney	Spleen	Lungs	Testes	Heart
Control	0.52 $\pm$ 0.05 <sup>a</sup>	1.40 $\pm$ 0.41 <sup>a</sup>	0.33 $\pm$ 0.05 <sup>a</sup>	0.19 $\pm$ 0.03 <sup>a</sup>	0.33 $\pm$ 0.04 <sup>a</sup>	0.05 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>a</sup>
450	0.52 $\pm$ 0.03 <sup>a</sup>	1.53 $\pm$ 0.24 <sup>a</sup>	0.32 $\pm$ 0.04 <sup>a</sup>	0.20 $\pm$ 0.04 <sup>a</sup>	0.34 $\pm$ 0.06 <sup>a</sup>	0.10 $\pm$ 0.02 <sup>a</sup>	0.11 $\pm$ 0.02 <sup>a</sup>
670	0.52 $\pm$ 0.07 <sup>a</sup>	1.54 $\pm$ 0.20 <sup>a</sup>	0.32 $\pm$ 0.04 <sup>a</sup>	0.22 $\pm$ 0.02 <sup>a</sup>	0.34 $\pm$ 0.04 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>
1000	0.53 $\pm$ 0.02 <sup>a</sup>	1.65 $\pm$ 0.20 <sup>a</sup>	0.34 $\pm$ 0.04 <sup>a</sup>	0.24 $\pm$ 0.05 <sup>a</sup>	0.35 $\pm$ 0.02 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analyzed by ANOVA followed by Tukey's post hoc test.

**Table 4:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on organ weights of laboratory mice.

of *Lippia javanica* at a dosage level of 1000 mg/kg body weight in both routes showed a significant ( $p \leq 0.05$ ) increase in the weight of testes and spleen (orally) relative to those of the control mice. The intraperitoneal route depicted higher organ weight values than the oral route.

Tables 5 and 6 shows that the intraperitoneal and oral administration of the extracts did not significantly change ( $p \leq 0.05$ ) the percent organ to body weight of brain, kidney, lungs and the heart in the four treatments. Moreover, in both routes the extracts indicated a dose dependent significant increase ( $p \leq 0.05$ ) in the percent organ to body weight that picked at 450 mg/kg body weight of the liver, spleen and testes relative to the control.

### Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* on some end point hematological parameters in mice

Tables 7 and 8 indicate that intraperitoneal and oral administration of aqueous leaf extracts of *Lippia javanica* to mice for 28 days significantly increased ( $p \leq 0.05$ ) RBC, Hb, MCV and PCV levels and

decreased MCH and MCHC levels in a dose dependent manner that picked at 670 mg/kg body weight across the four treatments. The oral and intraperitoneal administration of 1000 mg/kg of aqueous leaf extracts recorded highest values of RBC, Hb, MCV and PCV and lowest values of MCH and MCHC relative to that of the control mice.

Tables 9 and 10 show that in both routes the extracts caused a significant dose dependent increase in WBC, LYM, MON, NEU, EOS, BAS, PLT and MPV levels across the four treatments at  $p \leq 0.05$ . However, oral administration of 1000 mg/kg body weight of the extracts in mice indicated a significant difference ( $p \leq 0.05$ ) in eosinophiles and basophiles from other doses. A treatment dosage of 1g/kg of aqueous leaf extracts recorded highest values for WBC, LYM, MON, NEU, PLT and MPV relative to that of the control mice in both routes.

### Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* on some end point biochemical parameters in mice

Tables 11 and 12 show the effect of intraperitoneal and oral

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	Liver	Kidney	Spleen	Lungs	Testes	Heart
Control	2.06 ± 0.22 <sup>a</sup>	4.84 ± 0.70 <sup>b</sup>	1.71 ± 0.52 <sup>a</sup>	0.99 ± 0.27 <sup>b</sup>	1.49 ± 0.44 <sup>a</sup>	0.93 ± 0.12 <sup>b</sup>	0.49 ± 0.04 <sup>a</sup>
450	2.22 ± 0.10 <sup>a</sup>	8.65 ± 1.30 <sup>a</sup>	1.78 ± 0.35 <sup>a</sup>	1.21 ± 0.24 <sup>ba</sup>	1.56 ± 0.31 <sup>a</sup>	1.33 ± 0.11 <sup>a</sup>	0.50 ± 0.18 <sup>a</sup>
670	2.28 ± 0.27 <sup>a</sup>	9.39 ± 1.30 <sup>a</sup>	1.84 ± 0.34 <sup>a</sup>	1.34 ± 0.22 <sup>ba</sup>	1.87 ± 0.22 <sup>a</sup>	1.43 ± 0.07 <sup>a</sup>	0.51 ± 0.07 <sup>a</sup>
1000	2.26 ± 0.26 <sup>a</sup>	9.81 ± 0.67 <sup>a</sup>	1.94 ± 0.34 <sup>a</sup>	1.38 ± 0.08 <sup>a</sup>	1.94 ± 0.31 <sup>a</sup>	1.57 ± 0.33 <sup>a</sup>	0.59 ± 0.10 <sup>a</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test.

**Table 5:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on relative organ weights of laboratory mice.

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	Liver	Kidney	Spleen	Lungs	Testes	Heart
Control	2.03 ± 0.13 <sup>a</sup>	5.95 ± 1.60 <sup>a</sup>	1.31 ± 0.18 <sup>a</sup>	0.76 ± 0.11 <sup>b</sup>	1.36 ± 0.20 <sup>a</sup>	0.49 ± 0.07 <sup>b</sup>	0.45 ± 0.06 <sup>a</sup>
450	2.12 ± 0.12 <sup>a</sup>	6.13 ± 1.09 <sup>a</sup>	1.31 ± 0.18 <sup>a</sup>	0.82 ± 0.15 <sup>ba</sup>	1.38 ± 0.25 <sup>a</sup>	0.88 ± 0.11 <sup>a</sup>	0.45 ± 0.09 <sup>a</sup>
670	2.19 ± 0.09 <sup>a</sup>	6.26 ± 0.99 <sup>a</sup>	1.35 ± 0.22 <sup>a</sup>	0.92 ± 0.06 <sup>ba</sup>	1.39 ± 0.08 <sup>a</sup>	0.95 ± 0.09 <sup>a</sup>	0.49 ± 0.03 <sup>a</sup>
1000	2.21 ± 0.35 <sup>a</sup>	6.85 ± 0.94 <sup>a</sup>	1.43 ± 0.19 <sup>a</sup>	1.02 ± 0.20 <sup>a</sup>	1.47 ± 0.10 <sup>a</sup>	1.19 ± 0.35 <sup>a</sup>	0.49 ± 0.10 <sup>a</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test.

**Table 6:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on relative organ weights of laboratory mice.

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC ( $\times 10^6/\mu\text{L}$ )	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	9.28 ± 0.9 <sup>c</sup>	7.96 ± 0.78 <sup>c</sup>	39.22 ± 3.12 <sup>b</sup>	12.40 ± 3.30 <sup>a</sup>	35.22 ± 2.04 <sup>a</sup>	27.22 ± 2.38 <sup>c</sup>
450	11.44 ± 1.43 <sup>cb</sup>	13.84 ± 2.38 <sup>b</sup>	42.40 ± 1.44 <sup>b</sup>	10.27 ± 2.20 <sup>a</sup>	32.08 ± 1.49 <sup>a</sup>	39.84 ± 2.90 <sup>b</sup>
670	13.04 ± 1.58 <sup>b</sup>	13.26 ± 2.48 <sup>b</sup>	43.20 ± 3.29 <sup>b</sup>	8.96 ± 0.87 <sup>a</sup>	25.08 ± 3.00 <sup>b</sup>	41.82 ± 2.59 <sup>ba</sup>
1000	19.50 ± 0.68 <sup>a</sup>	17.44 ± 1.25 <sup>a</sup>	49.28 ± 3.12 <sup>a</sup>	8.65 ± 1.26 <sup>a</sup>	20.46 ± 3.38 <sup>b</sup>	44.84 ± 1.92 <sup>a</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. RBC=Red Blood Cell Count; Hb=Hemoglobin; PCV=Packed Red Cell Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; MCV=Mean Corpuscular Volume.

**Table 7:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on erythrocytes and related parameters in mice.

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC ( $\times 10^6/\mu\text{L}$ )	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	6.40 ± 1.32 <sup>c</sup>	8.90 ± 0.60 <sup>b</sup>	37.20 ± 2.06 <sup>c</sup>	14.45 ± 3.54 <sup>a</sup>	34.24 ± 2.06 <sup>a</sup>	19.82 ± 1.94 <sup>c</sup>
450	7.76 ± 0.50 <sup>c</sup>	9.98 ± 0.25 <sup>b</sup>	40.28 ± 3.15 <sup>bc</sup>	12.90 ± 0.81 <sup>ba</sup>	32.70 ± 1.79 <sup>a</sup>	34.26 ± 1.64 <sup>b</sup>
670	10.00 ± 1.04 <sup>b</sup>	10.66 ± 1.11 <sup>b</sup>	42.12 ± 1.46 <sup>b</sup>	10.73 ± 1.43 <sup>bc</sup>	27.92 ± 3.02 <sup>b</sup>	37.02 ± 1.39 <sup>b</sup>
1000	19.32 ± 0.61 <sup>a</sup>	16.88 ± 1.49 <sup>a</sup>	48.94 ± 3.15 <sup>a</sup>	8.76 ± 0.96 <sup>c</sup>	21.72 ± 2.79 <sup>c</sup>	44.28 ± 2.45 <sup>a</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. RBC=Red Blood Cell Count; Hb=Hemoglobin; PCV=Packed Red Cell Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; MCV=Mean Corpuscular Volume.

**Table 8:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on erythrocytes and related parameters in mice.

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hematological indices							
	WBC ( $\times 10^3/\mu\text{L}$ )	LYM ( $\times 10^3/\mu\text{L}$ )	MON ( $\times 10^3/\mu\text{L}$ )	NEU ( $\times 10^3/\mu\text{L}$ )	EOS ( $\times 10^3/\mu\text{L}$ )	BAS ( $\times 10^3/\mu\text{L}$ )	PLT ( $\times 10^3/\mu\text{L}$ )	MPV (fL)
Control	8.99 $\pm$ 0.24 <sup>c</sup>	5.34 $\pm$ 0.18 <sup>b</sup>	0.50 $\pm$ 0.20 <sup>b</sup>	2.76 $\pm$ 0.21 <sup>c</sup>	0.36 $\pm$ 0.09 <sup>c</sup>	0.03 $\pm$ 0.02 <sup>b</sup>	209.00 $\pm$ 14.37 <sup>c</sup>	9.26 $\pm$ 0.11 <sup>b</sup>
450	11.04 $\pm$ 1.19 <sup>b</sup>	5.98 $\pm$ 1.08 <sup>ba</sup>	0.76 $\pm$ 0.18 <sup>ba</sup>	3.78 $\pm$ 0.30 <sup>b</sup>	0.46 $\pm$ 0.11 <sup>c</sup>	0.06 $\pm$ 0.02 <sup>b</sup>	234.00 $\pm$ 23.58 <sup>c</sup>	9.54 $\pm$ 0.18 <sup>b</sup>
670	12.08 $\pm$ 0.23 <sup>b</sup>	6.16 $\pm$ 0.32 <sup>ba</sup>	0.72 $\pm$ 0.12 <sup>ba</sup>	4.18 $\pm$ 0.36 <sup>b</sup>	0.90 $\pm$ 0.14 <sup>b</sup>	0.12 $\pm$ 0.03 <sup>b</sup>	279.20 $\pm$ 9.68 <sup>b</sup>	9.62 $\pm$ 0.08 <sup>b</sup>
1000	15.86 $\pm$ 0.16 <sup>a</sup>	6.84 $\pm$ 0.18 <sup>a</sup>	0.88 $\pm$ 0.08 <sup>a</sup>	5.88 $\pm$ 0.43 <sup>a</sup>	1.18 $\pm$ 0.11 <sup>a</sup>	1.01 $\pm$ 0.47 <sup>a</sup>	340.40 $\pm$ 23.67 <sup>a</sup>	10.52 $\pm$ 0.58 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. WBC=White Blood Cell Count; NEU=Neutrophils; EOS=Eosinophils; BAS=Basophils; MON=Monocytes; LYM=Lymphocytes; PLT=Platelets; MPV=Mean Platelet Volume

**Table 9:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice.

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ( $\times 10^3/\mu\text{L}$ )	LYM ( $\times 10^3/\mu\text{L}$ )	MON ( $\times 10^3/\mu\text{L}$ )	NEU ( $\times 10^3/\mu\text{L}$ )	EOS ( $\times 10^3/\mu\text{L}$ )	BAS ( $\times 10^3/\mu\text{L}$ )	PLT ( $\times 10^3/\mu\text{L}$ )	MPV (fL)
Control	8.78 $\pm$ 0.54 <sup>c</sup>	5.20 $\pm$ 0.49 <sup>b</sup>	0.40 $\pm$ 0.12 <sup>b</sup>	2.76 $\pm$ 0.29 <sup>c</sup>	0.40 $\pm$ 0.15 <sup>b</sup>	0.02 $\pm$ 0.01 <sup>b</sup>	187.24 $\pm$ 4.93 <sup>c</sup>	8.66 $\pm$ 0.33 <sup>b</sup>
450	10.22 $\pm$ 0.13 <sup>b</sup>	5.68 $\pm$ 0.15 <sup>ba</sup>	0.48 $\pm$ 0.15 <sup>b</sup>	3.78 $\pm$ 0.26 <sup>b</sup>	0.24 $\pm$ 0.13 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>b</sup>	240.00 $\pm$ 12.75 <sup>b</sup>	9.47 $\pm$ 0.15 <sup>a</sup>
670	11.04 $\pm$ 1.19 <sup>b</sup>	5.98 $\pm$ 1.08 <sup>ba</sup>	0.76 $\pm$ 0.18 <sup>a</sup>	3.78 $\pm$ 0.30 <sup>b</sup>	0.46 $\pm$ 0.11 <sup>b</sup>	0.06 $\pm$ 0.02 <sup>b</sup>	234.00 $\pm$ 23.58 <sup>b</sup>	9.54 $\pm$ 0.18 <sup>a</sup>
1000	14.82 $\pm$ 0.44 <sup>a</sup>	6.52 $\pm$ 0.19 <sup>a</sup>	0.76 $\pm$ 0.11 <sup>a</sup>	5.72 $\pm$ 0.41 <sup>a</sup>	1.06 $\pm$ 0.11 <sup>a</sup>	0.76 $\pm$ 0.62 <sup>a</sup>	319.20 $\pm$ 23.90 <sup>a</sup>	10.16 $\pm$ 0.69 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. WBC=White Blood Cell Count; NEU=Neutrophils; EOS=Eosinophils; BAS=Basophils; MON=Monocytes; LYM=Lymphocytes; PLT=Platelets; MPV=Mean Platelet Volume

**Table 10:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice.

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	$\alpha$ -AMYL (U/L)	AST/ALT
Control	27.60 $\pm$ 1.14 <sup>c</sup>	215.20 $\pm$ 9.52 <sup>b</sup>	1.93 $\pm$ 0.27 <sup>c</sup>	0.18 $\pm$ 0.05 <sup>b</sup>	638.60 $\pm$ 131.49 <sup>b</sup>	228.60 $\pm$ 20.80 <sup>b</sup>	615.20 $\pm$ 85.10 <sup>c</sup>	7.81 $\pm$ 0.48 <sup>a</sup>
450	30.40 $\pm$ 2.41 <sup>c</sup>	218.60 $\pm$ 8.85 <sup>b</sup>	2.08 $\pm$ 0.24 <sup>c</sup>	0.18 $\pm$ 0.08 <sup>b</sup>	649.60 $\pm$ 131.87 <sup>b</sup>	240.20 $\pm$ 19.14 <sup>b</sup>	624.40 $\pm$ 88.97 <sup>c</sup>	7.23 $\pm$ 0.61 <sup>a</sup>
670	35.60 $\pm$ 2.70 <sup>b</sup>	244.60 $\pm$ 53.91 <sup>b</sup>	2.88 $\pm$ 0.40 <sup>b</sup>	0.42 $\pm$ 0.26 <sup>b</sup>	828.80 $\pm$ 61.98 <sup>a</sup>	292.20 $\pm$ 9.34 <sup>b</sup>	922.60 $\pm$ 50.76 <sup>b</sup>	6.92 $\pm$ 1.65 <sup>a</sup>
1000	57.20 $\pm$ 2.59 <sup>a</sup>	354.40 $\pm$ 28.27 <sup>a</sup>	4.84 $\pm$ 0.25 <sup>a</sup>	1.86 $\pm$ 0.05 <sup>a</sup>	1282.40 $\pm$ 195.07 <sup>a</sup>	1310.60 $\pm$ 151.94 <sup>a</sup>	1358.60 $\pm$ 168.76 <sup>a</sup>	6.20 $\pm$ 0.55 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. ALT=Alanine Transaminase; AST=Aspartate Transaminase; ALP=Alkaline Phosphatase; GGT= $\gamma$ -Glutamyl Transferase; LDH=Lactate Dehydrogenase; CK=Creatine Kinase;  $\alpha$ -AMYL= $\alpha$ -Amylase; AST/ALT=the ratio of the activity of aspartate transaminase to alanine transaminase

**Table 11:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on enzyme markers of liver and kidney functions in mice.

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	$\alpha$ -AMYL (U/L)	AST/ALT
Control	32.20 $\pm$ 1.64 <sup>c</sup>	236.40 $\pm$ 5.55 <sup>b</sup>	2.44 $\pm$ 0.13 <sup>b</sup>	0.24 $\pm$ 0.17 <sup>cb</sup>	782.60 $\pm$ 63.30 <sup>c</sup>	249.20 $\pm$ 14.52 <sup>c</sup>	818.60 $\pm$ 50.19 <sup>c</sup>	7.35 $\pm$ 0.32 <sup>a</sup>
450	33.80 $\pm$ 1.48 <sup>cb</sup>	240.00 $\pm$ 5.96 <sup>b</sup>	2.48 $\pm$ 0.15 <sup>b</sup>	0.44 $\pm$ 0.40 <sup>c</sup>	807.40 $\pm$ 57.33 <sup>c</sup>	254.40 $\pm$ 15.57 <sup>c</sup>	834.20 $\pm$ 53.58 <sup>c</sup>	7.11 $\pm$ 0.27 <sup>a</sup>
670	38.60 $\pm$ 3.36 <sup>b</sup>	265.20 $\pm$ 49.07 <sup>b</sup>	3.14 $\pm$ 0.26 <sup>b</sup>	0.78 $\pm$ 0.16 <sup>b</sup>	954.00 $\pm$ 49.71 <sup>b</sup>	304.60 $\pm$ 10.67 <sup>b</sup>	959.20 $\pm$ 46.21 <sup>b</sup>	6.90 $\pm$ 1.25 <sup>a</sup>
1000	60.80 $\pm$ 4.32 <sup>a</sup>	364.60 $\pm$ 16.67 <sup>a</sup>	6.76 $\pm$ 0.98 <sup>a</sup>	2.30 $\pm$ 0.16 <sup>a</sup>	1801.40 $\pm$ 95.89 <sup>a</sup>	1609.60 $\pm$ 35.54 <sup>a</sup>	1680.60 $\pm$ 85.20 <sup>a</sup>	6.03 $\pm$ 0.63 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. ALT=alanine transaminase; AST=aspartate transaminase; ALP=alkaline phosphatase; GGT= $\gamma$ -glutamyl transferase; LDH=lactate dehydrogenase; CK=creatin kinase;  $\alpha$ -AMYL= $\alpha$ -amylase; AST/ALT=the ratio of the activity of aspartate transaminase to alanine transaminase

**Table 12:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on enzyme markers of liver and kidney functions in mice.

administration of the aqueous leaf extract on enzyme markers of liver and kidney functions in mice. Results indicates the in both routes the extracts caused a significant ( $p \leq 0.05$ ) dose dependent increase in ALT, AST, ALP, GGT, LDH, CK and  $\alpha$ -AMYL enzymes in the four treatments. In addition, the control treatment recorded the lowest measurements for most enzymes. The intraperitoneal and oral administration of aqueous leaf extracts did not significantly ( $p \leq 0.05$ ) change AST/ALT levels across treatments.

Tables 13 and 14 show that intraperitoneally and orally administered extracts in mice for 28 days showed a dose dependent significant ( $p \leq 0.05$ ) increase in CREAT, UREA, BUN, and UA blood analytes levels across the four treatments. However, the control treatment recorded lowest blood analytes values compared to plant extracts along the measured biochemical metabolites.

Tables 15 and 16 show that in both routes the extracts caused a dose dependent significant decrease ( $p \leq 0.05$ ) in T-BIL, D-BIL, I-BIL, TG, TC, HDL-C and LDL-C levels across the four treatments. The control treatment recorded the highest values for most lipid profiles. In addition, the intraperitoneal and oral administration of the aqueous leaf extracts demonstrated a significant ( $p \leq 0.05$ ) decline in blood glucose levels across treatments in a dose dependent manner with the intraperitoneal route indicating a greater decrease of glucose levels compared to the oral route.

### Qualitative analysis of the phytochemical composition of aqueous leaf extracts of *Lippia javanica*

Table 17 shows qualitative phytochemical composition of aqueous leaf extracts of *Lippia javanica*. Results show that *L. javanica* indicated the presence of alkaloids, sterols, terpenoids, flavonoids, tannins and saponins.

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	10.60 ± 0.89 <sup>b</sup>	3.18 ± 0.22 <sup>c</sup>	1.68 ± 0.52 <sup>b</sup>	56.40 ± 16.09 <sup>b</sup>
450	10.80 ± 1.30 <sup>b</sup>	3.24 ± 0.25 <sup>cb</sup>	1.74 ± 0.48 <sup>b</sup>	58.20 ± 15.56 <sup>b</sup>
670	12.40 ± 0.89 <sup>b</sup>	3.70 ± 0.28 <sup>b</sup>	1.82 ± 0.22 <sup>b</sup>	84.60 ± 25.77 <sup>b</sup>
1000	20.20 ± 1.64 <sup>a</sup>	6.84 ± 0.34 <sup>a</sup>	3.38 ± 0.08 <sup>a</sup>	187.80 ± 9.52 <sup>a</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. CREAT=Creatinine; UREA=Urea; BUN=Blood Urea Nitrogen; UA=Uric Acid

**Table 13:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on enzyme markers of liver and kidney functions in mice.

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	12.60 ± 1.67 <sup>b</sup>	3.22 ± 0.13 <sup>c</sup>	2.28 ± 0.16 <sup>b</sup>	70.00 ± 7.78 <sup>c</sup>
450	13.20 ± 2.28 <sup>b</sup>	3.32 ± 0.13 <sup>c</sup>	2.34 ± 0.18 <sup>b</sup>	74.60 ± 7.77 <sup>c</sup>
670	15.40 ± 1.14 <sup>b</sup>	4.06 ± 0.24 <sup>b</sup>	2.12 ± 0.08 <sup>b</sup>	107.60 ± 12.76 <sup>b</sup>
1000	21.40 ± 2.30 <sup>a</sup>	8.00 ± 0.27 <sup>a</sup>	3.70 ± 0.19 <sup>a</sup>	207.20 ± 9.99 <sup>a</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. CREAT=Creatinine; UREA=Urea; BUN=Blood Urea Nitrogen; UA=Uric Acid

**Table 14:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on non-enzyme markers of liver and kidney functions in mice.

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	11.38 ± 0.24 <sup>a</sup>	5.88 ± 0.22 <sup>a</sup>	5.50 ± 0.20 <sup>a</sup>	0.68 ± 0.08 <sup>a</sup>	1.56 ± 0.14 <sup>ba</sup>	1.38 ± 0.15 <sup>a</sup>	0.18 ± 0.02 <sup>a</sup>	5.50 ± 0.10 <sup>a</sup>
450	6.66 ± 0.23 <sup>b</sup>	3.28 ± 0.13 <sup>b</sup>	3.38 ± 0.16 <sup>b</sup>	0.47 ± 0.07 <sup>b</sup>	1.72 ± 0.15 <sup>a</sup>	1.56 ± 0.15 <sup>a</sup>	0.16 ± 0.02 <sup>a</sup>	4.08 ± 0.26 <sup>b</sup>
670	5.56 ± 0.42 <sup>c</sup>	2.92 ± 0.16 <sup>c</sup>	2.64 ± 0.34 <sup>c</sup>	0.30 ± 0.07 <sup>c</sup>	1.54 ± 0.08 <sup>ba</sup>	1.42 ± 0.08 <sup>a</sup>	0.12 ± 0.11 <sup>a</sup>	3.42 ± 0.13 <sup>c</sup>
1000	5.46 ± 0.42 <sup>c</sup>	2.80 ± 0.19 <sup>c</sup>	2.54 ± 0.34 <sup>c</sup>	0.24 ± 0.05 <sup>c</sup>	1.45 ± 0.11 <sup>b</sup>	1.34 ± 0.11 <sup>a</sup>	0.11 ± 0.01 <sup>a</sup>	3.20 ± 0.16 <sup>c</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. T-BIL=Total Bilirubin; D-BIL=Direct Bilirubin; I-BIL=Indirect Bilirubin; TG=Triacylglycerols; TC=Total Cholesterol; HDL-C=High Density Lipoprotein Cholesterol; LDL-C=Low Density Lipoprotein Cholesterol; GLU=Glucose

**Table 15:** Effect of intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* for 28 days on lipid profiles and glucose levels in mice.

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	12.24 ± 0.35 <sup>a</sup>	6.18 ± 0.22 <sup>a</sup>	6.06 ± 0.21 <sup>a</sup>	0.78 ± 0.08 <sup>a</sup>	1.82 ± 0.17 <sup>a</sup>	1.60 ± 0.16 <sup>a</sup>	0.22 ± 0.02 <sup>a</sup>	6.08 ± 0.26 <sup>a</sup>
450	7.04 ± 0.30 <sup>b</sup>	3.54 ± 0.11 <sup>b</sup>	3.50 ± 0.23 <sup>b</sup>	0.53 ± 0.03 <sup>b</sup>	1.86 ± 0.12 <sup>a</sup>	1.64 ± 0.11 <sup>a</sup>	0.22 ± 0.01 <sup>a</sup>	4.54 ± 0.11 <sup>b</sup>
670	6.12 ± 0.11 <sup>c</sup>	3.26 ± 0.11 <sup>c</sup>	2.86 ± 0.19 <sup>c</sup>	0.44 ± 0.10 <sup>cb</sup>	1.70 ± 0.07 <sup>ba</sup>	1.58 ± 0.08 <sup>a</sup>	0.12 ± 0.03 <sup>b</sup>	3.92 ± 0.19 <sup>c</sup>
1000	6.04 ± 0.11 <sup>c</sup>	3.24 ± 0.11 <sup>c</sup>	2.72 ± 0.22 <sup>c</sup>	0.33 ± 0.08 <sup>c</sup>	1.60 ± 0.08 <sup>b</sup>	1.48 ± 0.08 <sup>a</sup>	0.12 ± 0.02 <sup>b</sup>	3.82 ± 0.19 <sup>c</sup>

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at  $p \leq 0.05$ ; analysed by ANOVA followed by Tukey's post hoc test. T-BIL=Total Bilirubin; D-BIL=Direct Bilirubin; I-BIL=Indirect Bilirubin; TG=Triacylglycerols; TC=Total Cholesterol; HDL-C=High Density Lipoprotein Cholesterol; LDL-C=Low Density Lipoprotein Cholesterol; GLU=Glucose

**Table 16:** Effect of oral administration of aqueous leaf extracts of *Lippia javanica* for 28 days on lipid profiles and glucose levels in mice.

Phytochemicals	<i>Lippia javanica</i>
Alkaloids	+
Sterols	+
Terpenoids	+
Saponins	+
Tannins	+
Flavonoids	+

Key: Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

**Table 17:** Qualitative phytochemical screening of aqueous leaf extract of *Lippia javanica*.

## Discussion

That the lower rate of weekly weight gain significantly ( $p \leq 0.05$ ) decreased in a dose dependent manner relative to the control following the oral and intraperitoneal administration of *L. javanica* for 28 days in mice suggests that the extracts may have contained the phytonutrients that either do not promote feed intake or interfere with its metabolism

as well as enhances proteolysis of skeletal muscles retarding growth [5]. Phytochemicals are known to affect body weight through manipulating the energy expenditure, appetite suppression, satiety enhancement and fat-glucose absorption blocking [12]. The extracts may also have promoted weight reduction through suppression of fatty acid synthesis, increased lipid oxidation and reduced food intake [13].

That the organ weights and relative organ body weights of the liver, spleen and testes significantly ( $p \leq 0.05$ ) increased across the four treatments might be due to the fact that plant extracts promoted higher metabolic activity and facilitated protein synthesis in these organs [14]. This maybe either due to presence of phytochemical constituents or mineral elements present in the plant extracts [5]. However, the revitalization in the body organ weights and absolute organ weights may also indicate that poor oxygenation of rapidly respiring tissues could have caused inflammation to these organs resulting in increased weights [15].

The differential activity based on route of administration may be,

at least in part, due to the much higher bioavailability of the extract in systemic circulation when given intraperitoneally. In oral administration the active principles might have taken time to be transported across the intestinal wall intact or were hydrolyzed or broken down by the digestive enzymes along the alimentary canal thus making the extract's constituents biologically unavailable or reducing their activity [16].

A daily administration of the aqueous leaf extracts orally and intraperitoneally for 28 days in mice caused a significant ( $p \leq 0.05$ ) increase in RBC, Hb, PCV and MCV and a significant decrease in MCH and MCHC levels across the four treatments. The hematological parameters RBC, PCV, and Hb are associated with the total population of the red cells; MCV reflects the size of red blood cells while MCH and MCHC are used mathematically to define the concentration of hemoglobin and to suggest the restoration of oxygen carrying capacity of the blood [17]. These results therefore, are an indicative of secondary polycythemia caused by the excess production of immature reticulocytes and macrocytic hypochromic anemia caused by production of immature red blood cells that are large in size with reduced hemoglobin concentration from the bone marrow resulting in decreased blood flow and poor tissue oxygenation (tissue hypoxia) [18].

The immature reticulocytes could be as a result of the extract constituents that interfere with folate or vitamin B<sub>12</sub> absorption or by complexing with and making either of the vitamins or both biologically unavailable resulting in either folate or vitamin B<sub>12</sub> deficiency [19].

The elevated erythrocytes levels might have been due to myeloproliferative syndrome or a reaction to chronically low oxygen levels in body tissues [20,21]. It could also indicate that the plant extracts facilitates the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells [22].

The increased levels of free Hb in blood (hemoglobinemia) might be as a result of massive hemolysis of the red blood cells following inflammation or an antibody attack on the red blood cells as evidenced by high differential leucocyte counts in blood [23]. As indicated in the study, the elevated levels of hematocrit may be as a result of hyperosmotic conditions due to high dosage levels of extracts or elevated levels of WBCs [24].

Results from this study showed that in both routes the extracts caused an accelerated production of white blood cells across treatments indicating an enhanced immunity to mice [25,26]. This therefore indicates that the extracts might have had a good stimulation of hematopoietic regulatory elements by the macrophages and stromal cells in the bone marrow which regulate the proliferation, differentiation and maturation of committed stem cells necessary for the production of white blood cells [27].

However, the dose dependent significant increase in polymorpho- and mononuclear leukocytes levels might be a sign of body's response protective mechanisms following an inflammatory or infectious states, malnutrition, hypoxic, atrophic and necrotic states, ulcerative colitis and lymphomas or lymphocytic leukemia [28].

The rise in platelets count seen may suggest that the extracts have a stimulatory effect on thrombopoietin [29]. They, therefore, can be used in management of haemophilia. The cause of increased platelet count (thrombocytosis) and MVP in mice may be associated with inflammation and presence of a blood disease such as abnormal bleeding induced by toxic phytochemical substances such as tannins in the plant extracts [30].

The reduced levels of MHC and MCHC across the four treatments

reflect diminished oxygenation of tissues resulting in tissue hypoxia. Tissue hypoxia therefore, causes most body organs including brain, liver, lungs, heart, kidney, testis and intestines to initially enlarge and as the cells continue swelling, they rupture resulting in a reduced organ size (so-called organ atrophy) [31]. Moreover, this could also account for increased levels of measured biochemical profiles such as alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, GGT, alpha amylase, lactate dehydrogenase, aspartate aminotransferase, creatine kinase and uric acid following oral and intraperitoneal administration of the plant extracts in mice.

The liver and kidney release AST, ALT, ALP, LDH, GGT and  $\alpha$ -AMY and elevation in their plasma concentrations indicate liver and kidney damage [32]. At the doses used, the plant extracts therefore did not have hepato- and nephron- protective effects [33]. Results indicating a decline in AST/ALT levels across treatments are more marked for chronic hepatitis, myocardial necrosis, hepatic metastases and liver congestion [33]. A decline in the total bilirubin levels in blood suggests liver impairment following hepatocellular damage caused by the cytotoxic constituents of the plant extracts.

Increased blood analyte levels as indicated in this study are marked for kidney damage (creatinine) [34]; increased protein breakdown (blood urea nitrogen) [35]; liver atrophy and increased levels of triglycerides (uric acid) [24].

The decrease in levels of various lipid parameters and those of blood glucose indicates that the extracts contained phytochemical constituents such as tannins that reduces feed intake by decreasing palatability, enhance satiety, suppress appetite, cause ulcers, reduce digestion, and induce fat and glucose absorption blocking (malabsorption) [36]. This lowers the bioavailability of cholesterol, triglycerides,  $\alpha$ -lipoproteins and glucose [37]. The hepatocellular injury due to cytotoxic effects of the extracts may have halted the metabolic, excretory and defense functions of the liver hence reduced levels of serum TG, TC, LDL-C, HDL-C and glucose level [38].

The phytoconstituents that were qualitatively determined in this study includes saponins, alkaloids, terpenoids, flavonoids, sterols and tannins. Phytochemicals such as alkaloids, tannins, terpenoids, saponins and flavonoids have been reported to be toxic [39]. Saponins have been reported to hemolyse red blood cells and cause cell death of many tissues [40,41]. In the kidneys, saponins lead to haemorrhage in the glomeruli and focal destruction of the renal tubules [14]. Toxic levels of saponins cause cardiac failure, acute hypoglycemia and hepatorenal damage leading to death [40]. Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia [42]. Terpenoids have been reported to increase membrane permeability to divalent and monovalent ions [42]. Tannins reduce feed intake by decreasing palatability, reduce feed digestion [14], increase excretion of proteins and essential amino acids and alter the excretion of certain cations [14].

## Conclusion

From this study it can be concluded that:

- i. Qualitative screening of aqueous leaf extracts of the studied plant indicated the presence of alkaloids, flavonoids, tannins, sterols and saponins.
- ii. The intraperitoneal and oral administration of the plant extracts (at 450, 670 and 1000 mg/kg body weight doses) demonstrated

toxic effects as evidenced by hematological and biochemical parameters changes, weekly change in body weights, changes in organ weights and absolute organ to body weights of mice. Consequently, an increment in the plant extract dosage level administered was proportional to their respective toxicological effects in mice.

- iii. The observed toxicological effects could account for the presence of the phytoconstituents such as tannins, alkaloids saponins and terpenoids.

### Suggestions for Further Studies

- i. The organic solvent extraction for these plants should be done to compare the safety activities of both aqueous and organic fractions.
- ii. A study should be conducted at a low dosage levels than the ones studied for these plant extracts to establish the threshold dose above which toxicological effects occurs or is not safe for treatment/therapy.

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