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Acute toxicity, hepatotoxicity and renal-toxicity profile of the crude methanol extract of *Mallotus oppositifolius (Geisel.)* (Euphorbiaceae) combined with honey in albino rats

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Abstract

Background: *Mallotus oppostifollous* is a shrub used folklorically in traditional medicine for the treatment and management of different diseases including infections, wounds, and inflammation across tribes where they are found including South East Nigeria. This study was aimed to toxicity, renal-protective and hepato-protective effect of crude methanol extract of *M. oppositifolius* honey mixture in rats.

Methods: The powdered leaf extracts of *M. oppositifolius* was extracted by cold maceration using methanol. The acute toxicity, hepatotoxicity and renal-toxicity profile of the crude extract of *M. oppositifolius* were carried out using standard laboratory procedures.

Results: The result showed that *Mallotus oppostifollous* administered orally had LD_{50} above 5000mg/kg and showed no toxic effect on the blood, kidney and liver of the rats when used orally. It stabilized the blood hematology and biochemical parameters. It showed no significant elevation on the creatinine, blood urea nitrogen and uric acid and therefore showed that the honey combination could have a stabilizing effect on the vital organs of the rats.

Conclusion: We therefore conclude that the crude extract of *Mallotus oppostifollous* has no toxic effect against the kidney, liver and blood parameters and therefore is safe for consumption as a phytomedicine. Therefore, we recommend its continued usage for the treatment of diseases.

Keywords: *Mallotus oppostifollous*; Acute toxicity; Subacute toxicity; Renal protective; Hepatoprotective; Biochemical; Hematological

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1. Introduction

Phytomedicine continues to be a powerful alternative to conventional medicine for the treatment of many illnesses that have an impact on human health worldwide. Plants have a significant role in modern medicine since they are either employed as raw materials for medications or as a model for drug discovery and synthesis [1].

Globally, herbalists, nutritionists, pharmacists, and cosmetologists have used plant-based medicines and other plant-based products for a variety of purposes, including the creation of food supplements, vitamins, antioxidants, nutraceuticals, cosmetics, and pharmaceuticals. One of the main benefits of phytomedicine is that it is widely accessible, well-tolerated by local cultures, and relatively safe when compared to conventional medicine. It is right to say that more natural compounds or active moieties have reportedly been created and altered by nature than by humans since the dawn of time. Nature is a master craftsman of molecules who has produced a nearly limitless variety of molecular things [2]. It serves as an endless source for drug development, novel chemotypes and pharmacophores, scaffolds for amplification into effective medications for a variety of illness indications, and other priceless bioactive molecules [2]. It has enormous potential that has yet to be realized.

Although just a small portion of the world's plant species have been assessed for possible pharmacological application, it is estimates that more than 25% of prescription medications contain chemicals derived from plants [3]. In order to prove the efficacy and safety of herbal formulations, standardization and toxicity studies must be evaluated scientifically in order to advise those that are using it folklorically. Due to its acceptance, the use of herbal remedies by traditional doctors to cure illnesses remains a mainstay of the healthcare system in developing countries. Herbal medicine is gaining popularity, especially among the rural populace in these countries [4]. The rise in demand for herbal drugs and herbal preparations in the recent past has been attributed to factors like the misconception that plant products are natural and free of the negative and toxic effects associated with conventional medicine [1] however, its use as an alternative form of treatment has increased dramatically in modern society due to its therapeutic efficacy.

Mallotus oppositifolius (Geisel.) Müll. (*M. oppositifolius*) belongs to the family Euphorbiaceae is a shrub known widespread in tropical Africa for its medicinal applications. The herb has been used therapeutically to cure a variety of illnesses. The stick is chewed for dental hygiene and teeth cleansing, while the root is decocted to treat anemia, pneumonia, and as an aphrodisiac [5]. More specifically, the ethanolic and aqueous leaf extracts of *M. oppositifolius* have been reported in the literatures to have antifungal, antibacterial, and antimalarial properties [6-7], antioxidant [8] and antidiabetic activities respectively [9]. It is reported to contain phytochemicals (alkaloids, glycosides, flavonoids, tannins, phlobatanins, saponins, steroids, proteins, carbohydrates, anthraquinones, reducing sugars, and anthocyanins), appreciable amount of minerals (magnesium, calcium, iron, Manganese, Zinc, and essential vitamins (Pyridoxine, Biotin, Ascorbic acid, Retinol, and Riboflavin [10]. Also, previous researcher has reported the extract of the plant for anti-inflammatory, antioxidant, antidiarrheic, antibacterial, antifungal and antitrypanosomal properties. [11]. About 32 compounds was isolated from the study of the leaf, stem and bark by [11] of which was described for *Trypanosoma brucei* and *Leishmania donovani*. The plant has also been reported for activity against pathogenic yeasts while three compounds including betulinic acid, quercetine and quercitin were isolated from methanol leaf extract [12]

Honey has not been reported for any toxicities except allergies associated with the pollen from the sources. Bees produce honey naturally through the processes of regurgitation, enzymatic activity, and water evaporation from floral nectar (a sugar-rich liquid generated in the plant gland known as nectarines or nectarines). Due to the medicinal nature of its components, which include enzymes, peptides, phenolic compounds, and organic acid, honey is a golden amber sweet liquid used as food and medicine. Numerous research teams have investigated honey in lab and clinical settings, and it now has a place in contemporary medicine [13].

Numerous human ailments, including bacterial and viral infections, gastrointestinal problems, and cardiovascular diseases, can be treated with honey. It has been claimed that honey taken orally can prevent and treat gastrointestinal disorders like stomach ulcers [14-15]. According to a study evaluating the cytoprotective effects of honey on the stomach, administering honey significantly reduced the size of ethanol-induced lesions [16]. Similarly, findings from a study revealed that honey may be used as sucralfate to treat peptic ulcer disease and has therapeutic effects for the healing of antral ulcers [17]. It has been reported that Carob honey has protective effect against kidney and liver [18]. Honey and crude extracts plants have been reported to have an additive impact as an anti-ulcer agent, according to a recent trend in combination therapy used to treat ulcers. Previous studies have reported antiulcer activities of a mixture of honey combined with *Euphorbia hirta* [19], honey combined with *Desmodium velutinum* [20]. These studies have proven that honey when combined with plant extract enhanced plant activities for antiulcer activities and should be exploited in therapeutic formulations.

There is need to carry out the toxicity studies of phytomedicines to ascertain their safety and therapeutic activity before its continued use. There is no information on the subchronic of toxicity of *M. oppositifolius* combined with honey in rats therefore the need to evaluate the toxicity of *M. oppositifolius* combined with honey in rats to ascertain their safety. This study was carried out to ascertain the toxicity effect of the crude extract of *M. oppositifolius* combined with honey in rats. Therefore, this study tends to study the toxicity, renal-protective and hepato-protective effect of crude methanol extract of *M. oppositifolius* honey mixture in rats.

2. Material and methods

2.1. Collection and identification of plant leaves

Fresh leaves of *Mallotus oppostifollous* were collected in June 2019 from their habitat Amawbia, Awka Local government Area, Anambra State. The plant was identified by a plant taxonomist a voucher number PCG/UNN/0337/ Euphorbiaceae was obtained and deposited in the herbarium. The plant leaves were air dried at room temperature for 14 days. The dry leaves were pulverized using pestle and mortar, and extracted by cold maceration using methanol. The mixture was covered in order to prevent solvent evaporation and was allowed to stand for 74 hours. Thereafter, it was filtered using Whatman filter paper No. 1. The filtrate was concentrated to dryness using rotary evaporator at 40 °C . The concentrated extract was scrapped into a sterile bottle, covered properly with foil paper and kept in refrigerator.

2.2. Experimental Animal

Adult albino rats weighing between 150 to 170 g were purchased from Pharmacology and Toxicology Department, University of Nigeria Nsukka Opi LGA Nsuka. The animals were acclimatized for 7 days, under 12 hours dark and light cycles. They were fed with water and standard pellet ad libitum until required for study.

2.3. Collection of Honey

The honey used in this study was a multifloral honey and was obtained from Opi Nsukka in Nsuka Local Government Area of Enugu State.

2.4. Acute Toxicity Studies

The acute toxicity test to determine the LD_{50} as an index of safety of the extract was done in 2 phases as described by [1]

2.5. Sub-chronic toxicity test

The subacute toxicity studies of the most active crude extract were done according to the modified method of [1]. Adult wistar albino rats of either sex were used. Thirty albino rats were divided into 6 groups of 5 animals each. Rats in group 1& 2 received crude extract at 250 and 500mg/kg; group 3 and 4 were given the extract combined with honey at doses of 250 and 500 mg/kg combined with 1 ml of honey. The rats in group 5 and 6 received distilled water (5ml/kg) and honey (1 ml per rat) respectively. The rats were administered through the oral route for 28 days. The animals were observed for sign of toxicity (mortality, nervousness and physiological changes) for 28 days. Weight of the rats were taken every other day from the start of experiment while park cell volume of the rats was measured every week within the period of 28 days. On the 28th day post treatment rats were fasted for 24 hours but allowed free access to water to water. Thereafter, animals were anaesthetized with chloroform and sacrificed. About 3 ml blood was collected and put into container with EDTA as an anticoagulant while the remaining 5 ml was collected in sample bottle, allowed to cloth and centrifuged to obtain serum. The serum was used to determine the biochemical parameters while the blood in the EDTA bottle was used to obtain the haematological parameter.

2.6. Estimation of blood hematological parameters

The blood hematological parameters to determine PCV, Hb, RBC, WBC, MCV, MCH and MCHC were all carried out according to the method demonstrated in [21].

2.7. Kidney function tests

Creatinine, blood uric acid, total protein, and urea rates were measured in plasma samples by according to the methods of [22].

2.8. Liver function test

2.8.1. Aspartate Amino Transferase (AST) Estimation

This test was carried out by method described by [23]. The substrate in the reaction are alpha ketoglutaric acid and L-aspartate. The product formed by the enzyme action are glutamate and oxaloacetate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 530 nm wavelength using spectrophotometer.

2.8.2. Alanine Amino Transferase (ALT) Estimation.

This test was carried out according to the method described by [22]. The substrate in the reaction are alpha ketoglutaric acid and L-aspartate. The product formed by the enzyme action are glutamate and pyruvate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550nm wavelength using spectrophotometer.

2.9. Data analysis

All statistical analysis was done using SPSS version 20.0 Data obtained were analyzed using one-way analysis of variance. Where significant differences were observed, the Turkey Post Hoc-test was used to identify and compare differences between groups. Values were considered significant if $P<0.05$. Duncan multiple range test was used to compare the means across each treatment group with untreated group.

3. Results

3.1. Acute Toxicity studies

The result showed that the LD50 were above 5000 mg/kg and this implied that the crude extract of the shrub *M. oppositifolius* are safe for consumption at acute administration because no death was observed at doses upto 5000 mg/kg.

3.2. Body Weight

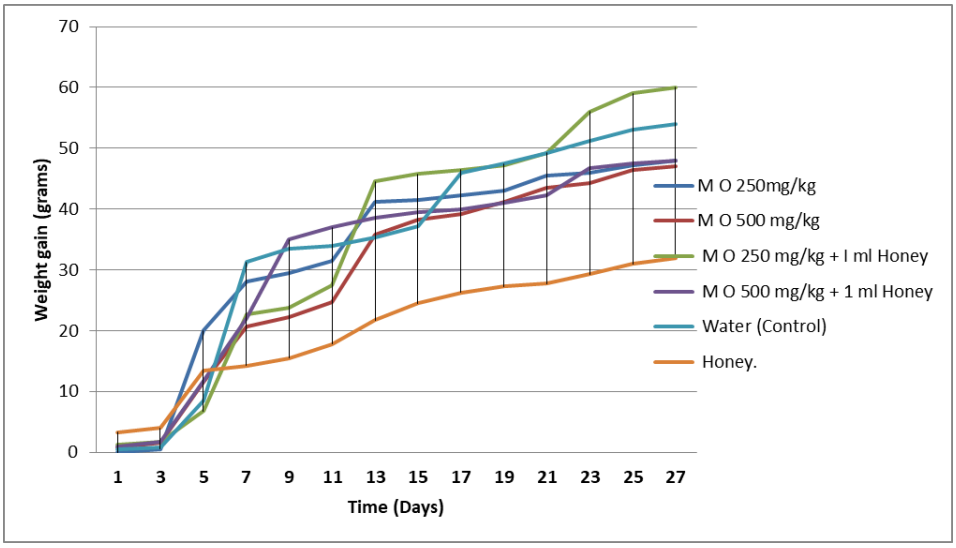

Figure 1 Effect of *M. oppositifolius* combined with honey at 250 and 500 mg/kg on the body weight of rats. Means with asterisk are significantly different ($P<0.01$) when compared with the control that received distilled water (M.O = *M. oppositifolius*)

The result of the effect of *M. oppositifolius* combined with honey on the body weight of rats is shown in Figure 1. All the rats (both the treated and control) had normal growth without any retardation, as depicted in Figures 1, however on 11th day all the treatments (250 mg/kg, 500 mg/kg, 1 ml honey , 250 plus 1ml honey) all had retardation in growth

except the group that received 500 mg/kg plus honey. The extract of *M. oppositifolius* combined with honey at doses of 250 and 500 mg/kg did not produce any significant change in body weight of rats up to 4 days post-administration of the treatment. However, there were slight significant (P<0.05) reduction in body weight of rats treated with extract and honey combination at 500 mg/kg on day 21st post administration. Group administered with honey had slow gain in weight constantly from day 6 and this could be attributed to lose stool observed in their cage when compared to other treatment groups and the control.

3.3. Organ weight

The result of the toxicity effect of the *M. oppositifolius* combined with honey on the internal organs of the rats is shown in Table 1. The effect of the treatment (plant extract combined with honey) on the vital organs of the liver, and the kidney different doses of 250 mg/kg and 500 mg/kg body weights combined with honey revealed that, there was no significant differences (P<0.01) in weight of the liver and kidney between the treatments and the control group (positive control). This implied that there was no inflammation or swelling of the liver and kidney as result of the treatment with the crude extract of *M. oppositifolius* and honey and their combination.

The result showed that the weight of liver of rats pre-treated with crude extract of *M. oppositifolius* at 250 and 500 mg/kg had 4.45 and 4.18 grams respectively while honey and water had 4.13 and 4.85 grams respectively. The result further showed that *M. oppositifolius* at 250 and 500 mg/kg combined with 1 ml of honey had 5.6 and 4.5 respectively. Also, the result showed that the weight of kidney of rats pre-treated with crude extract of *M. oppositifolius* at 250 and 500 mg/kg had 0.30 and 0.4 grams respectively while honey and water had 0.3 and 0.4 grams respectively. The result further showed that *M. oppositifolius* at 250 and 500 mg/kg combined with 1 ml of honey had 0.4 gram respectively.

Table 1 Effect of different doses of methanol extract of *M. oppositifolius* combined with honey on liver and kidney of rats

Organ Weight	Treatments					
	M O 250 mg/kg	**M O 500 mg/kg**	**M O 250 mg/kg + 1 ml Honey**	**M O 500 mg/kg + 1 ml Honey**	**Honey (1 ml)**	**Water (5ml/kg)**
liver weight	4.45 ± 0.6 [a]	4.18 ± 0.2 [a]	5.6 ± 0.5 [a]	4.5 ± 0.5 [a]	4.13 ± 0.4 [a]	4.85 ±0.5 [a]
kidney weight	0.37 ± 0.1 [a]	0.30 ± 0.0 [a]	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	0.33 ± 0.0 [a]	0.38 ± 0.1[a]

Note: mean with the same alphabets are not statistically significant (P<0.05) when compared with the control (a=a), (M.O = *M. oppositifolius*)

3.4. Biochemical parameters

3.4.1. Hepato-toxicity effect of M. oppositifolius

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on liver of rats are shown in Table 2. The extract combined with honey showed no significance effect (P>0.05) on blood Alanine Aminotransferase (ALT) of the treated rats at doses of 250 and 500 mg/kg body weight respectively (Table 2) except the 250mg/kg plus 1 ml honey (11.3) that had significant reduced ALT when compared with the control that received only 5ml/kg of distilled water (16.7).

Similarly, the crude extract of *M. oppositifolius* and its combination with honey had no significant effect on the Aspartate Aminotransferase (AST) of the rats (P>0.05) though the at 500mg/kg of the crude extract plus 1 ml honey the extract had significantly reduced AST (P<0.05) when compared with the control. Thus, this implied that the crude leaf extract of *M. oppositifolius* when administered alone and in combination with honey has no hepatotoxicity effect in rats and therefore are safe as alternative drug for treatment of diseases.

3.4.2. Nephro-toxicity effect of M. oppositifolius

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on kidney of rats are shown in Table 2. The crude extract of *M. oppositifolius* and its combination with honey showed significance elevation (P>0.05) in blood urea when compared with the control. The result showed that Urea concentration was higher at 250mg/kg and 250 mg/kg plus honey respectively. At 250 mg/kg of the crude extract and 250 mg/kg plus 1 ml honey had 81and 73 mg/dL respectively followed by 500mg/kg crude (70 mg/dL) and honey (63 mg/dL) while 500mg/kg plus 1ml honey had the least Urea content (53 mg/dL).

Similarly, creatinine had the same elevated effect as the creatinine when compared with the untreated control. The crude extract of *M. oppositifolius* and its combination with honey showed significance elevation (P<0.05) in blood creatinine concentration when compared with the control (1.8 mg/dL). The result showed that Urea concentration was higher at 250mg/kg and 250 mg/kg plus honey respectively. At 250 mg/kg of the crude extract and 250 mg/kg plus 1 ml honey had 2.53 mg/dL respectively followed by 500mg/kg crude (2.23 mg/dL) and honey (2.0 mg/dL) while 500mg/kg plus 1ml honey had the least creatinine content (1.9 mg/dL) when compared with the control with 1.8 mg/dL.

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on blood uric acid of rats are shown in Table 2. The crude extract of *M. oppositifolius* and its combination with honey showed no significance different (P>0.05) in blood uric acid when compared with the control (8 mg/dL) though they were slightly elevated. At 250 mg/kg of and 500 mg/kg the crude extract had Uric acid of 6.5 and 7.2 mg/dL respectively while at 250 mg/kg of and 500 mg/kg the crude extract combined with honey, the Uric acid concentration was approximately 7 and 8 mg/dL respectively but honey had uric acid concentration of 6 mg/dL.

3.5. Other Blood Biochemical Parameters

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on blood protein of rats are shown in Table 2. The extract combined with honey showed significance reduction (P<0.05) in blood protein of the treated rats at doses of 250 and 500 mg/kg body weight respectively and combination with honey (Table 2) when compared with the control (6.27 mg /dL). Protein content was least in the blood of rats that received 250 mg/kg plus 1 ml honey (3.57) and 250 mg/kg (4.0) respectively followed by rats pre-treated with 500mg/kg (4.6) and honey (4.9) while 500mg/kg plus 1 ml honey (5.27) had the higher protein content when compared with the control (6.27) respectively.

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on blood sodium concentration of rats are shown in Table 2. The crude extract alone showed no difference (P>0.05) in blood sodium concentration of all the treated rats at doses of 250 and 500 mg/kg body weight respectively and combination with honey (Table 2) when compared with the control (**118.53 mmol/L**). The result showed that the crude extract of *M. oppositifolius* alone had lesser mean concentration than the crude extract combined with honey. The result showed that the crude extract at 250 and 500 mg/kg had blood sodium concentration of 116 and 117 mmol/L, the crude extract at 250 and 500 mg/kg combined with 1 ml of honey respectively had blood sodium concentration of 118 and 123 mmol/L while 1 ml of honey and water had 120 and 119 mmol/L respectively. The result showed that the crude extract when administered alone had reduced blood sodium concentration but when combined honey the sodium concentration increased with increased in concentration of the dosage.

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on blood potassium concentration of rats are shown in Table 2. The crude extract alone showed no difference (P>0.05) in blood potassium concentration of all the treated rats at doses of 250 and 500 mg/kg respectively and its combination with honey (Table 2) when compared with the control (4.4mmol/L) except at 500 mg/kg combined with 1 ml Honey with 3.6 mmol/L that was significantly lower than the control (4.4mmol/L). The result further showed that blood sodium concentration reduced with increase in dosage of the crude extract (Table 2) and further reduced when the crude extract at 250 and 500 mg/kg is combined with honey. The result showed that at 250 and 500 mg/kg the blood potassium concentration was 4.8 and 4.6 mmol/L respectively while at 250 and 500 mg/kg combined with 1 ml of honey the blood potassium concentration was 4.17 and 3.6 mmol/L respectively and this was not significantly (P>0.05) different with the control (4.4mmol/L).

The results of the effect of the crude methanol extract of *M. oppositifolius* extract combined with honey on blood chloride concentration of rats are shown in Table 2. The crude extract of *M. oppositifolius* and their combination with honey showed significant difference (P<0.05) in blood potassium concentration of all the treatment at doses of 250 and 500 mg/kg respectively and its combination with honey (Table 2) when compared with the control (108 mmol/L). This implies that the crude extract and their combination with honey stabilize the blood chloride concentration. At 250 and 500mg /kg of the crude extract alone had blood chloride concentration of 106 mmol/L respectively while at 250 and 500mg /kg of the crude extract combined with honey and 1 ml of honey alone had blood chloride concentration of approximately 107 mmol/L each respectively.

The results of the effect of the crude methanol extract of *M. oppositifolius* combined with honey on blood calcium concentration of rats are shown in Table 2. The crude extract of *M. oppositifolius* and their combination with honey showed no significant difference (P>0.05) in blood potassium concentration of all the treatment at doses of 500 mg/kg

respectively and its combination with honey (Table 2) when compared with the control (8.8 mmol/L) but at 250 ml/kg (11 mmol/L) of the crude extract alone the extract had elevated calcium concentration. This implied that blood calcium concentration decreased with increase in dosage of the crude extract and honey combination respectively.

Table 2 Effect of different doses of methanol extract of *M. oppositifolius* combined with honey on blood biochemical parameters of rats

Biochemical Parameter	**Treatments**					
	MO250 mg/kg	**MO500 mg/kg**	**MO250 mg/kg+1 ml Honey**	**MO500 mg/kg+1 ml Honey**	**Honey (1 ml)**	**Water (5ml/kg)**
AST (U/L)	13.33 ± 0.3 ^{ab}	16.00 ± 1.5 ^{ab}	11.33 ± 1.2 ^{a}	12.67 ± 1.2 ^{ab}	14.67 ± 1.7 ^{ab}	16.67 ± 2.7 ^{b}
ALT (U/L)	10.33 ± 0.3 ^{ab}	11.67 ± 1.7 ^{b}	9.0 ± 0.6 ^{ab}	7.33 ± 0.9^{a}	10.0 ± 0.6 ^{ab}	11.67 ± 0.9 ^{b}
Urea mg/dL	81.00 ±3.5 ^{c}	69.67 ± 8.3^{bc}	73.33 ± 3.2 ^{bc}	53.0 ± 2.9 ^{a}	63.00 ± 3.5 ^{ab}	51.67 ± 4.9 ^{a}
Creatinine mg/dL	2.53 ± 0.1^{c}	2.23 ± 0.2 ^{bc}	2.53 ± 0.1 ^{c}	1.90 ± 0.1 ^{ab}	2.10 ± 0.1 ^{ab}	1.80 ± 0.1^{a}
Uric acid mg/dL	6.50 ± 0.7 ^{a}	7.23 ± 0.4 ^{a}	7.63 ± 0.4 ^{a}	7.97 ± 0.5 ^{a}	6.23 ± 1.4 ^{a}	7.57 ± 0.2 ^{a}
Total Protein g/dL	4.07 ± 1.0 ^{a}	4.64 ± 0.4 ^{ab}	3.57 ± 0.6 ^{a}	5.27 ± 0.4 ^{ab}	4.87 ± 0.1 ^{ab}	6.27 ± 0.8^{b}
Sodium mmol/L	116.33 ± 1.7 ^{a}	117.12 ± 1.9 ^{a}	118.37 ± 2.3 ^{a}	122.47 ± 3.0 ^{a}	120.73 ± 3.7 ^{a}	118.53 ± 3.7 ^{a}
Potassium mmol/L	4.80 ± 0.4 ^{b}	4.60 ± 0.2^{b}	4.17 ± 0.4 ^{ab}	3.60 ± 0.1 ^{a}	4.43 ± 0.2 ^{ab}	4.40 ± 0.2 ^{ab}
Chloride mmol/L	105.67 ±0.9 ^{a}	106.00 ± 0.6 ^{a}	106.67 ± 0.3 ^{ab}	107.00 ± 0.6 ^{ab}	106.67 ± 0.6 ^{ab}	108.00 ± 0.0^{b}
Calcium mg/dL	10.87 ± 0.9^{c}	10.00 ± 0.2 ^{ab}	8.67 ± 1.3 ^{ab}	7.30 ± 1.3 ^{a}	8.93 ± 0.5 ^{ab}	8.80 ± 0.3 ^{ab}

Note: Mean with the same alphabet is not statistically different (P<0.05) when compared with untreated control while mean with different alphabets are statistically different. M.O means *M. oppositifolius*

3.5.1. Effect of M. oppositifolius crude extract on Blood Hematological parameters of rats

The result of effect of crude extract of *M. oppositifolius* on the blood hematological parameters such as Packed Cell Volume (PCV), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) of rats are presented in table 3 and figure 20 as follows:

3.5.2. Park cell volume

The results of effect of crude extract of *M. oppositifolius* on blood park cell volume are presented in table 3 and figure 2. The result showed that there was no significant different (P>0.05) in the park cell volume of the blood of rats across all the treatments when compared with the positive control (50 %) across all the weeks (figure 2) from week 1 to week 4 (table 3 and figure 2). The result showed also that at 250 mg/kg crude methanol extract *M. oppositifolius* there was consistent elevation of PCV of rats from week 1 to week 4 (figure 2), at 500mg there was fluctuations in the PCV of rats from week 1 to week four (figure 2), at 250 mg/kg plus 1 mil of honey there was consistent increase in PCV of rats from week 1 to week four, at 500 mg/kg plus 1 mil of honey there was also consistency increase in PCV of rats from week 1 to week four except in week 3 that showed slight reduction (figure 2). Also 1 ml of honey showed consistent increase in the PCV of rats from week 1 to week four except week four (figure 2). Water (positive control) also had or showed consistent increase in PCV of rats (figure 2).

However at week four at week four, the crude extract of *M. oppositifolius* at 250 mg/kg had PCV of 47%, at 500 mg/kg the PCV was 42% while at 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey the park cell volume was 45 and 44 % respectively whereas honey and water (control) had 43 and 50 % respectively (see table 3). This implied that the blood PCV thought were reduced across all the treatment there was no significant different in the effect of *M. oppositifolius* on PCV of rats when treated orally.

3.5.3. Heamoglobin concentration

The results of effect of crude extract of *M. oppositifolius* on blood heamoglobin concentration are presented in table 3. The result showed that there was no significant different (P>0.05) in the heamoglobin concentration of the blood of rats across all the treatments when compared with the positive control (12 mg/dL). The result showed that *M. oppositifolius*

at 250 mg/kg had 11mg/dL, 500mg/kg had 11 mg/dL, 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey had 11 mg/dL respectively while honey 11 mg/dL. This implied that the crude extract of *M. oppositifolius* and its combination with honey as well as honey alone had no significant effect in the blood heamoglobin concentration of rats.

3.5.4. Red blood cell

The results of effect of crude extract of *M. oppositifolius* on red blood cell concentration of rats are presented in table 3. The result showed that there was significant different ($P < 0.05$) in the red blood cell concentration of the blood of rats across the treatments when compared with the positive control (5 x1012/ L) except at 500 mg/kg and its combination with honey respectively with RBC concentration of 5 x1012/ L each respectively .

The result showed that *M. oppositifolius* at 250 mg/kg had 4.0 x1012/ L, 500mg/kg had 5 x1012/ L, 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey had 4 and 5 x1012/ L respectively while honey had 4 . This implied that the crude extract of *M. oppositifolius* and its combination with honey as well as honey alone had increased significant effect in the red blood concentration of rats and this is dose dependent.

3.5.5. White Blood Cell

The results of effect of crude extract of *M. oppositifolius* on white blood cell concentration of rats are presented in table 3. The result showed that there was no significant different ($P >0.05$) in the white blood cell concentration of rats across the treatments when compared with the positive control (7 $x10^9$/ L). The result showed that *M. oppositifolius* at 250 mg/kg had 8 $x10^9$/ L, 500mg/kg had 7 $x10^9$/ L, 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey had 6 and 7 $x10^9$/ L respectively while honey had 7 $x10^9$/ L. This implied that the crude extract of *M. oppositifolius* and its combination with honey as well as honey alone had no significant effect in the white blood cell concentration of rats.

3.5.6. Mean corpuscular volume (MCV)

The results of effect of crude extract of *M. oppositifolius* on mean corpuscular volume of rats are presented in table 3. The result showed that there was no significant different ($P > 0.05$) in the MCV of rats across the treatments when compared with the positive control (1f L). The result showed that *M. oppositifolius* at 250 mg/kg had 1 f L, 500mg/kg had 1 f L, 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey had 1f L respectively while honey had 1 f L. This implied that the crude extract of *M. oppositifolius* and its combination with honey as well as honey alone had no significant effect in the MCV of rats.

3.5.7. Mean corpuscular heamoglobin Concentration (MCHC)

The results of effect of crude extract of *M. oppositifolius* on mean corpuscular heamoglobin concentration of rats are presented in table 3. The result showed that there was no significant different ($P > 0.05$) in the mean corpuscular heamoglobin concentration of rats across the treatments when compared with the positive control (23%). The result showed that *M. oppositifolius* at 250 mg/kg had 24%, 500mg/kg had 24 %, 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey had 24 % respectively while honey had 25 % f L. This implied that the crude extract of *M. oppositifolius* and its combination with honey as well as honey alone had no significant effect in the MCHC of rats.

3.5.8. Mean corpuscular heamoglobin (MCH)

The results of effect of crude extract of *M. oppositifolius* on mean corpuscular heamoglobin of rats are presented in table 3. The result showed that there was no significant different ($P > 0.05$) in the mean corpuscular heamoglobin of rats across the treatments when compared with the positive control (27 Pg / cell). The result showed that *M. oppositifolius* at 250 mg/kg had 27 Pg / cell, 500mg/kg had 23 Pg / cell, 250 mg/kg plus 1 ml honey and 500 mg/kg plus 1 ml honey had 26 and 24 Pg / cell respectively while honey had 24 Pg / cell. This implied that the crude extract of *M. oppositifolius* and its combination with honey as well as honey alone had no significant effect in the MCH of rats.

Table 3 Effect of different doses of methanol extract of M. oppositifolius combined with honey on blood hematological parameters of rats

Haematological parameters	Treatments					
	M O 250 mg/kg	MO500 mg/kg	MO 250 mg/kg + 1 ml Honey	MO500 mg/kg + 1 ml Honey	Honey (1 ml)	Water (5ml/kg)
PCV (%)	46.7 ± 0.1 [a]	42.3 ± 0. 2 [a]	45.0 ± 0.5 [a]	44.3 ± 0.5 [a]	42.7 ± 0.4 [a]	50.0 ± 0.5 [a]
Hb mg/dL	11.1 ± 0.6 [a]	10.6 ± 0.5 [a]	10.8 ± 0.9 [a]	10.9 ± 1.0 [a]	10.6 ± 0.8 [a]	11.7 ± 0.6 [a]
RBC ($x10^{12}$/L)	4.0 ± 0.1 [a]	4.6 ± 0.1 [c]	4.1 ± 0.1 [ab]	4.5 ± 0.1 [c]	4.4 ± 0.1 [b]	4.5 ± 0.1 [c]
WBC ($x10^{9}$/L)	7.5 ± 0.5 [a]	7.3 ± 0.5 [a]	6.3 ± 0.3 [a]	7.4 ± 0.5 [a]	6.6 ± 0.5 [a]	7.3 ± 0.9 [a]
MCV (fL)	1.2 ±0.0 [b]	0.9 ± 0.1 [a]	1.1 ± 0.1 [ab]	1.0 ± 0.1 [ab]	1.0 ±0.1 [ab]	1.1 ±0.1 [ab]
MCHC (%)	23.6 ± 0.5 [a]	25.0 ± 0.5 [a]	24.1 ± 0.9 [a]	24.4 ± 0.7 [a]	25.0 ± 0.8 [a]	23.3 ± 0.9 [a]
MCH Pg/cell	27.4 ± 0.1 [a]	22.9 ±0.1 [a]	26.1 ±0.2 [a]	24.1 ± 0.2 [a]	24.2 ± 0.1 [a]	27.0 ±0.2 [a]

Note: Mean with the same alphabet is not statistically different (P<0.05) when compared with untreated control while mean with different alphabets are statistically different. M.O means M. oppositifolius

4. Discussion

The study revealed that the plant had LD50 above 5000 mg/kg and this was in agreement with the studies of [10] and [12] who reported that the LD_{50} were above 5000 mg/kg and upto 12,000 mg/kg respectively. This implied that the plant had no toxic effect at acute administration of the plant crude extract. The result showed that the ALT and AST are within the normal range which implied that the combination and individual doses of the crude extract of *Mallotus oppostifollous* and honey had no pathological injury on the liver function and therefore safe for consumption.

Also, the result of the subchronic toxicity studies showed that the crude extract had no toxic effect on the blood, kidney and liver of the rats when. It stabilized the blood hematology and biochemical parameters. It showed no significant elevation on the creatinine, blood urea nitrogen and uric acid and therefore showed that the honey combination could have a stabilizing effect on the vital organs of the rats. This therefore was shown to be relatively non-toxic with high dose and this implied that the crude extract is safe for treatment of diseases and therefore should be exploited for its therapeutic activity.

5. Conclusion

We therefore conclude that the crude extract of *Mallotus oppostifollous* has no toxic effect against the kidney, liver and blood parameters and therefore is safe for consumption as a phytomedicine. Therefore, we recommend its continued usage for the treatment of diseases.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of ethical approval:

All experimental procedures were approved by the Faculty of Pharmaceutical Sciences research and ethical committee.

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Appendix