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RESEARCH ARTICLE

ACUTE TOXICITY AND HEPATO-RENAL PROTECTION OF LIME JUICE, HONEY AND THEIR FLAVONOID-RICH FRACTIONS IN HIGH FAT-DIET INDUCED OBESE RAT MODEL

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Abstract

Introduction: A major consideration for the use of alternative herbal medicine from natural compounds is the concern of safety due to possible toxicity. This study evaluated the safety and hepato-renal protection of fresh lime juice (FLJ), raw honey (RH) and their flavonoid-rich fractions in obese rat induced high fat-diet (HFD).
Methods: Oral acute toxicity (LD_{50}) study involved 24 female Wistar rats, divided into 8 groups of 3 rats, administered 300 mg/kg and 2000 mg/kg of FLJ, RH, methanol flavonoid rich fraction of lime juice (MFLJ) and ethyl acetate flavonoid rich fraction of honey (EAFH) respectively, for 14 days. Simultaneously, for the anti-obesity study, 24 neonate wistar rats of 21-days old, divided into 2 groups of 12 rats (for obesity induction phase-1, for two weeks), and regrouped into 4 groups of 4 rats (14 days treatment phase-2), were used.
Results: Result of LD_{50} on FLJ, RH, MFLJ, and EAFH showed no toxicity, no motility, and body weight of rats was not adversely affected evenup to 2000 mg/kg.The increased body weight of the HFD-obese rats was significantly ($p<0.05$)reduced compared to control. There was significant ($p<0.05$) decrease in activities of aspartate aminotransferase and alanine aminotransferase after MIX, MFLJ and EAFH administration, compared with control. Also, total protein and bilirubin concentrations was not significantly ($p<0.05$) different compared to control. Administration of EAFH significantly ($p<0.05$) reduced the concentrations of creatinine, urea, potassium, and chloride; while MIX and EAFH significantly ($p<0.05$) increased their concentrations compared to control.
Conclusion: It may be concluded that FLJ, RH, MFLJ, and EAFH are safe for consumption and also possess liver and renal protection.
Keywords: Acute toxicity, high fat diet, honey, lime juice, liver function, renal function.

INTRODUCTION

Plant use and applications as alternative medicine by traditionists have experienced bias of late, owing to the arrival of orthodox or conversional medicine[1]. However, it is reported that phytochemicals derivatives of plants and herbs make up greater than 25% chemical structure of pharmacological drugs, thus revealing the unparallel potentials of plants' biomolecule for targeted disease and drug development[2]. In traditional medicine practice, therapeutic formulations are usually made by combining plants parts, with promising phytochemicals of medicinal health benefits, for the management and treatment of diseases[2-5]. These include mixture of honey and *Desmodium velutinum* as anti-ulcer therapy[6], honey and lime juice mixture as anti-hypercholesterolemia[3], and as anti-obesity[5] therapies in rat's studies.

The rampant exploitation of natural medicinal plants and herbs in treating many diseases, against synthetic drug is due to the unbearable side effects synthetic drugs potentiate, making room for plant as alternative safe medicine[7]. Lemon juice was used to neutralize the toxic effect of veeram, during its preparation. Veeramis composed of mercury chloride (Hg_2Cl_2) employedin the treatment of gonorrhea, syphilis, stomach ulcer and[8]. Honey in combination with extract of *Mallotus oppostifollous* was reported to be a safe phytomedicine,

possessing protective effects on the kidney and liver[4]. Currently, one crucial anchor of the patronage of herbal medicine as alternative to conventional medicine is antioxidant and anti-inflammatory defense mechanism, found in most phytocompounds, in tackling diseases[5]. *Citrus aurantifolia* fruit juice and honey, are reported to contain several phytochemicals including alkaloids, flavonoid, carbohydrates, terpenoids, proteins, glycosides, saponins, tannins, phlobtannins, phenols and amino acids[9,10]. These phytochemicals are the agents responsible for the bioactivities in plants as alternative medicine for treatment, management and prevention of several diseases[11]. These bioactivities include antioxidant and anti-hyperlipidemic[5], anti-inflammatory, antifungal, anti-microbial[9], anti-diabetes[12], and enzyme inhibitory and inductive activities[11]. Flavonoids in citrus and honey, including neohesperidin, quercetin, coumarin, epigallocatechin, caffeic acid, sinapic acid, hesperidin, naphthoresorcinol, gallic acid, rutin, nobiletin, hesperitin, and apigenin and kaempferol are known for their significant antioxidant, anti-obesity, antihyperlipidemia, antiasth-matic, anti-tumor, anti-prostatitis, anti-allergic, anti-inflammatory and anticancer[5,13]. Flavonoids impact their anti-obesity effect by their operation on the activity of sympathetic nervous system to control appetite, improve hepatic fatty acid oxidation by enzyme regulation and improvement of energy expenditure by lowering nutrient absorption[14]. Although, majority of natural product medicine are reported safe for use, however, some are said to be toxic, with potential hideous side effects, depending on the quantity consumed[8]. In order to ascertain the safe dose and use of natural and synthetic medicinal products, researchers employ the use of oral acute toxicity and sub-acute toxicity study on animals. The median lethal oral dose, known as LD_{50} is established in this study[11]. The LD_{50} of a substance is the dose of that substance given orally, that result in 50 percent death of the test animals[4,11]. It is statistically calculated and expressed in terms of weight of test substance per unit weight of test animal (mg/kg)[11]. Thus, this study investigated and validated the speculated safety use of lime juice, honey, and their flavonoid rich fractions with hepatic and renal protectionin HFD induced obese rat model.

MATERIALS AND METHODS

Experimental Design and Induction of Obesity

At phase I, male neonate albino Wistar rats (twenty-four, of 21 days old) were divided into 2 groups of 12 rats each for induction of obesity, for two weeks. In phase II, animals were regrouped into 4 groups of 4 rats each, to commence treatment orally, with (50% FLJ and 50% honey mixture) MIX, MFLJ, and EAFH, for two weeks. Rats were sacrificed and blood samples and organs collected for biochemical analysis at the end of each phase.

Phase I

Group 1: Control, fed a normal diet and clean water (12 rats).

Group 2: Fed High-Fat diet (12 rats).

Phase II

Group 1: Control, fed normal feed + clean water.

Group 2: High-Fat diet-obese rats[a]+ 200 mg/kg oral administration of 50% FLJ and 50% honey mixture (MIX)

Group 3: High-Fat diet-obese rats[b] + 200 mg/kg oral administration of MFLJ

Group 4: High-Fat diet-obese rats[c]+ 200 mg/kg oral administration of EAFH

Body weight and length of rats was measured and used for the determination of Lee indices by the method of Nakagawa *et al*.[15], using the formula;

$$\text{Lee indices of animal} = \frac{\sqrt[3]{Body\ Weight\ (g)}}{\text{Nose to Anus Lenght (cm)}}$$

Obesity was established if a rat had Lee index ≥ 0.3.

% Adiposity index (% AI) was calculated by the formula expressed below[5,15].

$$\text{Adiposity index (\% AI)} = \frac{\text{Total weight of epididymal, visceral and retroperitoneal fat}}{\text{Body weight}} x100$$

Oral Acute Toxicity (LD_{50}) study of FLJ, RH, MFLJ and EAFH

Twenty-four female rats of 10 weeks old (75– 167 g) were used to determine the LD_{50} of fresh lime juice (FLJ), raw honey (RH) and their flavonoid rich fractions (MFLJ and EAFH) (Table 1). Animals were allowed to fast, by withholding food, not water overnight. After fasting, rats were weighed and extracts administered; and thereafter, weight was taken weekly. This study was done following the method described by the organization for economic co-operation development (OECD)[16], with little modification, and 300 mg/kg was selected as the starting dose. Thus, 300 mg/kg and 2000 mg/kg of extracts were given to 3 rats each, and food was withdrawn for 2 hours. Doses were given after 24 hours and animals were individually observed every 30 minutes of the 1st 24 hours, especially within the 1st 4 hours, and then daily for 14 days. Observation of toxic symptoms was done, such as changes in skin and fur, behavioral changes, tremors, eyes and mucous membranes, loco-motion, salivation, diarrhea, convulsion, lethargy, sleep, coma and mortality, immediately after oral administration of single dose. The LD_{50} of a substance is the dose of that substance given orally, that result in 50 percent death of the test animals. It is statistically calculated and expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Blood and tissue collection

Rats were made to fast overnight and anesthesized via subjection to mild chloroform before blood was collected into plain and EDTA tubes by cardiac puncture. Serum was separated by a retro fraction and plasma the erythrocytes after centrifuging the whole blood at 5000 rpm for ten minutes. All samples were stored at -20°C until analyzed.

Preparation and composition of high-fat diet in g/1000 g

High fat diet was prepared following the method of Idoko *et al*., with a little modification: pelletized

commercial feed: 300, Chichen skin: 84, Skin of pork: 161, Butter: 85, and Yoke of egg: 370.

Body weight measurement of rat

The neonate's rat average body weight prior to and after obesity induction was recorded to be 25 – 133 g. During treatment with MIX, MFLJ and EAFH, (67 – 130 g) were the measured average body weight on day 1, day 7 and day 11.

Liver and kidney function tests

Randox Kit was used to carry out the liver and renal function tests. Alanine aminotransferase (ALT) activity, by method of Reitman and Frankel[17], aspartate aminotransferase (AST) activityby method of Reitman and Frankel[17], alkaline phosphatase (ALP) activity by Englehardt[18] method, concentrations of total protein (TP) and bilirubin (Bil) byJendrassik and Grof[19] method. Urea and creatinine concentrations by Bartels and Bohmer[20], concentrations of chloride and potassium by Henry *et al.* [21].

Statistical analysis

Data was analyzed by T- test pare wise comparison of means, using both one and two-ways analysis of variance (ANOVA) in Statistical Product and Service Solutions (SPSS), version 20 and results were presented as Mean ± SD. Mean value with $p<0.05$ was considered significant and Duncan's new multiple range test was used to separate significant means at 95% level of probability.

RESULTS

Oral Acute Toxicity Study (LD_{50})

Table 1 shows the result of oral acute toxicity test on FLJ, RH, MFLJ and EAFH. No sign of toxicity and no mortality observed at doses of 300 mg/kg and 2000 mg/kg. This confers a level of safety on FLJ and RH, and MFLJ and EAFH. Hence, it implies that the dose of 2000 mg/kg of the fraction is safe. Therefore, 1/10th (200 mg/kg) and 1/8th (250 mg/kg) doses of the fractions were considered to evaluate the biological activity.

Table 1: Result of acute oral toxicity study (LD_{50}) on FLJ, RH, MFLJ and EAFH at 300 mg/kg and 2000 mg/kg.

Group	No of Rat	Dose (mg/kg)	Duration	No of Death	No of Survival	Effect on Body Weight
FLJ	3	300	14 days	0	3	SI
	3	2000	14 days	0	3	SD
RH	3	300	14 days	0	3	SI
	3	2000	14 days	0	3	SD
MFLJ	3	300	14 days	0	3	SD
	3	2000	14 days	0	3	SD
EAFH	3	300	14 days	0	3	SI
	3	2000	14 days	0	3	MD

SI: Slightly Increased; SD: Slightly Decreased; MD: More Decrease; FLJ=Fresh lime juice; EAFH= Ethyl acetate flavonoid rich fraction of honey; MFLJ=Methanol flavonoid rich fraction of lime juice.

The body weight (Table 2) of rats in this oral acute toxicity study was not adversely affected as no significant ($p<0.05$) declined weight loss (Tables 1 and 2), when either 300 mg/kg or 2000 mg/kg dosage group was compared with control group. Rather, rats significantly ($p<0.05$) gained weight, compared to control group. However, the effects of EAFH and MFLJ on body weight of rats in LD_{50} study dosing at 300 and 2000 mg/kg significantly ($p<0.05$) reduced bodyweight compared with FLJ and RH.

Body weight, Lee indices, and adiposity indices of treated rats

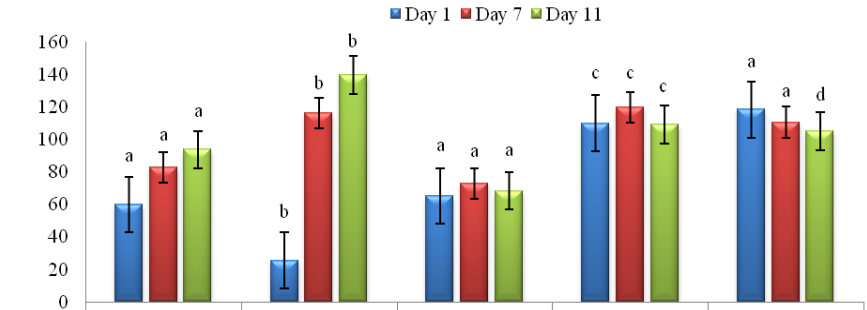
Figure 1 and Figure 2 show the body weight of rats both after obesity induction (AOI) and treatment with MIX, MFLJ and EAFH. In HFD – obese treated rats, which was significantly ($p<0.05$) reduced (especially on day 11) when each treatment group was compared with either control group or AOI group. Figure 2reveal the weight gain after treatment with MIX, MFLJ and EAFH.

The HFD– obese rats gained more weight (Figure 2) and consumed more feed (Table 3) than the control rats, but after treatment with MIX, MFLJ and EAFH, weight gain was significantly ($p<0.05$) reduced. Lee index and adiposity index (Table 2) were significantly ($p<0.05$) increased in HFD– obese rats when compared with control and treated rats, establishing obesity.

Table 2: Effects of FLJ, RH, MFLJ and EAFH on body weight of rats after LD_{50} study at 300 and 2000 mg/kg doses (n=3, $p<0.05$).

Group	Arrival Day	Day 1	Day 7	Day 14
Control	27.00±3.56^{a}	97.25±4.29^{a}	112.25±11.59^{a}	114.75±3.86^{a}
FLJ 300 mg/kg	91.50±10.60^{b}	107.50±3.53^{b}	110.00±4.24^{a}	124.00±7.07^{b}
FLJ 2000 mg/kg	99.50±9.19^{c}	115.00±7.07^{b}	113.00±14.14^{a}	110.50±6.36^{a}
RH 300 mg/kg	86.00±11.31^{b}	105.00±7.07^{b}	111.00±11.31^{a}	124.00±7.07^{b}
RH 2000 mg/kg	101.00±4.24^{c}	120.50±3.53^{c}	128.50±26.16^{b}	117.50±3.53^{a}
MFLJ 300 mg/kg	111.50±13.44^{c}	129.50±20.51^{d}	128.50±19.09^{b}	124.00±14.14^{b}
MFLJ 2000 mg/kg	75.50±4.95^{d}	119.00±7.07^{c}	115.00±1.41^{a}	107.00±2.83^{a}
EAFH 300 mg/kg	125.50±0.70^{e}	134.50±0.71^{d}	98.00±1.41^{c}	113.00±2.82^{a}
EAFH 2000 mg/kg	161.50±54.44^{f}	143.50±33.23^{e}	125.00±28.28^{b}	78.50±2.12^{c}

FLJ=Fresh lime juice; EAFH= Ethyl acetate flavonoid rich fraction of honey; MFLJ=Methanol flavonoid rich fraction of lime juice.

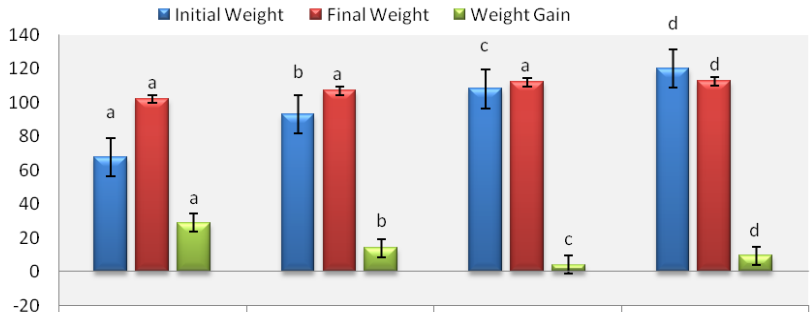


	Control	AOI	MIX	MFLJ	EAFH
Day 1	60	25.5	65.33	110	118.33
Day 7	82.67	116.25	72.66	119.66	110.5
Day 11	93.66	139.75	68.33	109	105

Figure 1: Effects of MIX, MFLJ, and EAFH on body weight of HFD-obese treated rats (n=4, $p<0.05$).
HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: Ethyl acetate Flavonoid Rich Fraction of Honey.

Liver function and kidney function tests

In HFD-obese treated rats (Figure 3 and Figure 4), MIX, MFLJ and EAFH treated rats had their TP concentrations significantly ($p<0.05$) lower than control and higher than AOI group. AST activity was significantly ($p<0.05$) lowered in rats treated MIX and MFLJ than both control and AOI groups. AST activity was significantly ($p<0.05$) higher in EAFH rats than control, but significantly ($p<0.05$) lowered than AOI group. There was significant ($p<0.05$) reduction in ALT activity in rats treated MIX and MFLJ, and increase in rats treated EAFH than control and AOI groups. D-BIL and T-BIL were significantly ($p<0.05$) reduced in rats treated MIX, MFLJ and EAFH than control and AOI groups. ALP was significantly ($p<0.05$) increased in rats treated MIX, MFLJ and EAFH than control and AOI rats.



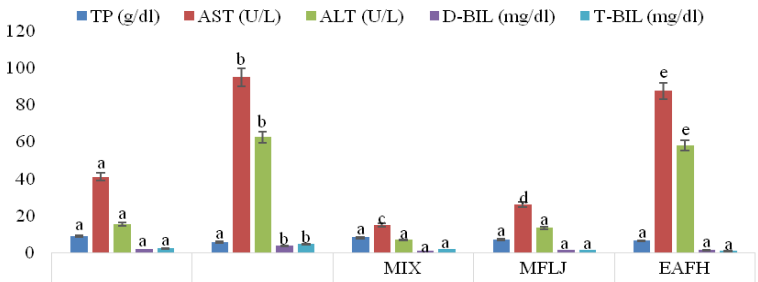
	Control	MIX	MFLJ	EAFH
Initial Weight	67.5	93	108	120
Final Weight	102	107	112	112.5
Weight Gain	29	14	4	9.5

Figure 2: Effects of MIX, MFLJ and EAFH on weight gain of HFD diets obese treated rats (n=4, $p<0.05$).
HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: Ethyl acetate Flavonoid Rich Fraction of Honey.

In HFD-obese treated rats, administration of MIX and MFLJ significantly ($p<0.05$) increased the concentrations of creatinine, urea and potassium than in control and AOI groups. There was significant ($p<0.05$) high concentration of urea in rats treated EAFH than control and AOI groups. Rats treated EAFH had their creatinine and potassium concentrations reduced than control and AOI groups, in a significant ($p<0.05$) level (Figure 5). Chloride concentration in HFD-obese treated rats (Figure 6), was reduced by MIX and MFLJ treatment than in control and AOI groups, and was increased by EAFH than AOI group but reduced than control group, in significant ($p<0.05$) way.

DISCUSSION

In the oral acute toxicity study (Table 1), it was found that there was no sign of toxicity or mortality observed at doses of 300 mg/kg and 2000 mg/kg of administered FLJ, RH, MFLJ and EAFH, within two weeks of LD_{50} study, and thus LD_{50} was not determined or calculated for FLJ, RH, MFLJ and EAFH administered to rats in this study. This implied that the LD_{50} value would be higher than 2000 mg/kg body weight. Based on the Globally Harmonized System of Classification and Labeling of chemicals (GHSCLC), FLJ, RH, MFLJ and EAFH at 2000 mg/kg body weight may be classified as Category 5[16].



	Control	AOI	MIX	MFLJ	EAFH
TP (g/dl)	8.89	5.75	8.19	7.14	6.46
AST (U/L)	41	95	15	26	87.5
ALT (U/L)	15.5	62.5	7	13.5	58
D-BIL (mg/dl)	1.92	3.49	0.72	1.26	1.39
T-BIL (mg/dl)	2.23	4.61	1.68	1.46	0.89

Figure 3: Effects of MIX, MFLJ and EAFH on serum TP, AST, ALT, D-BIL and T-BILHFD-obese treated rats (n=4, p<0.05).

AOI: After Obesity Induction; HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: Ethyl acetate Flavonoid Rich Fraction of Honey; TP: Total protein; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; D-BIL: Direct Bilirubin; T-BIL: Total Bilirubin.

Table 3: Lee index, adiposity index and diet intake of rat

	LB	LA	Adipose Index	Diet intake
Control	0.25±0.035[a]	0.28±0.01[a]	1.30±1.13[a]	46.07±9.04[a]
HFD	0.26±0.01[a]	0.51±0.00[b]	5.93±0.58[b]	184.00±13.39[b]

The safety of FLJ, RH, MFLJ and EAFH in this study is confirmed by recent studies, which reported that the LD_{50} of honey was up to 5000 mg/kg for two days[4], and lime juice was up to 5000 mg/kg for 14 days[12]. In the course of this study, administration of FLJ, RH, MFLJ and EAFH does not cause any observable abnormal physical toxicological symptoms such as loss of fur, diarrhea, sleep, aggressiveness, fatigue, coma or mortality. For EAFH, the studies of Samat *et al.*[22], and Suhana *et al.*[23], agree with this observation, who both reported that 2000 mg/kg dose of Gelam and Acacia honey administered to rats did not result in any toxicological signs. However, for MFLJ, the report of Obiajulu *et al.*[24], does not agree with the observation of this study. In their report, rats which received dose of 60 and 100 ml/kg showed signs of toxicity and eventually, resulted in mortality. Meanwhile, 300 and 2000 mg/kg MFLJ were administered to rats in this study, which was far higher than that of Obiajulu *et al.*[24], and no mortality was recorded. The conferred safety on EAFH and MFLJ could be due to the anti-toxicity, anti-inflammatory and antioxidant property of flavonoids in the MFLJ flavonoid rich fraction[8].

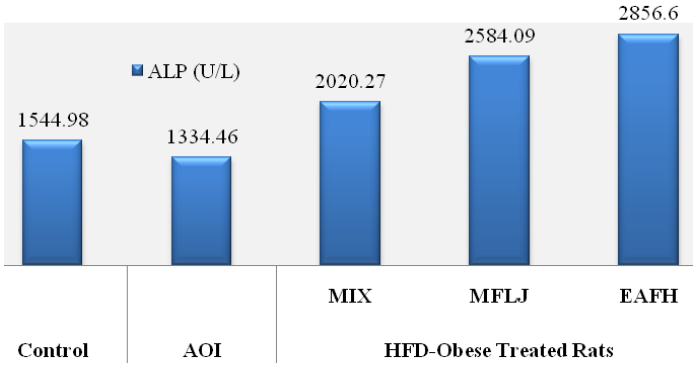


Figure 4: Effects of MIX, MFLJ and EAFH on serum ALP in HFD-obese treated rats (n=4, p<0.05).

AOI: After Obesity Induction; HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: Ethyl acetate Flavonoid Rich Fraction of Honey; ALP: Alkaline Phosphatase.

During the LD_{50} study (Table 2), the body weight of rats administered FLJ, RH, MFLJ and EAFH was not adversely affected. Judging from the last day of the study, rats significantly (p<0.05) gained weight, compared to control in all 300 mg/kg dose and significantly (p<0.05) decreased in weight compared to control in all 2000 mg/kg dose of FLJ, RH, MFLJ and EAFH administered. However, on the contrary, Zulkhairi *et al.*[25], reported that in either dose case, there was no significant (p<0.05) change in body weight of rats in their acute toxicity study. The body weight of HFD-obese treated rats was significantly (p<0.05) reduced compared with the HFD-obese rats (AOI), as seen in Figure 1 and Figure 2 and there was significant (p<0.05) increase of body weight in MFLJ and EAFH treated rats compared with control. Again, the studies of Samat *et al.*[22], and Suhana *et al.*[23], supported this observation, were Gelam and Acacia honey were

observed to increase the body weight of rats when test group was compared with normal control group. The gain in body weight could be attributive to the fact that honey is a rich source of amino acids and other nutrients, which could support buildup of body mass[22]. The Lee index values (Table 3) which established obesity in rats in this study is consistent with the report of Idoko *et al.*[5]. In this study, HFD-obese rats ate food the most, compared with control. This model of obesity in association with hyperphagia (excessive eating) in neonatal fed high fat diet is connected with increased lipogenesis, decreased adipose tissue lipolysis and raised plasma corticosterone concentration[26].

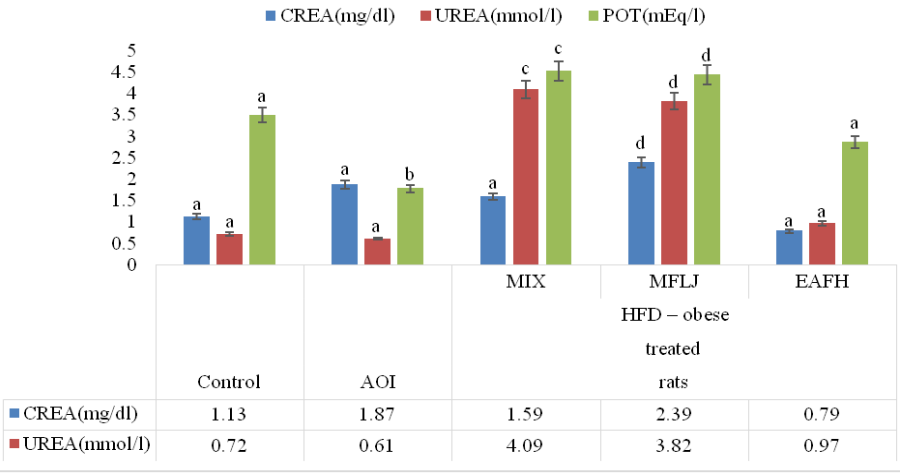


Figure 5: Effects of MIX, MFLJ, and EAFH on serum CREA, Urea, and POT in HFD-obese treated rats (n=4, $p<0.05$).

AOI: After Obesity Induction; HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: Ethyl acetate Flavonoid Rich Fraction of Honey; CREA: Creatinine; POT: Potassium.

The percentage adiposity index of rats treated HFD (Table 3) was found to be higher than normal control rats, but treatment with MIX, MFLJ and EAFH, reduced the % adiposity index as compared with normal control and AOI. This result agrees with the report of Suhana *et al.*[23], as the relative organ weight of high fat diet group was reduced by treatment with Gelam honey, Acacia honey and orlistat.

Figure 3 and Figure 4 reveal the result of liver function test. There was no significant ($p<0.05$) decrease in concentration of TP in rats treated MIX, MFLJ and EAFH compared with control, but was higher compared with AOI group (HFD-obese rats). This study agrees with the report of Úrsula *et al.*[27], who reported the concentrations of total protein and albumin to be higher in healthy rats than in obese rats treated fruit purees. Obiajulu *et al.*[24], reported a non-significant ($p<0.05$) effect of *C. aurantifolia* fruit juice on serum concentrations of total protein, albumin, total bilirubin, K^+, Na^+, Cl^- and HCO^{3-} for the 3 doses tested when compared to the normal control in a toxicological study. In obese and overweight individuals, serum total protein concentration was reported to be associated with the onset of prehypertension and hypertension[28]. While ALT activity in HFD-obese treated EAFH rats was significantly ($p<0.05$) higher. The observations of this study are in agreement with El-Haskoury *et al.*[29], who reported reduced activities in serum AST, ALT and ALP in aqueous and ethyl acetate extract of carob honey in streptozotocin -induced diabetic rats; and Obiajulu *et al.*[24], who also reported significant ($p<0.05$) decreased in serum activities of AST, ALT and ALP for the three groups of rats administered different doses of *C. aurantifolia* fruit juice.

Damage to the liver by a substance or a plant extract (fractionated or crude) is determined by assaying the cerum activities/concentrations of liver function parameters which include ALT, AST, ALP, TP, Abumin, T-Bil and D-Bil[30].

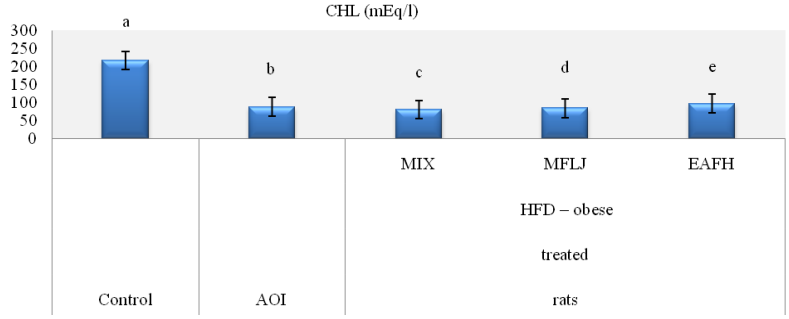


Figure 6: Effects of MIX, MFLJ and EAFH on serum CHL in HFD-obese treated rats (n=4, $p<0.05$).

AOI: After Obesity Induction; HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: Ethyl acetate Flavonoid Rich Fraction of Honey; CHL: Chloride.

The healthy and functional state of the liver is specifically linked to the cellular cytoplasmic release of ALT[24]. Thus, the higher serum activities of ALT, AST and ALP in the various obesity models in this study suggest hepatocellular injury, as compared to the non-obese control rats. Meanwhile, treatment with MIX, MFLJ and EAFH reduced the higher levels of AST, ALT and ALP as revealed in obese rats[29]. The hepatoprotective ability demonstrated by MIX, MFLJ and EAFH could be attributed to the antioxidant compounds (flavonoids) present in both *C. aurantifolia* fruit juice and honey and was found to reduce damage to tissue in the treated obese rats by antioxidant free radical scavenging of free radicals; thus, decreased amount of free radical in tissue could also implies restoration of cellular architecture due to decreased amount of metabolites[27]. The serum concentrations of D-Bil and T-Bil HFD-obese rats treated MIX, MFLJ and EAFH was significantly (p<0.05) decreased than in control and AOI rats. The result of this study is consistent with the study of Úrsula *et al.*[27], who reported that the serum concentrations of T-Bil and D-Bil in obese rats was higher than in healthy control rats and rats treated with fruit purees. But on the contrary, results from Obiajulu *et al.*[24], revealed that serum T-Bil and D-Bil concentrations was reduced in *C. aurantifolia* fruit juice treated rats than in normal control rats. It was reported that obesity and serum concentration of bilirubin are bi directionally related[31], and serum concentration of bilirubin is not dependently and directly associated with adiposity or body mass index[32]. Obesity and bilirubin are bidirectionally related such that in obesity, serum total bilirubin concentration may decrease by gut microbiota modification[33], and decrease in serum total bilirubin concentration may prevent insulin resistance by improving visceral obesity and adipose tissue inflammation[31].

Administration of MIX and MFLJ significantly (p<0.05) increased the concentrations of creatinine, urea and potassium than in control and AOI groups. EAFH had significant (p<0.05) elevated effect on the concentration of urea than control and AOI groups. Rats treated EAFH had their creatinine and potassium concentrations significantly (p<0.05) reduced than control and AOI groups (Figure 5). There was significant (p<0.05) decrease in chloride concentration in HFD-obese rats treated MIX and MFLJ (Figure 6),compared with control and AOI groups, and increase in EAFH treated rats than AOI group, but decrease than control group. Akpevwoghene *et al.*[34], gave a different report, of significant (p<0.05) rise in serum chloride concentration in rats treated honey than control. No significant (p<0.05) change in serum chloride in rats administered lime fruit juice compared with control[24]. A physiological factor that determines a balance homeostasis of chloride concentration is the balance of sodium concentration[35]. In obesity, chlorine imbalance may result from the following; excess fats accumulation, resulting in high blood circulation volume, heart beats faster and raised cardiac output, caused by hemodilution from high blood pressure in obese state[36]. The result of serum creatinine and urea concentrations in this study is consistence with Akpevwoghene *et al.*[34], where excess Bee honey, fed rats had their creatinine and urea concentrations significantly (p<0.05) higher in test rats than in control. Similarly, Suhana *et al.*[23], reported that serum urea was significantly (p<0.05) lower in high fat diets obese rats, treated with Gelam and Acacia honey than in normal control while serum creatinine concentration was significantly (p<0.05) higher in high fat diets obese rats, treated with Gelam and Acacia honey. Renal function is mostly measured by creatinine and urea concentrations, and not necessarily a measure of renal toxicity[37]. An indication of creatinine in the blood suggests the ability of the kidney to remove and produce same[38]. The glomerular filtration rate (GFR), a more reliable measure of kidney function than independent estimation of creatinine or urea, measures the ration of urea: creatinine, and it is reported to be reduced in elevated creatinine concentration, resulting in renal disease (chronic and acute renal disease)[38,39]. Honey and lime juice contain appreciable content of amino acid and protein, and this could support the increased serum concentration of urea, as breakdown product of protein[39]. Thus, urea concentration might be high in the blood, in none renal disease state due to the amount produced by the liver and cleared by the kidney via excretion in urine[37]. This study is consistent with the report of Brurya *et al.*[40], where increase in dietary intake of K was significantly associated with loss of weight and reduced BMI. Thus, MIX, MFLJ and EAFH, confer electrolyte-protective ability, supporting cellular electrolyte balance[24].

Limitations of the study

Isolation of these flavonoid rich fractions should be done, and molecular docking and simulation studies may be carried out to investigate the anti-obesity potential of these flavonoids on a targeted obesity receptor protein, for drug development.

CONCLUSIONS

In this study, the oral acute toxicity study on lime juice, honey and their flavonoid rich fractions, revealed they can be consumed safely, without toxic side effect. Also, the anti-obesity study on high fat-obese rats reveal they were also found to possess liver and kidney protective effects, due to the rich flavonoids they contain. Thus, MFLJ and EAFH could be potential source of anti-obesity agents.

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AUTHOR'S CONTRIBUTION

Idoko A: writing original draft, methodology, investigation. **Parker JE:** conducted the fieldwork as part of his PhD studies. **Njoku OU:** writing, review

and editing, methodology. **Ugwudike PO:** formal analysis, supervision. **Jennifer NC:** writing, review, and editing, methodology, data curation. All authors revised the article and approved the final version.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

There is no conflict of interest in connection to this paper.

REFERENCES

1. de Barros NF, Fiuza AR. Evidence-based medicine and prejudice-based medicine: The case of homeopathy. Public Health Notebooks 2014;30(11):2368-2376
2. Miller JS. The global importance of plants as sources of medicines and the future potential of Chinese plants. Lin Y. (eds) Drug Discovery Trad Chinese Med 2001; 33-42. *https://doi.org/10.1007/978-1-4615-1455-8_4*
3. Idoko A, Ikpe VPO, Nelson NO, *et al*. Effects of lime juice and honey on lipid profile of cholesterol enriched diet fed rat model. Annual Res Rev Biol 2017; 20(3):1-10. *https://doi.org/10.9734/ARRB/2017/37213*
4. Ifeanyi PO, Chioma VA, Omoirri MA, *et al*. Acute toxicity, hepatotoxicity and renal-toxicity profile of the crude methanol extract of *Mallotus oppositifolius* (Geisel.) (Euphorbiaceae) combined with honey in albino rats. GSC Biol Pharm Sci 2023;23(01):182–192.e *https://doi.org/10.30574/gscbps.2023.23.1.0120*
5. Idoko A, Parker JE, Njoku OU. Ethyl acetate flavonoid biocompounds of honey with mitigating anti-hyperlipidemic and antioxidant properties in carbohydrate and lipid enriched diets – Obese rats. Annual Res Rev Bio 2023; 38(9): 1-23. *https://doi.org/10.9734/arrb/2023/v38i930603*
6. Onyeka IP, Onyegbule FA, Ezugwu CO, *et al*. Gastroprotective effects of methanol leaf extract of *Desmodium velutinum* (Fabaceae) and honey on ethanol induced gastric ulcer in albino rat: The concept of combination therapy. GSC Biol Pharma Sci 2022; 20 (1): 167-181.*http://dx.doi.org/10.30574/gscbps.2022.20.1.0262*
7. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, *et al*. Discovery and resupply of pharmacologically active plant derived natural products: A review. Biotechnol Adv 2015; 33(8):1582-1614. *https://doi.org/10.1016/j.biotechadv.2015.08.001*
8. Madhavan R, Muthukumar NJ, Savariraj SC, *et al*. Studies on the safety profiles of a Siddha preparation-Thirithodamathirai. Biomed 2022; 42(3): 605-611. *http://dx.doi.org/10.51248/.v42i3.1242*
9. Bukola CA, Temitayo OA, Olubusola AO. Phytochemical composition and comparative evaluation of antimicrobial activities of the juice extract of *Citrus aurantifolia* and its silver nanoparticles. Nig J Pharma Res 2016; 12(1):59-64.
10. Asokan S, Jayanthi I. Phytochemical analysis of various honey samples obtained from Theni District, South India. Int J Curr Res 2017;9(1):45387-45390.
11. Ahmed AJ, Kamaran KA, Parween A, Sharoukh M, Gülda M, Abdullah SS. Phytochemical profile, antioxidant, enzyme inhibitory and acute toxicity activity of *Astragalus bruguieri*. Baghdad Sci J 2023; 20(1): 157-165. *http://dx.doi.org/10.21123/bsj.2022.6769*
12. Deborah D, Ifedolapo AA, Ismail I, Moshood A, Margaret OS, Olufunmilayo OA. The antidiabetic effect of the lime juice (*Citrus aurantifolia*) extract of *Ficus exasperata* in Streptozotocin induced rats. The Nig J Pharm 2023; 57(2): 602-612. *http://dx.doi.org/10.21123/bsj.2022.6769*
13. Shagun J, Poonam AA, Harvinder P. A comprehensive review on *Citrus aurantifolia* essential oil: Its phytochemistry and pharmacological aspects. Brazilian J Nat Sci 2020;3(2):354–364. *http://dx.doi.org/10.31415/bjns.v3i2.101*
14. Wang S, Moustaid-Moussa N, Chen L, *et al*. Novel insights of dietary polyphenols and obesity. The J Nutr Biochem 2014; 25(1):1–18. *https://doi.org/10.1016/j.jnutbio.2013.09.001*
15. Nakagawa T, Ukai K, Ohyama T, Gomita Y, Okamura H. Effects of chronic administration of sibutramine on body weight, food intake and motor activity in neonatally monosodium glutamate-treated obese female rats: Relationship of antiobesity effect with monoamines. Exp Animals 2000; 49:239–249. *https://doi.org/10.1538/expanim.49.239*
16. OECD. Acute Oral Toxicity – Fixed Dose Procedure. Acute Oral Toxic Class Method Guideline 423 adopted 17.12.2001. In: Eleventh Addendum to the OECD guidelines for the testing of chemicals organization for economic co-operation development, Paris, June, 2000.
17. Reitman S, Frank S. Transaminases. Am J Clin Path 1957; 28: 56.
18. Englehardt A. Measurement of alkaline phosphatase. Aerztl Lab 1970; 16:42-43.
19. Jendrassik L, Grof P. *In vitro* determination of total and direct bilirubin. Biochemica 1938; 297: 81.
20. Bartels H, Bohmer M. *In vitro* determination of creatinine and urea. Clin Chem 1972; 2: 37-193.
21. Henry JB. Clinical Diagnosis and Management by Laboratory Methods, Philadelphia, W.B. Saunders 1984; 1434.
22. Samat S, Mohd NN, Hussein FN, Eshak Z, Ismail WIW. Short-term consumption of gelam honey reduces triglyceride level. Int Food Res J 2017; 24(4): 1519-1524.
23. Suhana S, Francis KE, Fuzina NH, Wan IWI. Four-week consumption of Malaysian honey reduces excess weight gain and improves obesity-related parameters in high fat diet induced obese rats. Evidence Based Compl Alter Med 2017; 2017: 1-9.*http://dx.doi.org/10.1155/2017/1342150*
24. Obiajulu CE, Onuabuchi NA, Michael CO. Toxicological studies of *Citrus aurantifolia* fruit juice in wistar rats. Asian J Biochem Gen Mol Biol 2022;10(4): 38-47. *http://dx.doi.org/10.9734/ajbgmb/2022/v10i430251*
25. Zulkhairi AFA, Shafiq CMZ, Sabri S, *et al*. *In vivo* toxicity assessment of the *Probiotic Bacillus* amyloliquefaciens HTI-19 isolated from stingless bee (*Heterotrigona itama*) Honey. Nutrients 2023; 15:2390. *https://doi.org/ 10.3390/nu15102390*
26. Guimaraes RB, Telles MM, Coelho VB, *et al*. Adrenalectomy abolishes the food-induced hypothalamic serotonin release inboth normal and monosodium glutamate-obese rats. Brain Res Bull 2002; 58:363–369. *https://doi.org/10.1016/S0361-9230(02)00799-2*
27. Úrsula MM, Eduardo MB, Sonia GS, John PT, Efigenia M. Anti-obesity and hepatoprotective effects in obese rats fed diets supplemented with fruit purees. Food Sci Tech 2020; 40(1): 33-41.*https://doi.org/10.1590/fst.31618*
28. Malhotra R, Cavanaugh KL, Blot WJ, *et al*. Higher protein intake is associated with increased risk for in cident end-stage renal disease among blacks with diabetes in the southern community cohort study. Nutr Metab Cardio Dis 2016; 26:1079-1087. *https://doi.org/10.1016/j.numecd.2016.07.009*
29. El-Haskoury R, Al-Waili N, El-Hilaly J, Al-Waili W, Lyoussi B. Antioxidant, hypoglycemic, and hepatoprotective effect of aqueous and ethyl acetate extract of carob honey in streptozotocin-induced diabetic rats. Vet World 2019;12(12):1916-1923. *https://doi.org/10.14202/vetworld.2019.1916-1923*
30. Ezeigwe OC, Nzekwe FA, Nworji OF, Ezennaya CF, Iloanya EL, Asogwa KK. Effect of aqueous extract of *F capensis*

leaves and its combination with *C. aconitifolius* leaves on essential biochemical parameters of phenylhydrazine-induced anemic rats. J Exp Pharma 2020; 12:191-201. *https://doi.org/10.2147/jep.s254003*

31. Takei R, Inoue T, Sonoda N, *et al*. Bilirubin reduces visceral obesity and insulin resistance by suppression of inflammatory cytokines. PLoS ONE, 2019; 14(10):e0223302. *https://doi.org/10.1371/journal.pone.0223302*
32. Seyed KN, Grindel A, Wallner M, *et al*. Mild Hyperbilirubinaemia as an endogenous mitigator of overweight and obesity: Implications for improved metabolic health. Atherosclerosis 2018; 269:306–311. *https://doi.org/10.1016/j.atherosclerosis.2017.12.021*
33. Khan MJ, Gerasimidis K, Edwards CA, Shaikh MG. Role of gut microbiota in the aetiology of obesity: Proposed mechanisms and review of the literature. J Obesity 2016; 2016:7353642. *https://doi.org/10.1155/2016/7353642*
34. Akpevwoghene A, Jerome NA, Olusegun GA. Liver and renal cell damage following excess bee honey consumption in male wistar rat. Biol Med Nat Prod Chem 2022;11(1):35-43. *https://doi.org/10.14421/biomedich.2022.111.35-43*
35. Joseph J, Sunil B, Andrew LC, Joseph J, Sunil B, Andrew LC. Hypochloraemia in patients with heart failure: causes and consequences. Cardiol Ther 2020; 9:347-351. *https://doi.org/10.1007/s40119-020-00194-3*
36. Abebe T, Kassahun H. Patterns of Calcium- and Chloride-ion disorders and predictors among obese outpatient adults in Southern Ethiopia. Diabet Metab Synd Obesity Target Ther 2021; 14(2021):1349–1358. *https://doi.org/10.2147/dmso.s300434*
37. Rock RC, Walker WG, Hennings CD. Nitrogen metabolites and renal function. In: Tietz NW, ed. Fundamentals of Clinical Chemistry, 3rd ed. Philadelphia: WB Saunders1987; 669-704.
38. Nisha R, Srinivasa KSR, Thanga MK, Jagatha P. Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. J Clin Path Lab Med 2017; 1(2): 1-5.
39. Idoko A, Philip OC, Nwali ON, *et al*. Effects of raw and cooked aqueous and methanol extracts of *Phaseolus vulgaris* (Kidney Beans) on renal function in albino wistar rats. Universal J Pharm Res 2020; 5(3):6-11. *https://doi.org/10.22270/ujpr.v5i3.408*
40. Brurya T, Jessica S, Marianna Y, Gabi S, Assaf B, Limor BH, *et al*. Nutrients 2019;11(1256):1-11.