**Original Research Article**

**ACUTE TOXICITYAND HEPATO-RENAL PROTECTION OF LIME JUICE, HONEY AND THEIR FLAVONOID-RICH FRACTIONS****IN HIGH FAT-DIET INDUCED OBESE RATMODEL**

**ABSTRACT**

**Introduction:** A major consideration for the use of alternative herbal medicinefrom natural compounds is the concern of safety. This study evaluated the safety andhepato-renal protectionof fresh lime juice (FLJ), raw honey (RH) and their flavonoid-rich fractions in high fat-diet induced obese rat model.

**Methods:** Oral acute toxicity (LD50) 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 ethylacetate flavonoid rich fraction of honey (EAFH) respectively, for 14 days.Simultenously, 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 LD50onFLJ, RH, MFLJ, and EAFHshowed no toxicity, nomotality, 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 andalanine 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.

**Keyword:**Acute toxicity, Lime juice, Honey, High fat diet, Renal function, Liver function

**INTRODUCTION**

Plant use and applications as alternative medicine by traditionists have experienced bias of late, owing to the arrival of orthodox or conversional medicine1. 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 development2.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 diseases2-5. Theseinclude mixture of honey and Desmodiumvelutinum as anti-ulcer therapy6, honey and lime juice mixture as anti-hypercholesterolemia3, and as anti-obesity5 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 medicine7.Lemon juice was used to neutralize the toxic effect of veeram, during its preparation. Veeramis composed of mercury chloride (Hg2Cl2) employedin the treatment of gonorrhea, syphilis, stomach ulcer and8. Honey in combination with extract of Mallotusoppostifollous was reported to be a safe phytomedicine, possessing protective effects on the kidney and liver4. 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 diseases5. Citrus aurantifolia fruit juice and honey, are reported to contain several phytochemicals such as flavonoid, alkaloids, carbohydrates, amino acids, proteins, glycosides, phenols, saponins, tannins, phlobtannins and terpenoids9,10. These phytochemicals are the agents responsible for the bioactivities in plants as alternative medicine for treatment, management and prevention of several diseases11. These bioactivities include antioxidant and anti-hyperlipidemic5, anti-inflammatory, antifungal, anti-microbial9, anti-diabetes12, and enzyme inhibitory and inductive activities11.Flavonoids in citrus and honey, including quercetin, p. coumarin, epigallocatechin, caffeic acid, sinapic acid, naphthoresorcinol, gallic acid, apigenin, rutin, kaempferol, nobiletin, hesperidin, hesperitin, and neohesperidin are known for their significant antioxidant, anti-obesity, anti-hyperlipidemia, anti-inflammatory, anti-tumor, anticancer, anti-prostatitis, anti-allergic and antiasthmatic5,13. Flavonoids impact their anti-obesity effectbytheir 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 absorption14.

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 consumed8. 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 toxicitystudy on animals. The median lethal oral dose, known as LD50 is established in this study11.It is the statistically derived dose of a substance expected to cause death in 50 % animals when administered by oral rout4,11. The LD50 value is 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 fractionswith hepatic and renal protectionin high fat-diet 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. At the end of each phase, rats were sacrificed and blood samples and organs were collected for biochemical analysis.

**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-Fatdiet-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 methanol flavonoid rich fraction of lime juice (MFLJ)

Group 4: High-Fat diet-obese rats**c+** 200 mg/kg oral administration of ethylacetate flavonoid-rich fraction of honey (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;

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

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

Adiposity index was determined by the total weight of epididymal, visceral, and retroperitoneal fat divided by body weight × 100 and expressed as adiposity percentage (% AI).

$$Adiposity index (\% AI)=\frac{Total weight of epididymal, visceral and retroperitoneal fat }{ Body weight}×100$$

**Oral Acute Toxicity (LD50) Study of** **FLJ, RH, MFLJ and EAFH**

Twenty-four female rats of 10 weeks old (75– 167 g) were used to determine the LD50 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 over-night. After fasting, rats were weighed and extracts administered; and thereafter, weight was taken weekly. This study was done following the method described by OECD16, with little modification, and 300 mg/kg was selected as the starting dose. Thus, 300 mg/kg and 2000 mg/kg of extracts were administered to 3 rats each. After the administration, food was further withheld for 2 hours. Doses were administered after 24 hours and animals were individually observed every 30 minutes during the first 24 hours, with special attention in the first 4 hours, and daily for a period of 14 days. A single dose of extract was administered orally and observed from the time of administration, for toxic symptoms, such as changes in skin and fur, eyes and mucous membranes, behavioral changes, tremors, loco-motion, salivation, diarrhea, convulsion, lethargy, sleep and coma/mortality. LD50 (median lethal oral dose), is the statistically derived dose of a substance expected to cause death in 50 % animals when administered by oral rout. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg). This was calculated by the formula below;

LD50= $\sqrt{Min}. Conc. that caused death ×Max. conc. that result to no death$

**Blood and Tissue collection**

Blood was collected into plain and EDTA tubes by cardiac puncture under light chloroform anesthesia after an overnight fast. The serum was separated by a retro fraction. The plasma was separated from the erythrocytes by centrifuging the whole blood at 5000 rpm for 10 minutes. The liver organ and adipose tissue were excised, rinsed with normal saline, blotted dry, and weighed immediately. All samples were stored at -20oC until analyzed.

**Preparation and Composition of High-Fat diet in g/1000 g**

High fat diet was prepared according to the method of Idoko et al. (2023), with a little modification: Normal feed:300, Chichen skin:84, Skin of pork:161, Butter:85, and Yoke of egg:370.

**Body Weight Measurement of Rat**

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

**Liver Function and Kidney Function Tests**

Randox Kit was used to carry out the liver function and kidney function tests. Alanine aminotransferase (ALT) activity,by method of Reitman and Frankel17, aspartate aminotransferase (AST) activityby method of Reitman and Frankel17, alkaline phosphatase (ALP) activity by Englehardt18 method, concentrations of total protein (TP)and bilirubin (Bil) byJendrassik and Grof19 method. Urea and creatinine concentrations by Bartels and Bohmer20, concentrations of chloride and potassium by Henry et al.21.

**RESULTS**

**Acute oral toxicity study (LD­50)**

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.

The body weight (Table 2) of rats in this oral acute toxicity study was not adversely affected as there was no significant (p < 0.05)declined weight loss (Tables1 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 LD50 study dosing at 300 and 2000 mg/kg significantly(p < 0.05)reduced body compared with FLJ and RH.

**Table 1: Result ofAcute oral toxicity study (LD­50)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 |

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

**Table 2: Effects of FLJ, RH, MFLJ and EAFH on body weight of rats after LD50 study at 300 and 2000 mg/kg doses.**

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

Results are Mean ± SD; comparing normal control with groups of rats treated various doses (300 and 2000 mg/kg) of FLJ, RH, MFLJ and EAFH respectively. Values with different alphabets in a column, comparing control with a group are significantly (p < 0.05) different, and (n=3). Key: **FLJ**=Fresh lime juice; **EAFH**= Ethylacetate flavonoid rich fraction of honey;**MFLJ**=Methanol flavonoid rich fraction of lime juice.

**Body Weight, Lee Indices, and Adiposity Indices of Treated Rats**

Figures 1 and 2show the body weight of rats both after obesity induction (AOI) and treatment withMIX, MFLJ and EAFH.In HFD – obese treated rats, the body weight of rats 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 withMIX, 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.

**Figure 1:Effects of MIX, MFLJ and EAFH on body weight of HFD-obese treated rats**

Results are Mean ± SD; Comparing control with HFD-obeserats, treated MIX, MFLJ and EAFH. Bars with different alphabets comparing control and a group are significantly (p< 0.05) different, and (n=4). Key: HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: EthylacetateFlavonoid Rich Fraction of Honey.

**Figure 2:Effects of MIX, MFLJ and EAFH on weight gain of HFD diets obese treated rats**

Results are Mean ± SD; Comparing control with HFD-obeserats, treated FLJ, RH, MIX, MFLJ and EAFH. Bars with different alphabets comparing control and a group are significantly (p< 0.05) different, and (n=4). Key: HFD-obese: High fat diet-obese rats; MFLJ: Methanol Flavonoid Rich Fraction of Lime Juice; MIX: Mixture of FLJ (50%) and RH (50%); EAFH: EthylacetateFlavonoid Rich Fraction of Honey.

**Table 3: Lee index, adiposity index and diet intake of Rat**

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

Results are Mean ± SD; Comparing control with HFD-obese rats. n = 12. Values within the same column with different alphabets are significantly (p < 0.05) different from each other.

**Liver Function and Kidney Function Tests**

In HFD-obese treated rats (Figures 3 and 4), MIX, MFLJ and EAFH treated rats had their TP concentrations significantly (p < 0.05) lower than control but significantly (p < 0.05) 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 concentration was significantly (p < 0.05) higher in EAFH rats than control, but significantly (p < 0.05) lowered than AOI group. ALT concentration was significantly (p < 0.05) reduced in rats treated MIX and MFLJ, and significantly (p < 0.05) raised in EAFH rats 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.

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. EAFH significantly (p < 0.05) increased 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). Chloride concentration in HFD-obese treated rats (Figure 6), was significantly (p < 0.05) reduced by MIX and MFLJ treatment than in control and AOI groups, and was significantly (p < 0.05) increased by EAFH than AOI group but significantly (p < 0.05) reduced than control group.

**Figure 3: Effects of MIX, MFLJ and EAFH on serum TP, AST, ALT, D-BIL and T-BILHFD-obese treated rats**

Results are Mean ± SD; comparing normal control with AOI group and HFD-obese rats, treated MIX, MFLJ and EAFH. Bars with different alphabets comparing control and a group are significantly (p < 0.05) different, and (n=4). Key: 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: EthylacetateFlavonoid Rich Fraction of Honey; TP: Total protein; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; D-BIL: Direct Bilirubin; T-BIL: Total Bilirubin.

**Figure 4: Effects of MIX, MFLJ and EAFH on serum ALP in HFD-obese treated rats**

Results are Mean ± SD; comparing normal control with AOI group and HFD-obese rats, treated MIX, MFLJ and EAFH. Bars with different alphabets comparing control and a group are significantly (p < 0.05) different, and (n=4). Key: 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: EthylacetateFlavonoid Rich Fraction of Honey; ALP: Alkaline Phosphatase.

**Figure 5: Effects of MIX, MFLJ and EAFH on serum CREA, Urea and POT inHFD-obese treated rats**

Results are Mean ± SD; comparing normal control with AOI group and HFD-obese rats, treated MIX, MFLJ and EAFH. Bars with different alphabets comparing control and a group are significantly (p < 0.05) different, and (n=4). Key: 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: EthylacetateFlavonoid Rich Fraction of Honey;CREA: Creatinine; POT: Potassium.

**Figure 6: Effects of MIX, MFLJ and EAFH on serum CHLinHFD-obese treated rats**

Results are Mean ± SD; comparing normal control with AOI group and HFD-obese rats, treated MIX, MFLJ and EAFH. Bars with different alphabets comparing control and a group are significantly (p < 0.05) different, and (n=4). Key: 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: EthylacetateFlavonoid Rich Fraction of Honey; CHL: Chloride.

**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 LD­50 study, and thus LD50 was not determined or calculated for FLJ, RH, MFLJ and EAFH administered to rats in this study. This implied that the LD50 value would be higher than 2000 mg/kg body weight. Based on the Globally Harmonized System of Classification and Labelling of chemicals (GHSCLC), FLJ, RH, MFLJ and EAFH at 2000 mg/kg body weight may be classified as Category 516.The safety of FLJ, RH, MFLJ and EAFH in this study is confirmed by recent studies, which reported that theLD50 of honey was up to 5000 mg/kg for two days4, and lime juice was up to 5000 mg/kg for 14 days12.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, this observation is consistent with the studies of Samat et al.22 and Suhana et al.23, 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 flavonoids rich fraction8.

During the LD50 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) reducedcompared with the HFD-obese rats (AOI), as seen in Figures1 and 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 mass22.

The Lee index values (Table 3) which established obesity in rats in this study is consistent with the reportofIdoko 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 fedhigh fat diet, is connected with increased lipogenesis, decreased adipose tissue lipolysis and raised plasma corticosterone concentration26. 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 observation is consistent with the report of Suhana et al.23, where the Relative organ weight of high fat diet group was reduced by treatment with Gelam honey, Acacia honey and orlistat.

Figures 3 and 4 reveal the result of liver function test. The concentration of total protein (TP) was not significantly (p <0.05) lower in rats treated MIX, MFLJ and EAFH than control, but was significantly (p <0.05) higher than AOI group (HFD-obese rats). This study is consistent 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 *citrus aurantifolia* fruit juice on serum concentrations of total protein, albumin, total bilirubin, K+, Na+, Cl- and HCO3- 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 hypertension28.The serum AST and ALT activities in HFD-obese rats treated MIX and MFLJ was significantly (p<0.05) lower than control and AOI groups. AST activity in EAFH treatedrats was significantly (p<0.05) lower than AOI group. While ALT activity in HFD-obese treated EAFH rats was significantly (p<0.05) higher. The observations of this study are consistent with the work of 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 *citrus 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-Bil30. The healthy and functional state of the liver is specifically linked to the cellular cytoplasmic release of ALT24. 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 rats29. The hepatoprotective ability demonstrated by MIX, MFLJ and EAFH could be attributed to the antioxidant compounds (flavonoids) present in both citrus 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 metabolites27. 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 *citrus aurantifolia* fruit juice treated rats than in normal control rats. It was reported that obesity and serum concentration of bilirubin are bidirectionally related31, and serum concentration of bilirubin is not dependently and directly associated with adiposity or body mass index32. Obesity and bilirubin are bidirectionally related such that in obesity, serum total bilirubin concentration may decrease by gut microbiota modification33, and decrease in serum total bilirubin concentration may prevent insulin resistance by improving visceral obesity and adipose tissue inflammation31.

Administration of MIX and MFLJ significantly (p < 0.05) increased the concentrations of creatinine, urea and potassium than in control and AOI groups. EAFH significantly (p < 0.05) increased 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). Chloride concentration in HFD-obese treated rats (Figure 6), was significantly (p < 0.05) reduced by MIX and MFLJ treatment than in control and AOI groups, and was significantly (p < 0.05) increased by EAFH than AOI group but significantly (p < 0.05) reduced than control group.Akpevwoghene et al.34 gave a different report, where serum chloride concentration was significantly (p < 0.05) increased in rats treated honey than control. There was no significant (p < 0.05) change in serum chloride in rats administered lime fruit juice compared with control24. A physiological factor that determines a balance homeostasis of chloride concentration is the balance of sodium concentration35. 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 state36.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 toxicity37. An indication of creatinine in the blood suggests the ability of the kidney to remove and produce same38. 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 protein39. 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 urine37. 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 balance24.

**CONCLUSION**

In this study, the oral acute toxicity study onlime 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.

**CONFLICT OF INTEREST** No conflict of interest is associated with this work.

**ACKNOWLEDGEMENTS**

The authors extends appreciation to the Department of biochemistry, University of Nigeria, Nsukka and Department of Biochemistry Caritas University, Enugu, Nigeria to provide necessary facilities for this work.

**REFERENCES**

1. de Barros NF, Fiuza AR. Evidence-based medicine and prejudice-based medicine: The case of homeopathy. Cadernos de SaúdePública. 2014;30(11):2368-2376
2. Miller JS. The Global Importance of Plants as Sources of Medicines and the Future Potential of Chinese Plants. In: Lin Y. (eds) Drug Discovery and Traditional Chinese Medicine. 2001 pp 33-42. Springer, Boston, MA. <https://doi.org/10.1007/978-1-4615-1455-8_4>.
3. Idoko A, Ikpe VPO, Nelson NO, Effiong JU, Alhassan AJ, Muhammad IU, *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. DOI: 10.9734/ARRB/2017/37213
4. Ifeanyi PO, Chioma VA, OmoirriMA, Nnamdi MA, Gabriel OO, Felix AO, *et al.* Acute toxicity, hepatotoxicity and renal-toxicity profile of the crude methanol extract of Mallotusoppositifolius (Geisel.) (Euphorbiaceae) combined with honey in albino rats, GSC Biol Pharm Sci 2023;23(01):182–192.e DOI: https://doi.org/10.30574/gscbps.2023.23.1.0120.
5. Idoko A, Parker JE, Njoku OU. Ethylacetate 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.
6. Onyeka IP, Onyegbule FA, Ezugwu CO, Dingwoke EJ, Ike CJ, Ogbue CO, *et al.* Gastroprotective effects of Methanol leaf extract of Desmodiumvelutinum (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
7. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant derived natural products: A review. Discovery and resupply of pharmacologically active plant derived natural products: a review. Biotechnol Adv 2015; 33(8): 1582–1614.
8. Madhavan R, Muthukumar NJ, Savariraj SC, Davidraj C, Sriram S, Rajalakshmi P,*et al.* Studies on the safety profiles of a Siddha preparation -Thirithodamathirai. Biomed2022; 42(3): 605-611
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
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. TheNigJPharm 2023; 57(2): 602-612.
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.
14. Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, *et al.*Novel insights of dietary polyphenols and obesity. The J NutrBiochem 2014;25(1):1–18.
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 antiobesityeffect with monoamines. Exp Animals 2000;49:239–249.
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 ClinPath1957; 28: 56.
18. Englehardt A. Measurement of alkaline phosphatase. Aerztl Lab1970;16:42-43.
19. Jendrassik L, Grof P. In vitro Determination of total and Direct Bilirubin. Biochemica1938;297: 81.
20. Bartels H, Bohmer M. *In vitro* determination of creatinine and urea. Clin Chem1972;2: 37-193.
21. Henry JB. Clinical Diagnosis and Management by Laboratory Methods, Philadelphia, W.B. Saunders1984; p. 1434.
22. Samat S, Mohd NN, Hussein FN, Eshak Z, Ismail WIW. Short-term consumption of Gelam honey reduces triglyceride level. Int Food Res J2017; 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 Med2017;2017: 1-9.
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
25. Zulkhairi AFA, Shafiq CMZ, Sabri S, Ismail N, Chan KW, *et al.*In Vivo Toxicity Assessment of the Probiotic Bacillus amyloliquefaciens HTI-19 Isolated from Stingless Bee (Heterotrigonaitama) Honey. Nutrients 2023; 15:2390. https://doi.org/ 10.3390/nu15102390
26. Guimaraes RB, Telles MM, Coelho VB, Mori RC, Nascimento CM, Ribeiro EB. Adrenalectomy abolishes the food-induced hypothalamic serotonin release in both normal and monosodium glutamate-obese rats. Brain Res Bull2002; 58:363–369
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 Tech2020; 40(1): 33-41.
28. Malhotra R, Cavanaugh KL, Blot WJ, Ikizler TA, Lipworth L*et al.* Higher Protein Intake Is Associated with Increased Risk for Incident End-Stage Renal Disease among Blacks with Diabetes in the Southern Community Cohort Study. NutrMetab Cardio Dis2016; 26:1079-1087.
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 World2019;12(12):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 Pharma2020;12:191-201.
31. Takei R, Inoue T, Sonoda N, Kohjima M, Okamoto M, Sakamoto R,*et al.*Bilirubin reduces visceral obesity and insulin resistance by suppression of inflammatory cytokines. *PLoS ONE,*2019; 14(10):e0223302
32. Seyed KN, Grindel A, Wallner M, Molzer C, Doberer D, MarculescuR,  *et al.*Mild hyperbilirubinaemia as an endogenous mitigator of overweight and obesity: implications for improved metabolic health. Atherosclerosis 2018;269:306–311.
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 Obesity2016; 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 Chem2022;11(1):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 Ther2020; 9:347-351.
36. Abebe T, Kassahun H. Patterns of Calcium- and Chloride-Ion Disorders and Predictors among Obese Outpatient Adults in Southern Ethiopia. DiabetMetabSynd ObesityTarget Ther2021; 14(2021):1349–1358.
37. Rock RC, WalkerWG, 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 Med2017; 1(2): 1-5.
39. Idoko A, Philip OC, Nwali ON, Ugwudike PO, Blessing NO, Ani PN,*et al.* Effects of raw and cooked aqueous and methanol extracts of *Phaseolus vulgaris* (kidney beans) on renal function in albino wistar rats. Univ J Pharm Res2020;5(3):6-11.
40. Brurya T, Jessica S, Marianna Y, Gabi S, Assaf B, Limor BH,*et al.*Nutrients2019;11(1256):1-11.