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Research Article

Biochemical, hematological and histological effect of *Spondias mombin* L fruit juice on some physiological properties of Wistar rats

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ABSTRACT

Ripe fruits of *Spondias mombin* are eaten as mild laxative, diuretic, ice cream, cool beverages, jams, wine, and also of medicinal and industrial uses world widely. Different concentrations (15, 50, 80 and 100%) of crude fruit juice of *S. mombin* and *E. coli* Serotype 0157:H7 were separately fed to rats for three days. The blood of rats was taken for haematological analysis, liver and kidney for biochemical assay and histopathological study. Positive control rats were allowed only to rat feed and water while negative control group of rats were infected with pathogenic *Escherichia coli*. The juice was found not toxic to the rats even at concentration of 100%. Significant increase ($P<0.05$) in biochemical and haematological levels were observed in the positive control. The crude juice on the biochemical and haematology of rats proved beneficial and also proved save on the liver and kidney of rats.

Keywords: Acute toxicity. Physiological properties. Rats. *Spondias mombin*. Health.

INTRODUCTION

Fruits are special food sources that are rich in potassium (K), calcium (Ca) and magnesium (Mg). These minerals among others are nutrient requirements for which their intake is associated with reduced risk of diseases such as cardiovascular, cataracts and age-related functional decline [1; 2]. However, some edible fruits contain parts that are injurious to human health. For example, the asparagus bright red berries do cause diarrhea and vomiting in human and also the pip of apricots, cherries, peaches and plums contain hydrogen cyanide that is poisonous to humans. In view of this, emphasis was laid on *Spondias mombin* hence it is a common fruit that is favourite for many people. *S. mombin* is a fruit well known and consumed world widely, but has different names around the world as Jobo (in Spanish speaking Caribbean and Mexico), Yellow mombin (in the English speaking Caribbean, Gully or Coolie Plum (Jamaica), Caja in Brazil. The juice of *S. mombin* besides its good taste has medicinal values as diuretic and antipyretic properties. *S. mombin* L.) belongs to the Anacardiaceae family; this fruit is found in the tropical areas of America, Asia, Africa and in Brazil [3]. The taste and aroma of the exotic tropical fruits, produced in a large variety, become an attractive characteristic, which is responsible for their good acceptance by a diversified public [4]. *S. mombin* fruit is small and elliptical in shape. In Nigeria, it is one of the edible and useful wild fruits but in some countries they are cultivated for their useful purposes. The consumption of commercial products of regional fruits such as *S. mombin*, *Ananas comosus*, *Dacryo des edulis* and many more has increased in the few years because of their importance in the health care of man. Decreased risks of heart disease and cancer have been found to be associated with the consumption of fruits and vegetables. The antioxidants in the fruits act by inactivating oxygen species involved in initiation or progression of these diseases [5]. Apart from its medicinal use, it is also a highly appreciated fruit, presenting good characteristics for industrialization [6].Till date no study has embarked on the effect and mechanism of *S. mombin* fruit juice on physiological properties for validity of the juice as safe for human consumption. This study therefore was aimed to

study the effects *S. mombin* crude fruit juice could have on haematology, biochemical indices and histopathology of vital organs using animal model.

Materials and Methods

Acute toxicity test

The methods of World Health Organization [7], guideline for the evaluation of safety and efficiency of herbal medicine and Organization of Economic Co-operation and Development [8], guide for testing of chemicals were adopted for this test. Sixty wistar rats (male and female) were used for this study whereby they were fasted for 6 h and then divided into six groups of ten per group. The rats in group 1 were administered with 10 ml/kg orally with normal saline. Groups 2-5 were orally administered with a single daily dose of *S. mombin* crude juice concentrations (15, 50, 80 and 100%) respectively as low, medium, high and overdose. The dosed rats were allowed to normal feed rats and clean water. The rats were observed for obvious toxic symptoms and mortality in each group at every 24 h for 14 days. The median lethal dose (LD_{50}) of the juice concentrations were estimated with probit analysis [9]. Meanwhile, weight of the rats was measured with a balance before and after experiment for inference.

Fruit juice preparation

Ripe harvested fruits of *S. mombin* were washed with 3% hypochlorite and thoroughly rinsed with distilled water. The fruits were juiced by pressing and filtered through Whatman No 1 filter paper. The filtered juice was pasteurized and kept for use in sterile samples bottle at 4 °C.

Experimental animals

Wistar albino rats of healthy status with weights between 40 to 43 g were used. The rats were confined in rat cages but with access to feed and water under standard laboratory condition. The rats were left under this condition for two weeks to acclimatize. Before experiment, the rats were deprived of feed but only access to water and were conducted in compliance with NIH guide for care and use of laboratory animals.

Juice administration to rats

Thirty Wistar rats were divided into six groups of five. Group 1 served as the negative control experiment and the rats were only allowed to feed and water. Group 2 of rats which were dose with pathogenic *Escherichia coli* served as positive control. Into each rat in groups 3-5, a single daily dose of 1 ml each of the varied juice concentrations of 15, 50, 80 and 100 ml/kg^{bw} was administered orally with a devised syringe and rubber tube dispenser of which the rubber tube was directly laid on the throat of rats. This experiment was conducted for 14 days before termination. During the 14 days of juice study on possible physiological changes in rats, signs of toxicity such as colour change in body surfaces, nature of movement, respiratory pattern, and aggressiveness among other clinical signs of toxicity were observed.

E. coli Serotype 0157:H7 inoculations

Pure reactivated colonies of *E. coli* Serotype 0157:H7 was grown in Brain Heart infusion broth and incubated for 72 h at 35 °C in a shaker water bath. The broth culture was centrifuged at 12000 rpm for 15 minutes and the supernatant was discarded. This washing with sterile distilled water was repeated for three times to obtain well washed bacterial cells. Five Wistar rats were each orally administered with 1 ml of 10^7Cfu/ml of the washed *E. coli* cells using a devised dispenser in a single daily dose for 3 days. The rats were allowed to normal rat feed and clean water; and were observed for gross changes in autonomic, neurological, behavioural profiles and mortality. The test bacteria cell (*E. coli*) was confirmed in the faeces of mice by plating 1 ml of emulsified faeces on Eosin methylene blue agar for green metallic sheen colonial colour. Such colonies were purified and identified for confirmation. On noticing symptoms of illness in the rats, they were left untreated for 7 days to enable elaborate physiological changes manifestation in the rats' liver. The purpose of this experiment was to compare physiological changes by *E. coli* as a pathogen to the effects of *S. mombin* in the rats.

Preparation of serum and liver homogenate

Cotton wool saturated with chloroform was placed in a jar and the rats were placed in the jar and covered to be unconscious. Thereafter, the jugular veins were cut with sharp scalpel. The rats head were held downwards to bleed into a clean dry centrifuge tube. The tubes were left at room temperature for 10 min and centrifuged at 12000 rpm for 15 min. The sera were collected with needle and syringe into dry glass vials and kept at -20 °C until needed. The rats' liver and kidney were obtained and washed with 0.25 M sucrose solution. The obtained livers were chopped to pieces and homogenized with ice cold 0.25 M sucrose solution (1:5 v/v) according to the method of. Akanji [10]. The homogenate was centrifuged at 12000 rpm for 15 min. The supernatant was collected into glass vials and used for biochemical assay. The Randox and QCA Commercial Enzyme Kits were used for the biochemical assay.

Aspartate Aminotransferase Activity Assay

The method of Bergmeyer et al. [11] was adopted with little modifications. Briefly, aspartate aminotransferase activity was measured by

monitoring the following information of oxaloacetate hydrazone with 2, 4-dinitrophenylhydrazine. The AST substrate phosphate buffer of 0.5 ml each was pipette into the sample blank (B) and sample test (T) test tubes respectively. The serum sample of 0.1 ml was added to the sample test (T) only, mixed immediately and was incubated in a water bath for 30 minutes at 37 °C. After incubation, a volume of 0.5 ml of 2, 4- dinitrophenyldydrazine was added to each tube containing the sample blank (B) and sample test (T). In addition, 0.1 ml of the sample was added to the sample blank (B) only. The medium was mixed and allowed to stand for 20 min at 25°C. Finally, 5.0 ml of (NaOH) was added to both the sample blank (B) and sample test (T) tubes and mixed thoroughly. Absorbance of the test samples was read at a wavelength of 550 nm against the sample blank after 5 min.

Alanine Aminotransferase Activity Assay

The method of Bergmeyer *et al*. [13] was adopted but with little modifications. Alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. The ALT substrate phosphate buffer of 0.5 ml each was added into two sets of test tubes labelled B (sample blank) and T (sample test) respectively. The serum (0.1 ml) sample was added to the sample test (T) only and mixed properly. This was incubated for 30 min in a water bath regulated at 37 ° C. After incubation, 0.5 ml each of 2,4-dinitrophenylhydrazine was added to both test tubes labelled T (sample test) and B (sample blank). Also, 0.1 ml of serum sample was added to the sample blank (B) only. The mixtures was shaken properly and allowed to stand for 20 min at 25 °C. Thereafter, 5.0 ml of NaOH solution was added to both test tubes and mixed thoroughly. Absorbance of the test sample was recorded against the sample blank at a wavelength of 550 nm after 5 min.

Assay of Alkaline Phosphatase Activity

This method is based on the principle that serum alkaline phosphatase hydrolyses a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein, which at alkaline pH values, turns into a pink colour that can be photo metrically determined. Distilled water (1.0 ml) was pipette into 2 sets of test tubes labelled SA (sample) and ST (standard) respectively. Then one drop of chromogenic substrate was added to the distilled water in the two sets of test tubes. Their contents were mixed and incubated at 37 °C for 20 minutes in a water bath. After incubation, a standard solution of 0.1 ml was added to the standard test tube (ST) only; followed by the addition of the serum sample of 0.1 ml to the sample test tube (SA). The content was also mixed and incubated at 37 °C for 20 min in a water bath. A colour developer of 5.0 ml each was added to both sets of test tubes. Absorbance of the sample against the blank (water) was read at a wavelength of 550 nm. The activity of alkaline phosphatase in the serum was obtained from the formula (calculations) below:

$$\frac{\text{STO.D}}{\text{SAO.D}} \times 30 = \text{U/L of Alkaline phosphatase}$$

Where:

STO.D = Standard Optical Density

SAO.D = Sample Optical Density

For the kidneys function, uric acid was determined by the method of Schirmeister et al. [13]; urea by the criteria of Patton and Crouch,[14]; creatinine by the method of Young *et al*. [15]. Protein by the method of Peters [16] and albumin was determined by the method of Doumas and Giggs, [17]. Cholesterol was determined by the method of Wybenga et al. [18], bilirubin was determined by the method of Malloy and Evelyn, [19].

Haematological test

Blood was collected from the rats during sacrifice in EDTA bottles for the various haematological parameters. The white blood and red blood cells were enumerated with Neobauer haemocytometer, while haemoglobin was estimated with Sahli's Hemoglobinometer by standard procedures. Estimation of differential leukocytic counts was by the methods of Coles, [20].

Histopathological study

Pieces of rats' liver and kidney were collected from experimental controls (positive and negative) and the juice concentrations treated. The organs were cut to small sizes of about 1 cm and dehydrated with grades of ethanol starting from 50% to absolute. The tissues were cleared in xylene for two changes and impregnated with paraffin wax in oven regulated at 60 °C for 1 h. The tissues were then embedded in paraffin wax and sectioned with a microtome (Bright, England) at 3-5 μm. The sectioned tissue films were floated in water regulated at 40 °C and picked with glass slides previously rubbed with egg albumin. After drying at room temperature, the tissues were dewaxed with xylene, hydrated with water and passed through grades of ethanol from absolute to 20%, stained with haematoxylin and eosin. Excess stain was washed with 95% ethanol and then cleared twice in xylene and mount with DPX. The prepared slides were photographed, observed with microscope (Olympus) and

interpreted according to level of damages of safety.

Statistical analysis

Obtained data were expressed as mean ± standard deviation (SD) and subjected to one way analysis of variance (ANOVA). The least significant difference (LSD) was performed for the pair wise mean comparisons to determine the significant treatment dose at 95 level of confidence. Values were considered statistically significant at ($P<0.05$).

Results

Toxicity effect with *Spondias mombin* fruit juice was not observed in Wistar rats as abnormal behaviour and death was not recorded in the acute toxicity study. Decrease in weight loss was observed in the positive control group of rats while there was weight gain in the negative control and crude juice treated group of rats (figure 1).Significant increase ($P<0.05$) in biochemical levels was observed in the positive control values on comparism with the negative control values. In the crude juice concentrations treated groups of rats, significant difference was not observed in the high dose (80% kg^{bw}) and overdose (100% kg^{bw}). With this insignificant difference, the high dosed and over dosed rats' biochemical values were about the same with the negative control value. However, significant differences existed between the low juice dose (15% kg^{bw}), medium juice dose (50% kg^{bw}) and the negative control of biochemical values. Despite this significant differences observed, values of the low juice and medium juice doses were within permissive standard values for approval as nontoxic. Aspartate aminotransferase (AST) values are 68.46±4.05 IU/L and 15.40±1.54 IU/L in positive and negative control groups respectively, making a difference in value of 53.56±2.5 IU/L. With low dose of juice concentration, 25.68±2.71 IU/L was recorded, therefore, evaluating difference of 10.28±11.17 IU/L between the low dose and negative control values. AST value was 21.27±1.63 IU/L in medium dose, 18.54±1.34 IU/L in high dose and 16.70±1.26 in overdose. Values of alanine aminotransferase (ALT) in positive and negative control are 65.92±3.70 and 25.20±3.42 IU/L respectively. Meanwhile, Low dose value was 42.97±3.53 IU/L, medium dose (31.82±1.90 IU/L), high dose (28.74±3.10 IU/L and over dose with value of 27.26±3.45 IU/L. Alkaline phosphate (ALP) value was 219.12±2.19 IU/L in positive control and 66.21±2.50 IU/L in negative control, raising a difference of 152.90±0.40 IU/L. In crude juice treated mice, it was 144.14±2.57 with low dose, 112.70±1.90 IU/L with medium dose, 90.32±3.43 IU/L with high dose and 86.53±1.18 IU/L with over dose (table 1).However, this same trend of decreased value with crude juice treatment equating with the negative control value was observed also in bilirubin, urea, uric acid, creatinine and cholesterol contents. However, higher values in negative control were observed over the positive control, total albumin and total protein. Increase in value on dose dependant was as well observed in the crude juice concentrations treatment, all tending towards the negative control than the positive control for justifiable remarks (Table 2).The hematological indices of crude juice concentrations of *S. mombin* are related in table 2. The negative and positive control groups had mean values of 11.56±0.08 and 20.18±1.22% respectively. These two values indicated differences between normal health status and infection in the rats when compared with the permissive standard unit of 11-19%. The low dose juice (15%) results in haemoglobin status of 12.57±0.02%, medium dose (50%) with value of 12.74±0.23, high dose (80%) with value of 12.36±0.20% and over dose (100%) with value of 12.24±0.18%. These values showed indications of non-alteration of haemoglobin out or below standard range. The red blood cells count (RBC) in the negative control was 7.63±0.92 while positive control resulted at 4.46±0.18 m/cu.mm. The low juice concentration treatment was valued at 6.35±0.19 m/cu.mm, medium dose (7.42±0.26 m/cu.mm), high dose (7.52±0.16 m/cu.mm) and over dose (7.58±0.28 m/cu.mm). In white blood cells count (WBC) positive control value was 9.70±0.39 t/cu.mm and negative control with value of 4.73±0.20 t/cu.mm. Count in the groups of rats with low, medium, high and over dose of crude juice was 4.20±0.71, 3.42±0.50, 7.52±0.16 and 7.58±0.28 t/cu.mm respectively. The packed cells volume (PCV) in negative control was 43.68±0.15% while in the positive control, it was 27.18±0.10%. However, with the crude juice concentrations treatments, it was 42.44±0.63% with low juice dose, 45.47±0.52% with medium dose, 42.36±0.21 with high dose and 41.24±0.61% with over dose. Observations in the differential white blood cells count was dependant on the values obtained from RBC, WBC and haemoglobin. The higher value of RBC in negative control than the positive control paved way for the high value obtained from the differential monocyte count5 in the negative control. The higher value obtained from WBC and haemoglobin count in the positive control group of rats, also resulted to the high values in

differential counts of lymphocytes. Neutrophil and eosinophil in the positive control group of rats. The improvement observed in the differential and PCV counts, points an indication of non-interference in the physiological properties of rats treated with crude juice of *S. mombin* even at over dose concentration. The histopathological sections of liver tissue are shown in plate 1. The negative control liver had no cellular distortion. The liver cells appeared normal in architectural structures with prominent nucleus, a distinct central vein and a well preserved cytoplasm. The liver of rats treated with the juice concentrations revealed that cells of liver were intact with normal cellular architectural structure, central cellular vein and hepatocytes that are well separated by sinusoids. In the bacterial treated rats' liver, distortions were in sinusoids that were well not separated. Hepathocellular necrosis was observed as a result of the liver disorder established by *E. coli* 0157:H7 without treatment. Images also observed is wide necrosis across the cells resulting to high degeneration in hepatic plates and loss of cellular boundaries. The neutrophils were not surrounding the portal vein and hepatocytes were disrupted and sinusoids affected. The histopatho logical sections of kidney tissue are shown in plate 2. The negative control kidney had no cellular distortion. The kidney cells appeared normal in architectural structures with prominent glomeruli and interlobular veins. The kidney of rats treated with the juice concentrations revealed that cells of kidney were intact with normal cellular architectural structure, collecting duct, preserved bowman's capsules, glomeruli, urinary poles and glomerular capillaries. In the bacterial treated rats' liver, distortions were observed such as the lack of interlobular veins, hepatic drainage and macular densa were parts of the observed result of the kidney disorder established by *E. coli* 0157:H7 without treatment.

Discussion

The documentation of plants and plant derivatives in traditional medicine all over the globe is an aspect of making known valuable indigenous knowledge in solving problems of diseases for better health care in urban, rural and even developed communities. Some edible wild fruits such as *Adansonia digitala*, *Dacryode sedulis*, *Chrysobalanus icaco*, Tropical almond, *Persea americana* and host of others have been found very impressive in healing and prevention of diseases. This is as a result of their health benefit constituents such as minerals and antioxidants matched with vital phytochemicals. These edible wild fruits in urban and rural areas are consumed to quench thirst and supplement for food without knowledge of their health importance and medicinal values [21]. *S. mombin* fruit juice has been found to contain plant chemicals such as flavonoids, phenol, saponons, steroids, alkaloids, tannins [22], minerals such as sodium, magnesium, potassium, phosphorus etc [4] and antioxidants such as catalase, glutathione and lipid peroxidation [23]. The haemoglobin value obtained from the negative control group of rats which was within standard value of 11-90% proves that red blood cells were intact while the high haemoglobin value recorded from the positive control group of rats denotes alteration in the red blood cells, even as reflected in the low PCV value of this group of rats. This reduced level of haemoglobin was as a reduction in the disturbance in heme biosynthesis hence the linkage of iron with heme was inhibited and activities of enzymes necessary for heme biosynthesis reduced [23; 24]. This suggests that natural plant properties in their crude forms remain problem solving in health care of man as they work together to effect adorable performance in health care. This however could make them acceptable in some cases than the refined antibiotics hence they address both the problem in question and the minors which might contribute some factors to illnesses [25].The toxicity affected in the rats by *E. coli* 0157:H7 accounted for the increase of WBC seen in circulation of the Wistar rats. The neutrophil, lymphocytes and eosinophil counts in the positive rats were above standard. This denotes severe hematology defect in the WBC count. However, the results obtained from the crude juice treated rats (negative control) suggested non toxicity as record of hematological defects was not observed. This emphasized that natural products from plants or plants' derivatives have resolving effects in intracellular antioxidant in the cytomembranes responsible for cellular integrity maintenance. The membrane stabilization of the crude juice components such as phytochemicals could be responsible for the enhanced improvement observed in the cellular integrity of the differential counts, thereby inhibiting free radicals generated from damaging the rats' cells. Antioxidants are the first line of defense against free radical damage and are critical for maintaining optimum health. Therefore, the need for antioxidant becomes even more critical with increased exposure to free radicals [26]. Also, the recorded abnormalities in the physical properties of positive control rats collectively resulted in the increased values obtained from the biochemical enzyme markers of AST, ALT and ALP activities, cholesterol, bilirubin, urea, uric acid and creatinine levels; and the decrease values in total

albumin and total protein levels. Thereabout, stabilization activities of the biochemical parameters investigated with the crude fruit juice, manifested distinct improvement in the functional status of liver cells which may be due to its free radical scavenging activities. The ability of infectious dose of Serotype 0157:H7 to affect damages on liver and kidney tissues is in connection with the varieties of alteration in the biochemical indices, and this is capable to manifest intracellular constituents such as AST, ALT, ALP and cholesterol among others into circulation. The amount of these biochemical markers in circulation on analyses gave a predictive level of hepatocellular damages. Bilirubin is derived from the breakdown of haemoglobin and the poor processing of bilirubin by liver made this substance floating in high value observed in the body circulation of the positive control group of rats. Albumin is made in the liver and because of liver condition of the positive control; low albumin level was produced and high in negative control because of the non-effect *S. mombin* juice has on physiological properties of the rats, thus the observed state of good condition. Total protein level is a rough measure of protein status but reflects major functional changes in kidney and liver functions. The decrease in total protein level observed in the positive control could be as a result of the liver not able to build complex molecules from simple substances which are absorbed from the digestive tract; unable to manufacture bile which aids fat digestion and synthesis of protein due to degenerating factors of the liver attained from *E. coli* Serotype 0157:H7. Hence albumin is made in the liver; the poor condition of liver of the positive control group of rats could not allow much synthesis of albumin thus the low level of albumin recorded. The decrease in total protein level also from the positive control could be as a result of the liver not able to build complex molecules from simple substances which are absorbed from the digestive tract and also the inability of the liver to produce bile that helps in the digestion of fat and protein synthesis caused by the bacterial pathogen. However, increase in albumin and protein contents were observed in dose dependant of crude juice concentration. The liver is the sole site for synthesis of albumin, which makes up approximately 6% serum total protein concentration. Therefore, these increases in total albumin and protein concentrations in the juice treated rats indicates that the synthetic function of the hepatocytes were not impaired. The increased synthesis of albumin may also contribute in the reduction in overall unconjugated [27]. Increase in urea level also observed in the positive control, is an indication of pathological changes that resulted in the liver due to the effect of *E. coli* Serotype 0157:H7 bacterial pathogen. The crude juice of *S. mombin* treatment elevated the abnormally reduced biochemical markers and protected the abnormally depleted biochemical markers as the case may be for normal health of the rats. The abnormal values obtained from the biochemical and haematological activities were either below or above standard values in the positive control group of rats. This was elicited by the infectious dose (10 cells/injection) of *E. coli* serotype 0157:H7. Diseases are aimed with drugs only when there is illness or peradventure for prevention, but in most cases, the physiological changes imputed by a disease is neglected with the hope that recovery will set in when the disease is cured. This negligence has peradventure lead to many problems in human physiological properties above management and then be deduced to side effects of drugs. The management of diseases with plant natural products have the key to address this issue vehemently because of their valuable contents which both care for disease cure and quick recovery from damaged or altered physiological properties in an infection. The beneficial effects of the crude juice of *S. mombin* in the haematological and biochemical profiles in this study may be as a result of the presence of vital plant chemicals and minerals [21], vitamin C [4; 28] and other antioxidant properties. This may explain why the red blood cells were not quite altered [29] even with the overdose of the crude juice of *S. mombin*. The observed mild defects in the cellular architecture of the liver and kidney of positive control was due to *E. coli* Serotype 0157:H7 that caused the physiological damages which resulted in both the haematological and biochemical dysfunctions of the rats' physiological properties. The histological defects observed in the liver of positive group of rats includes blood flow, cellular infiltration, lack of cellular boundaries and necrosis while that observed in the kidney are intestertial space, collecting duct, hepatic drainage and macula densa. Hence the crude juice was not toxic, the juice treated groups of rats maintained quality health status in the negative control where good architectural structure was observed in the liver and kidney histology. Such structures in the rat's liver were central artery, ventral vein and bile duct while in the kidney structures as space of Bowman's capsule, glomeruli, loop of Helen, interlobular vein, urinary pole, distal tubes among others were observed.In histopathological investigation of sugammadex in experimental animals, Kiraz et al. [30], observed no histopathological defect in

negative control in low concentration dosed mice of which the histopathology observations in this study is corresponded. Emphasis has also been laid on *Irvingia gabonensis* fruits juice has been suitable in the biochemical and physiological properties of mice [31] as the fruit juice of *S. mombin* in this study has contributed an addition that some fruits could be good and cause no harm in human physiological properties

Conclusion

S. mombin fruit juice was effectively studies using animal model. However, the studies on the biochemical and haematology of Wistar rats proved beneficial as well as the histopatho logical study proved save on the liver and kidney of rats. The juice effects and mechanisms in the rats' health status could be related to the compounds that are beneficial to health care system. Such compounds are vitamins, plant chemicals and minerals of valuable in maintenance and improvement of health as well as cure and prevention of diseases.

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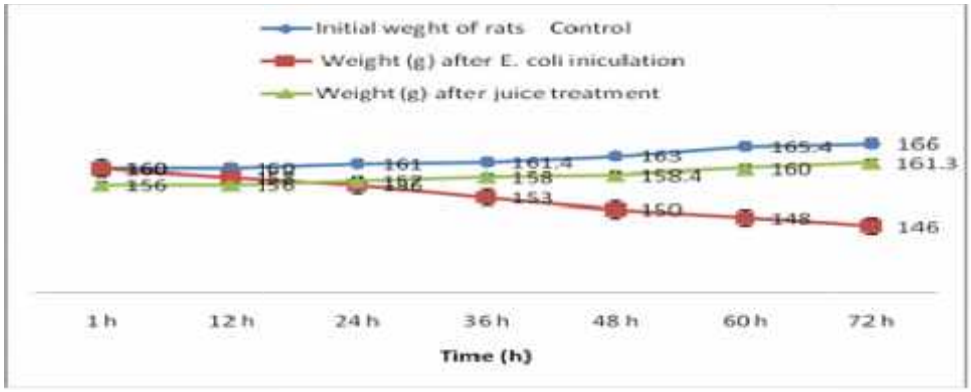

Figure 1: Weight(g) of rats during acute toxicity study

Table 1: Biochemical properties of mice fed with crude fruit juice of *S. mombin*

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Total Albumin (g/dL)	Total protein (g/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dl)	Cholesterol (mg/dl)
Control (-)	15.40 ±1.54	25.20 ±3.42	66.21± 2.50	1.07± 0.11	4.67± 0.10	7.16± 0.42	10.13 ±1.43	3.05± 0.54	0.87± 0.31	113.72 ±0.51
Control (+)	68.46 ±4.05	65.92 ±3.70	219.12 ±2.10	2.09± 0.60	2.18± 0.60	5.18± 0.60	23.43 ±0.14	8.01± 1.08	1.94± 0.07	216.12 ±0.61
15%	25.68 ±2.71	43.97 ±3.53	144.14 ±2.57	1.64± 0.23	3.52± 0.33	6.43± 0.37	14.55 ±0.35	5.31± 0.41	0.48± 0.32	134.23 ±3.60
50%	21.27 ±1.63	31.82 ±1.90	112.70 ±1.90	1.33± 0.24	4.34± 0.21	6.56± 0.30	12.60 ±0.75	4.28± 0.64	0.64± 0.43	123.19 ±0.37
80%	18.54 ±1.34	29.76 ±3.10	90.32± 3.43	1.16± 0.03	4.56± 0.34	6.63± 0.37	12.26 ±1.03	3.20± 0.47	0.70± 0.18	119.17 ±1.08
100 %	16.70 ±1.26	27.26 ±3.45	86.53± 1.18	1.10± 0.05	4.58± 0.44	6.81± 0.05	12.13 ±1.01	3.11± 0.32	0.74± 0.08	114.32 ±1.15

Table 2: Hematological profile of mice fed with crude fruit juice of *S. mombin*

	Hemoglobin (g%)	RBC (m/cu.mm)	WBC (t/cu.mm)	Monocyte %	Neutrophil %	Lymphocyte %	Eosinophil %	PCV %
Control (-)	11.56±0.08	7.63±0.92	4.73±0.20	3.18±0.02	14.63±0.18	52.40±0.22	2.43±0.02	43.08±0.15
Control (+)	20.18±1.22	4.46±0.18	9.70±0.39	0.50±0.62	57.40±0.15	88.16±0.26	5.43±0.21	27.18±0.10
15%	12.57±0.02	6.35±0.19	4.20±0.71	1.54±0.04	22.40±0.13	70.92±0.53	1.70±0.40	42.44±0.63
50%	12.72±0.23	7.42±0.26	3.42±0.50	1.80±0.40	16.04±0.17	56.91±0.27	2.25±0.23	45.47±0.52
80%	12.36±0.20	7.52±0.16	3.57±0.08	1.93±0.76	14.43±1.64	55.31±0.12	2.84±0.40	42.36±0.21
100%	12.24±0.18	7.58±0.28	3.62±0.43	1.95±0.48	14.32±0.26	55.36±0.04	2.18±0.36	41.24±0.16

References

1. Temple NJ. Antioxidant and diseases: more questions than answers. Nutritional Research, 2000; 20, 449-459
2. Aberounmand A, Deokule SS. Elements evaluation of some edible vegetables and fruit of Iran and India. Asian Journal of Agricultural Science, 2010; 2(1): 35-37
3. Mouraneto LG, Érica Milo DE, Freitas Felipe Rocha, Marcos Rodrigues Amorim Afonso,, Suel Rodrigues., José Maria, Correira da Costa. Physicochemical and sensory evaluation of yellow mombin (*Spondias mombin* L.) atomized powder. Revista Caatinga, Mossoró, 2015; 28: (4)244-252.
4. Tiburski JH, Amauri Rosenthal Rosires Deliza, Ronoel L, de Oliveira Godoy, Sidney Pacheco. Nutritional properties of yellow mombin (*Spondias mombin* L.) pulp. Food Research International. 2011; 44:2326–2331. http://dx.doi.org/10.1016/j.foodres.2011.03.037
5. Duthi, SJ, Jenkinson AMCE, Crozier A, Mullen W, Pirie L, Kyle J, Yap LS, Christen P, Duthie GG. The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers. European Journal of Nutrition, 2006; 45(2):113–122. https://doi.org/10.1007/s00394-005-0572-9.
6. Oliveira GS, Costa JMC, Afonso MRA. Caracterização e comportamentohigroscópico do pó da polpa de cajáliofilizada. Revista Brasileira de EngenhariaAgrícola e Ambiental, Campina Grande, 2014; 18(10): 1059-1064
7. World Health Organization (WHO). General guidelines for methodologies on research and evaluation of traditional medicine. Switzerland 2000;
8. The Organization of Economic Cooperation and Development (OECD). The OECD guideline for testing of chemical: 420 Acute Oral Toxicity. France, 2010;
9. Miller LC, Tainter MC. Estimation of LD50 and its error by means of logarithmic-probit graph paper. Proceedings of the Society of Experimental Biology and Medicine, 1994; 57: 261-264.
10. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of rat liver cellular system. Toxicology, 1993; 81:273-179.
11. http://dx.doi.org/2010.1016/0300-483X(93)90010-P
12. Bergmeyer HU, Horder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6 1:1.). Journal of Clinical Chemistry and Clinical Biochemistry1986a; 24: 497-510.
13. Bergmeyer HU, Horder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic

concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6 1:2.). Journal Clinical Chemistry and Clinical Biochemistry, 1986b; 24: 481-495
14. Schirmeister J, Willmann H, Kiefer H. Plasma kreatininalsgroberIndikator der Nierenfunktion Dtsch Medical Wschr. 1964: 89: 1018.
15. Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Analytical Chemistry, 1977; 49: 464-469. http://dx.doi.org/10.1021/ac50011a034.
16. Young DS, Pestamer LC, Gibber-Man,V. Effect of drugs on clinical laboratory tests. Clinical Chemistry, 1975; 21(5): 1D-432D.
17. Peters T. Protein colorimetric method. Clinical Chemistry, 1968; 14: 1149-1159
18. Doumas BT, Giggs HG. Albumin standard and measurement of serum albumin with bromocresol green. Clinical Chemistry Acta, 1971; 31:78
19. Wybenga DR, Pileggi VJ, Dirstine PI, Giorgio D. Direct manual determination of serum total cholesterol with a single stable reagent. Clinical Chemistry, 1980; 16: 980
20. Malloy HT, Evelyn KA. The determination of bilirubin. Journal of Biological Chemistry, 1997; 119: 481–5
21. Coles EH. Veterinary Clinical Pathology, pp. 279–297, WB Saunders, Philadelphia, Pa, USA, 4th edition, 1986.
22. Akharaiyi FC, Boboye BE, Adetuyi FC. Antibacterial, phytochemical and antioxidant activities of the leaf extract of *Gliricidia sepium* and *Spoathodea campanulata*. World Applied Sciences Journal.2012; 16(14): 523-530.
23. Ali FU, Mathias CO, Ogbanshi ME, Eze US. Phytochemical Analysis of *Spondias mombin*. International Journal of Innovative Research and Development, 2014; 3(9): 101-107
24. Afonso M., Silva AMO, Carvalho EBT, Rivelli DP, Barros SBM, Rogero MM. Phenolic compounds from Rosemary (*Rosmarinus officinalis* L.) attenuate oxidative stress and reduce blood cholesterol concentrations indirect-induced hypercholesterolemic rats. Nutrition and Metabolism. 2013; 10: 2–9.
25. Brandy PG. Iron deficiency anemia: a call for aggressive diagnostic evaluation. South African Medical Journal, 2007; 100(10): 966-967.
26. Akharaiyi FC, Boboye BE, Akinyosoye FA, Oso, BA. Antioxidant, phytochemicals and minerals evaluations of *Spondiasmombin* crude fruit juice. Acta Biological Szeg, 2016; 60(2):171-176.
27. Begun SKA, Madhuri M, Rajani A, Hemamalini K. Evaluation of in-vitro antioxidant activity of some synthesized novel L-arginine analogues. International Journal of Pharmaceutical Science Research, 2014; 5(5): 2051-2054.
28. Igwe CU, Onyeze GOC, Onwuliri VA, Osuagwu OG, Ajiako OA. Evaluation of the chemical compositions of the leaf of *S. mombin* Linn from Nigeria. Australian Journal of Basic & Applied Sciences. 2010; 4:706-710.
29. Guiter E, Simko V. Deficiency of vitamin D and vitamin C in the pathogenesis of bronchial asthma. Bratislava Medical Journal, 2016; 117(6):305-307.
30. http://dx.doi.org/10.4149/BLL_2016_060.
31. Igwe CU, Onwuliri VA, Osuagwu CG, Onyeze GOC, Ajiako OA, Biochemical and haematological studies on the ethanol leaf extract of *S. mombin* Linn. Biochemical and Analytical Biochemistry, 2011; 1(1): 1-5. http://dx.doi.org/10.4172/2161-1009.1000104.
32. Kiraz HA, Toman H, Erbas M, Yuyucu Karabulut Y, Simsek T, Hanci V, Uzun M. Investigation of histopathological effects after intra-arterial Sugammadex injection in an experimental animal model. Bratislavia Medical Journal, 2016; 117(5): 295-298.
33. http://dx.doi.org/10.4149/BLL_2016_058.
34. 31. Emejulu AA, Alisi CS, Asiwe ES, Iheanacho KM, Onwuliri VA. Hypolipidemic effect of *Irvingia gabonensis* fruits juice on sodium fluoride induced dyslipidemia in rats. African Journal of Biochemistry Research, 2014; 8(8):151-157.

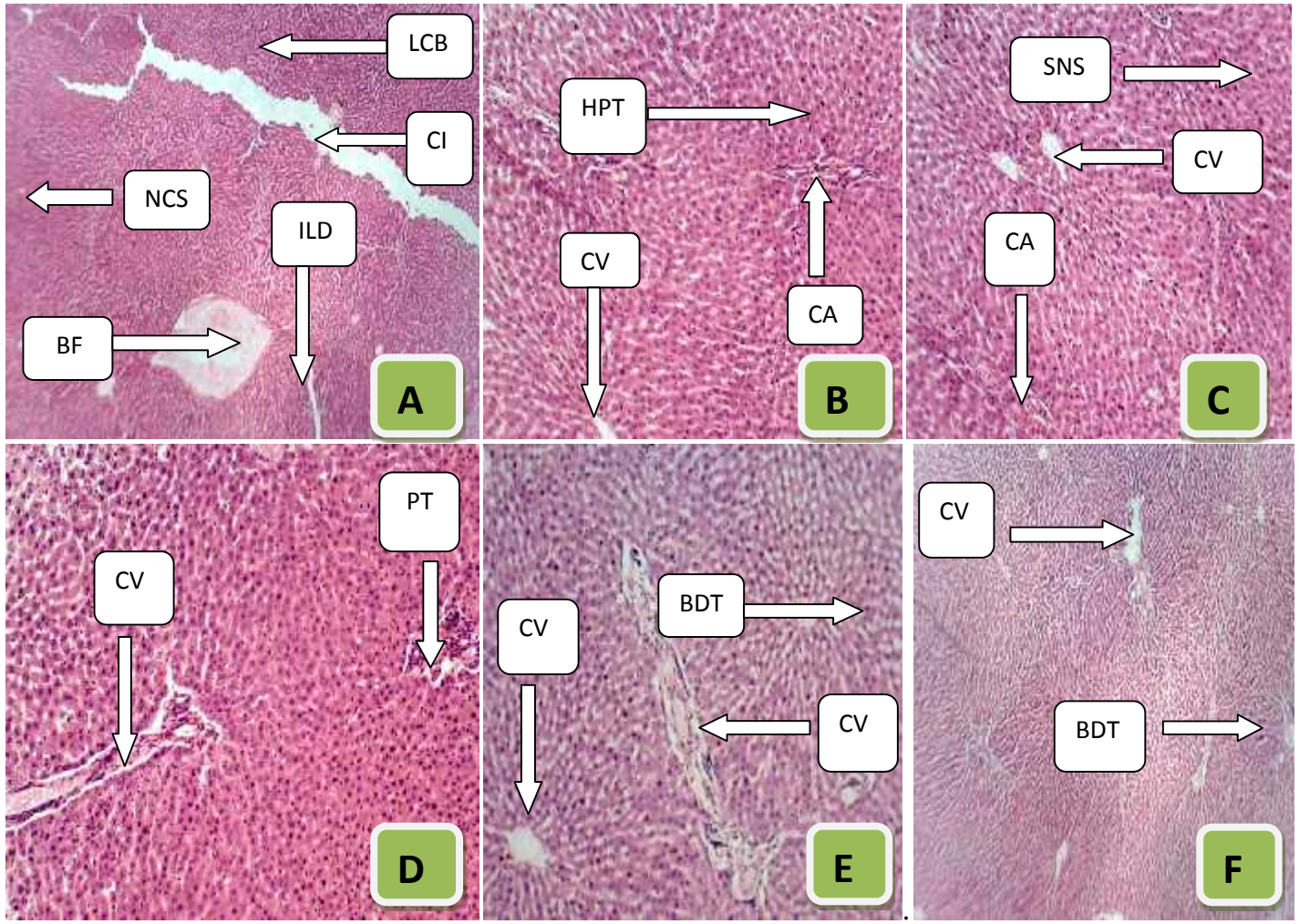

Plate 1: Photomicrograph of livers of rats inoculated with *E. coli* and juice concentrations

LEGEND: (A): Positive control- infected with *E. coli*, (B): Negative control, (C): Juice overdose, (D): High dose, (E): Medium dose, (F): low dose. HPT=Hepatocytes, CA=Central artery, BDT=Bile ductile, SNS=Sinusoids, BF=Blood flow, CV=Central vein, NCS=Necrosis, LAC= Lack of cellular boundaries, ILD=Intralobular duct, CI=Cellular infiltrate

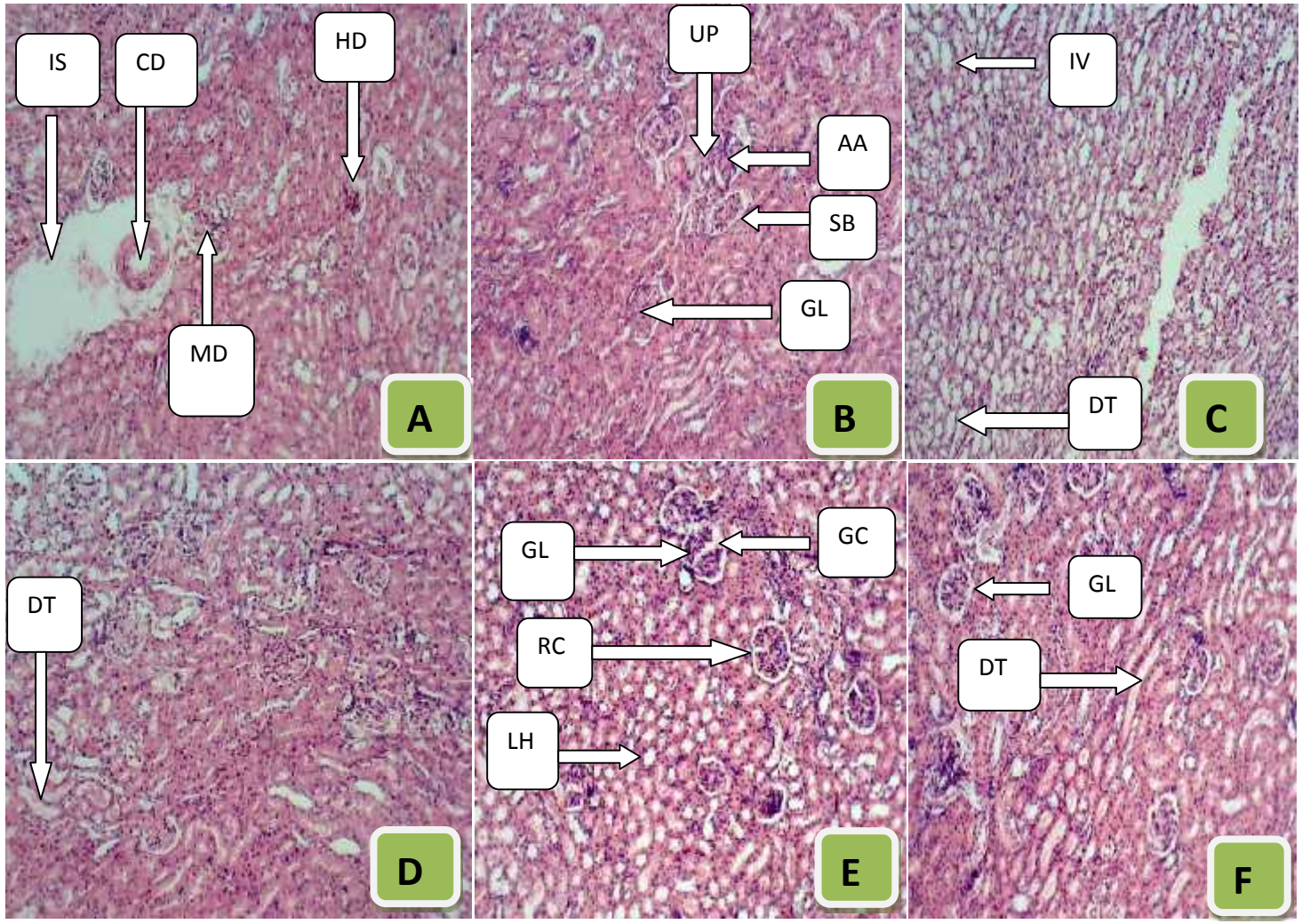

Plate 2: Photomicrograph of kidneys of rats inoculated with *E. coli* and juice concentrations

LEGEND: (A): Positive control infected with *E. coli*, (B): Negative control, (C): Juice overdose, (D): High dose, (E): Medium dose, (F): low dose. IS=Intestertial space, CD=Collecting duct, HD=Hepatic drainage, AA=Afferent arteriole, SB=Space of Bowmen's capsule, DT=Distal tubes, RC=Renal capsule, GL= Glomeruli, LH=Loop of Helen, IV=Interlobular vein, MD=Macular densa, UP=Urinary pole, GC=Glomerular capillary.