Full Length Research Paper

The hepato-rejuvinative and hepato-toxic capabilities of *Citrus paradisi* Macfad fruit juice in *Rattus Norvegicus*

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Citrus paradisi Macfad (Rutaceae) popularly called grapefruit is widely cultivated in tropical and subtropical countries. The juice of *C. paradisi* fruit is commonly used both as a beverage and in folkforic medicine for the treatment of several disease conditions. In the present study, the effects of *C. paradisi* fruit juice on the Wistar rat liver histology and oxidative status were investigated. 200, 400 and 600 mgkg^{-1} per day orally of the extract was administered on three groups of Wistar rats respectively for 60 days. A fourth group that served as control was given 5 mlkg^{-1} daily, orally of distilled water. Animals that had 200 mgkg^{-1} of the extract showed improved liver histology and a largely preserved liver oxidative status. However, the groups of rats that were treated with both 400 and 600 mgkg^{-1} of the extract exhibited poor histological profiles and increased evidence of liver oxidative stress. The results therefore indicate that, while the lower dose of *C. paradisi* fruit juice is hepato-protective, the higher doses indeed demonstrate hepato-toxicity in the rat. Thus, the dose of application of the extract in folk medicine and even in beverages should be regulated to forestall possible hepatic derangement.

Key words: *Citrus paradisi*, liver, histology, rat.

INTRODUCTION

Citrus paradisi (grapefruit) tree is a tropical and subtropical plant that grows up to 3 to 5 m high or even 3.7 m with age. It has a rounded top of spreading branches; the trunk may exceed 15 cm in diameter; that of a very old tree actually reaches nearly 2.4 m in circumference. Its fruit is mostly big and globular, with nipple at apex, and bright yellow or lemon colored. While some fruits are seedless or nearly so, there may be up to 90 white, elliptical, pointed seeds about 1.25 cm in length (Morton, 1987).

C. paradisi fruit (CPF) juice contains high levels of vitamin C, vitamin E, and bioflavonoids (Sachs, 1997; Halliwell, 2008). These compounds are known powerful antioxidants. Bioflavonoids form a class of benzo-gamma-pyrone derivatives that have high pharmacological potency. A great interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds (Diplock et al., 1998). This is due primarily to their radical-scavenging and iron-chelating properties (Cook and Samman, 1996). They are found naturally in the leaves, bark, roots, flowers, and seeds of plants (Cook and Samman, 1996).

One bioflavonoid in the juice of *C. paradisi* fruit with especially potent antioxidant capabilities is naringenin (Lee et al., 2002). It is the aglycone of the natural glycoside naringin present abundantly in grapefruit and which constitute its bitter principle. The antioxidant activities of bioflavonoids complement, extend, and sometimes synergize the antioxidant activities of vitamin C, vitamin E, and carotenoids, making them an important

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nutritional component in the body's defenses against free radical damage (Ho, 1994).

Grapefruit juice is mildly acidic and has slight bitter taste (Monsef-Esfahani et al., 2006). Like most plant extracts, *C. paradi* fruit juice has been shown to also contain highly toxic alkaloids and saponins (Morten, 1987; Monsef-Esfahani et al., 2006). Furthermore, and as has been variously demonstrated, plant extracts with strong antioxidative potentials could be both beneficial as well as toxic to tissues (Saalu et al., 2010a).

In spite of the aforementioned scenario and despite the fact that CPF is widely consumed in all parts of the world, in addition to its versatile ethno-medical usage, there is a dearth of information on its effects on the liver (the main organ where ingested substances are metabolized).

The present study is therefore, aimed at investigating the role of three doses of *C. paradisi* fruit (CPF) juice extract on the liver histology and oxidative status in rats.
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MATERIALS AND METHODS

Plant materials

Fresh grapefruits were purchased from a fruits shop in Bariga, Lagos, Nigeria. Botanical identification and authentification was done at the Botany Department, University of Lagos. The fruits were washed, cut into two halves and the juice extracted using a manual juice extractor. The extracted juice was then sieved to obtain a clear sample of the juice and to separate the squeezed pulp from the juice. The juice was evaporated and dried in a moisture oven at 40°C. The desired juice concentrate was stored in a refrigerator for the experiment. The volume of juice before evaporation was 2500 ml while the volume of juice after evaporation was 250 ml.

Phytochemical screening of *C. paradise*

The juice was evaluated for the presence of various phytoconstituents by performing different qualitative chemical tests reported. It showed the presence of anthraquinone glycosides, alkaloids saponins, tannins, flavonoids and resins (Monsef-Esfahani et al., 2006).

Animals

Male Wistar rats (*Rattus norvegicus*) weighing between 150 and 240 g were purchased from an animal house in Ladoke Akintola University of Technology (LAUTECH) Ogbomosho. The rats were bred in the laboratory animal house, Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos. The rats were subjected to a suitable temperature of 32 to 37°C, 24 h light supply, full aeration which was enhanced by wire gauzed cage properly partitioned into four chambers and roomy enough to allow for proper ventilation and free movement within it. The floor of the cage was lined with carpet pieces and sprayed with coarse saw dust which served as a cushion. The coarse saw dust was changed every day to dispose waste droppings and maintain cleanliness. The rats were fed with growers marsh (pellets), purchased from a feed store- Bendel feeds and flour mills, and water during the breeding period to acclimatize the rats. The rats went through an acclimatization period of 7 days.

The weights of the animals were estimated at procurement, during acclimatization, at commencement of the experiments and twice within a week throughout the duration of the experiment, using an electronic analytical and precision balance (BA210S, d = 0.0001 g) (Satorius GA, Goettingen, Germany). Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (CCAC, 1985). Further, the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002).

Acute oral toxicity study of *C. paradisi* fruit juice

The acute oral toxicity study for *C. paradisi* fruit (CPF) juice was conducted using the Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Humane End points that should reduce the overall suffering of animals used in this type of toxicity test. The test used was the limit dose test of the up and down procedure.

Briefly, 5 animals were weighed and individually identified. The first animal was given the test dose – *C. paradisi* extract 2000 mgkg[-1] body weight. The second and third animals were concurrently dosed and the fourth and fifth animals sequentially dosed.

The results were evaluated as follows (S = survival, X = death). The animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter for a total period of 14 days. All observations were systematically recorded with individual records maintained for each animal.

Animal grouping and experimental design

Forty adult male Wistar rats (11 to 13 weeks old) weighing 150 to 240 g were used for this research work. The rats were randomly divided into four groups (A to D) of ten rats each such that the average weight difference between and within groups did not exceed ± 20% of the average weight of the sample population.

Rats in group A which served as control were given 5 mlkg[-1] of distilled water for 60 days. Rats in groups B to D served as the treatment groups and were orally treated with 200, 400 and 600 mgkg[-1] of CPF, respectively, for 60 days.

The plant extract and distilled water were administered once daily by 12 pm for five days (Monday to Friday) within a week.

Animal sacrifice and sample collection

Each rat was at the time of sacrifice first weighed and then anaesthesized by placing it in a closed jar containing cotton wool sucked with chloroform anaesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the liver and the heart. Blood samples were collected from the apex of the heart into plain heparinized bottles for biochemical analysis, using a syringe. Then the liver was excised and trimmed of all fat. The liver weight of each animal was evaluated with an electronic analytical and precision balance (BA 210S, d=0.0001- Sartoriusen GA, Goettingen, Germany). The liver volume was measured by water displacement method. A portion of the median lobe of the liver was dissected and fixed in 10% formol-saline for histological examination. The remaining parts of the liver were frozen quickly in dry ice and stored at -25°C for biochemical analysis.

Histological procedures and analysis

This was done as described by Saalu et al. (2008). Briefly, the organs were cut on slabs about 0.5 cm thick and fixed in 10% formol saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Serial sections of 5 µm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven- dried. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex,UK).

Assay of liver enzymatic antioxidants

Assay of catalase (CAT) activity

Catalse activity was measured according to the method of Aebi (1983). 0.1 ml of the liver homogenate (supernatant) was pipetted into cuvette containing 1.9 ml of 50 mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30% (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of enzyme was expressed as units /mg protein.

Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured according to the method of Winterbourn et al. (1975) as described by Rukmini et al. (2004). The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). Briefly, the reaction mixture contained 2.7 ml of 0.067 M phosphate buffer, pH 7.8, 0.05 ml of 0.12 mM riboflavin, 0.1 ml of 1.5 mM NBT, 0.05 ml of 0.01 M methionine and 0.1 ml of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15 W fluorescent lamp for 10 min. Control without the enzyme source was included. The absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions. It was expressed as u/mg protein.

Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was measured by the method described by Rotruck et al. (1973). The reaction mixture contained 2.0 ml of 0.4 M Tris- HCl buffer, pH 7.0, 0.01 ml of 10 mM sodium azide, 0.2 ml of enzyme; 0.2 ml of 10 mM glutathione and 0.5 ml of 0.2 mM H_2O_2. The contents were incubated at 37°C for 10 min followed by the termination of the reaction by the addition of 0.4 ml 10% (v/v) TCA, centrifuged at 5000 rpm for 5 min. The absorbance of the product was read at 430 nm and expressed as nmol/mg protein.

Assay of liver non-enzymatic antioxidants

Assay of liver reduced glutathione (GSH) concentration

GSH was determined by the method of Ellman (1959). 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). 0.4 ml of distilled water was added. The mixture was thoroughly mixed and the absorbance was read at 412 nm, expressed as nmol/mg protein.

Estimation of lipid peroxidation (malondialdehyde)

Lipid peroxidation in the liver tissue was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) method of Buege and Aust (1978). A principle component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min and cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was calculated using the molar absorptive of malondialdehyde which is 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol/mg protein.

Statistical analysis

All data were expressed as mean ± SD of number of experiments (n = 5). The level of homogeneity among the groups was tested using analysis of variance (ANOVA) as done by Snedecor and Cochran (1980). Where heterogeneity occurred, the groups were separated using Duncan multiple range test (DMRT). A value of $p < 0.05$ was considered to indicate a significant difference between groups (Duncan, 1957).

RESULTS

Acute oral toxicity studies

There were no deaths of rats dosed 3000 mgkg[-1] body weight of the plants extract both within the short and long outcome of the limit dose test of up and down method (Table 1).

Body weight changes

Table 2 shows those rats in control and 200 mgkg[-1] CPF groups significantly ($p < 0.05$) increased in weight when compared to their initial mean live weight. Both the 400 and 600 mgkg[-1] CPF- administered groups lost weights when compared with their initial weights. However the weight loss by the 600 mgkg[-1] CPF-administered rats was higher than the losses by the group that received CPF 400 mgkg[-1] treatment.

Weights and volumes of liver

The liver weights, liver volumes and liver weight/body weight ratio of the 600 mgkg[-1] CPF-administered rats were the least, being significantly lower ($p < 0.001$) compared to the mean liver weights, liver weight/body weight ratio and volumes of the control rats and those that were given 200 mgkg[-1] CPF (Table 2).

Table 1. Results of acute toxicity test for *Citrus paradisi* (up and down procedure) in rats.

Test serial no	Animal identity	Dose of CPF (mg/kg)	Short term results (48 h)	Long term results (14 days)
1	REP	2000	S	S
2	LEP	2000	S	S
3	TC	2000	S	S
4	RLT	2000	S	S
5	I	2000	S	S

S = Survival; REP = right ear pierced; LEP = left ear pierced; TC = tail cut; RDC = right leg tagged; I = intact rat.

Table 2. The changes in gross anatomical parameters of Wistar rats.

Treatment group	Initial body weight (g)	Final body weight (g)	Body weight difference (g)	Liver weight (g)	Liver volume (ml)	Liver Wt/ Body Wt ratio
Control	190.5 ± 4.5	190.6 ± 4.3	0.1	10.43 ± 0.9	10.52 ± 0.5	0.006
CPF 200 mgkg^{-1}	191.2 ± 3.8	191.3 ± 2.2	0.1	10.51 ± 0.3	10.57 ± 0.2	0.006
CPF 400 mgkg^{-1}	201.5 ± 2.4	188.5 ± 1.3	13.1	8.31 ± 0.6*	9.36 ± 0.6	0.005
CPF 600 mgkg^{-1}	199.4 ± 5.3	146.6 ± 3.6**	52.7**	6.47 ± 0.5**	6.54 ± 0.3**	0.003**

* and **, Significant decreases at $p < 0.05$ and $p < 0.001$, respectively when compared to control values. wt, Weight. Values are means ± S.E.M. n = 10 in each group.

Table 3. Liver antioxidative enzymes and liver contents of GSH and MDA.

Treatment group	SOD (u/mg protein)	CAT (u/mg protein)	GPX (nmol/mg protein)	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Control	46.65 ± 4.50	14.75 ± 1.25	0.81 ± 0.65	2.33 ± 0.08	0.56 ± 0.04
CPF 200 mgkg^{-1}	47.86 ± 4.64	15.60 ± 3.35	0.78 ± 0.16	1.87 ± 0.06	0.67 ±0.05
CPF 400 mgkg^{-1}	29.55 ± 2.45*	9.35 ± 2.4*	0.54 ± 0.26*	0.88 ± 0.14*	1.15 ±0.6*
CPF 600 mgkg^{-1}	12.41 ± 2.35**	4.25 ± 0.25**	0.26 ±0.15**	0.55 ±0.07**	1.95 ±0.05**

* and **, Significant differences at $p < 0.5$ and $p < 0.001$, respectively compared to controls. Values are means ± S.E.M. n = 10 in each group.

Liver oxidative status

Activities of liver antioxidative enzymes- superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)

The activities of liver enzymes, SOD, CAT and GPx are shown in Table 3. Administration of distilled water 5 mlkg^{-1} per day orally (control) caused no significant ($p > 0.05$) changes in liver SOD, CAT and GPx activities. Similarly, 200 mgkg^{-1} day^{-1} CPF given orally did not elicit any statistically significant ($p > 0 .05$) change in SOD, CAT and GPx activities.

The animals that were administered with 400 and 600 mgkg^{-1} day^{-1} CPF given orally however, exhibited a statistically significant ($p < 0.05$) reduction in the activities of the liver antioxidative enzymes (SOD, CAT, and GPx).

### Liver content of glutathione (GSH) and malondialdehyde (MDA)

As shown in Table 3, the liver content of GSH and MDA of the Wistar rats that were treated with 200 mgkg^{-1} day^{-1} CPF orally fairly approximated the values for the control animals. However, the groups of rats that were administered 400 and 600 mgkg^{-1} day^{-1} CPF orally demonstrated a remarkable ($p < 0.05$) decrease in the liver content of GSH as well as a remarkable ($p < 0.05$) increase in the liver content of MDA.

Liver histology

The representative sections of the liver of control rats showed normal cytoarchitecture with visible central veins,
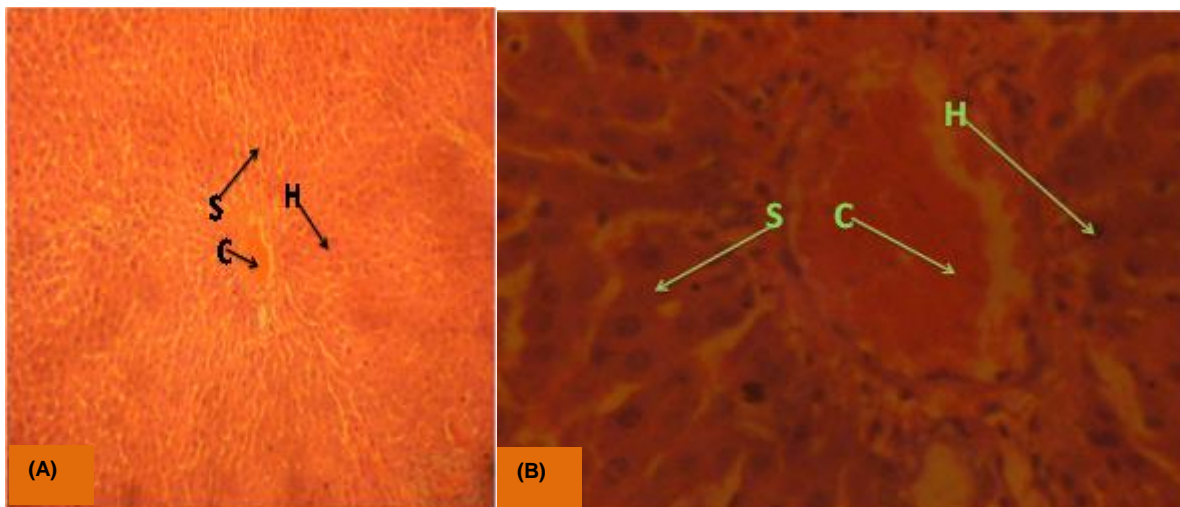
Plate 1. Photomicrograph showing a section of the liver of an adult male Wistar rat from the control group using H&E stain. The central vein (C), sinusoids (S) and hepatocytes (H) are clearly visible. A = x100, B = x400.
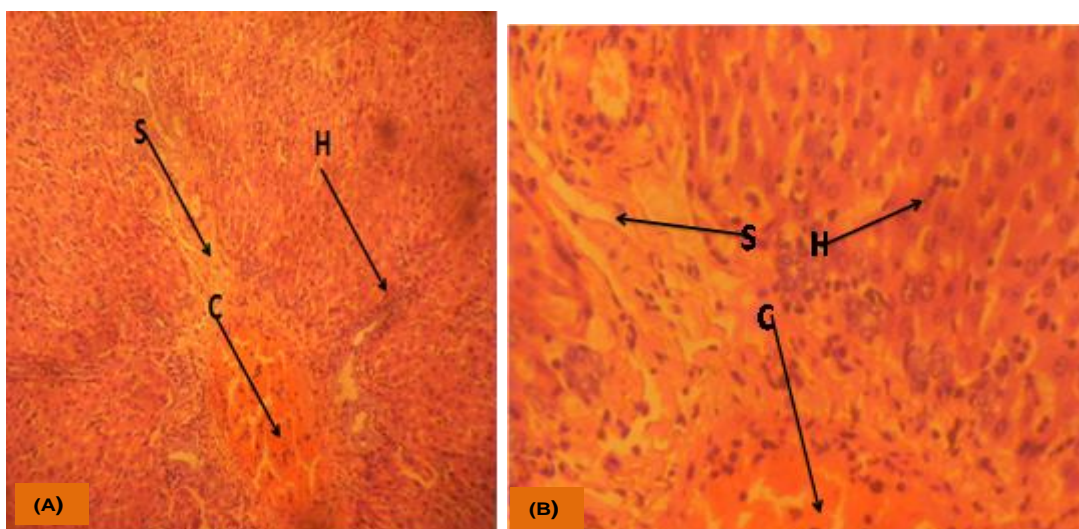
Plate 2. Photomicrograph of a section of the liver of a group of animals given 200 mgkg^{-1} of *C. paradisi* fruit juice, using H&E stain. The central vein (C) and sinusoids (S) are visible with a remarkably dense number of hepatocytes (H). A = x100, B = x400.
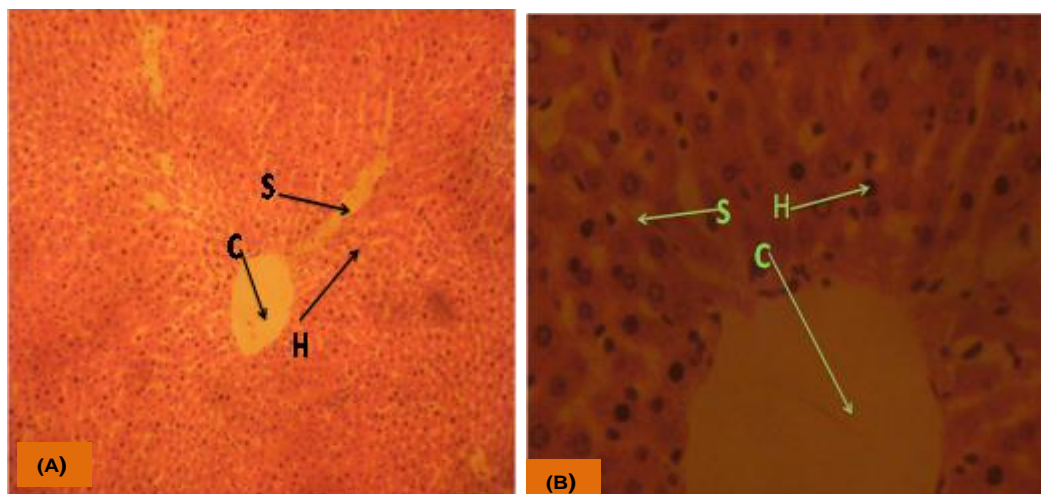
sinusoids and hepatocytes (Plate 1).

The histological profiles of the liver of animal that were treated with 200 mgkg^{-1} day^{-1} CPF given orally were largely similar to those of the control rats, except that there appeared to be increased hepatocytes density (Plate 2).

In the Wistar rats that were given CPF 400 and 600 mgkg^{-1} daily orally, there were evidences of degenerative changes in the liver characterized by reduced hepatocytes density and increased central veins sizes probably as a result of venous congestion. The highest dose of CPF exhibited a strikingly worst liver cytoarchitecture (Plates 3 and 4).

DISCUSSION

There is great interest currently on the use of medicinal plants for the treatment and prevention of a wide range of metabolic and infectious conditions. This stems partly from the rising cost of prescription drugs and partly from the awareness of the bio-prospecting of new plant-derived drugs ((Hoareau et al., 1999). It is a widely accepted fact that consumption of plant foods in adequate amounts is associated with numerous health benefits rooted in their various physiological effects as a result of their phytochemical and nutritional constituents (Hunter and Fletcher, 2002). While a good number of

Plate 3. Photomicrograph of a section of a liver of a group of animals given 400 mgkg^{-1} CPF, using H&E stain. The central vein (C) is very visible with few sinusoids (S) and a comparably sparse network of hepatocytes (H). A = x100, B = x400.
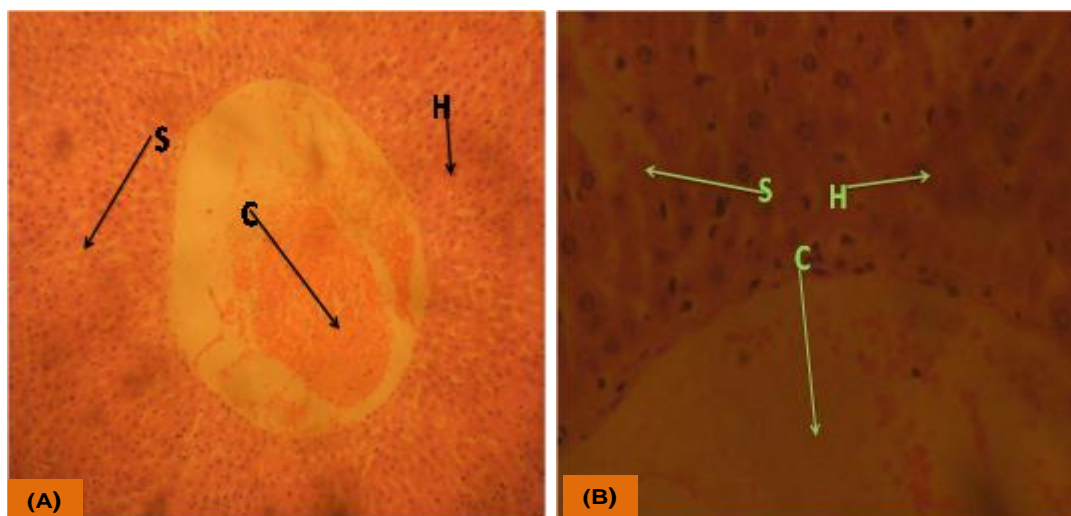
Plate 4. Photomicrograph of a section of a liver of a group of animals given 600 mgkg^{-1} CPF using H&E stain. The central vein (C) is wider with few sinusoids (S) and a very sparse network of hepatocytes (H). A = x100, B = x400.

locally used herbal remedies have been scientifically evaluated and validated but a large number of these remedies remain scientifically unevaluated. One of this is the juice of *C. paradisi* Macfad, which has ancestral use in the local management of obesity and heart diseases.

The results from our study demonstrated that the juice of *C. paradisi* fruit possesses both hepatoprotective and hepatotoxic capabilities in Wiatar rats. At a lower dose (200 mgkg^{-1} daily orally) CPF, the liver histlgical profiles and oxidative status parameters were preserved and even enhanced. This conforms to several reported

beneficial effects of another component of *C. paradisi* demonstrated in our previous studies (Saalu et al., 2009; Saalu, 2010). This could be due to the unique phytochemical composition of the juice of *C. paradisi*. Morten (1987) and Sachs (1997) have variously shown the chemical composition to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C and minerals such as zinc, iron, calcium and magnesium. It also contains phenolic compounds such as flavonoids.

Most of these active ingredients have well documented cellular growth enhancing capabilities (Mohan et al., 1997;

Ahmed et al., 2012). The administration of CPF could therefore be regarded as a steady supply of additional nutrients to the treated rats over control rats. As antioxidants, the flavonoids and vitamins in CPF could maintain liver morphology and liver oxidative status (Saalu et al., 2007, 2010b, Rahmat et al., 2012).

The results from this study indicate that higher doses of CPF (400 and 800 mgkg^{-1} daily, per oral) demonstrated hepatic injury as evidenced by histological alterations and liver oxidative stress. The molecular mechanism by which this toxicity is mediated remains unclear. However, the toxicity could be related to the high content of alkaloids in *C. paradisi* fruit juice. It has been postulated that these alkaloids could be bioactivated to release metabolites, which bind to cell molecules and cross-link DNA cause cytotoxicity (Badifu and Ogunsua, 1991). It would be plausible to hypothesize that this effect will be more prominent with higher doses of CPF as dose makes poison.

Higher doses of CPF given for a prolonged period of time as was the case with the present study may also provoke liver toxicity by increasing liver oxidative stress. This occurrence is in agreement with the well established fact that extracts from plants are pro-oxidants at high concentrations (Iwalewa et al., 2005; Saalu et al., 2010a). This may explain why the liver oxidative status of these groups of rats was severely altered as shown by the significant ($p < 0.05$) decrease in the activities of SOD, CAT and GPx; in addition to the significant ($p < 0.05$) reduction in the GSH level as well as the significantly ($p < 0.05$) enhanced lipid peroxidation measured as MDA.

Conclusion

It is concluded from the results obtained in this study that the juice of *C. paradisi* fruit (grapefruit) possesses protective and even rejuvinative effects on the liver of Wistar rats at a lower dose, but it is deleterious to the liver at higher doses.

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