

## Effect of Shaddock Citrus Fruit Juice on the Liver, Body Weight and Haematology of Male Wistar Albino Rats

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**Abstract:** The effect of shaddock citrus fruit juice on some liver function markers, body weight and some hematological parameters of Wistar albino rats were evaluated in 16 male albino rats. The rats were procured from Faculty of Veterinary Medicine, Animal House, University of Nigeria, Nsukka and were of average age of 12 weeks, weighing 130-170 grams. The rats were acclimatized for 7 days and randomly separated into 2 groups of eight rats each, kept in 2 different standard cages. The groups were as follows: A- rats fed with rat feed (control), B- rats fed with rat feed and shaddock juice. The study lasted for 28 days. The rats were weighed with electronic weighing balance at weekly intervals. At the end of 28 days feeding experiment, the rats were bled and blood collected used for liver and hematological parameters assays using standard biochemical methods. The result of the study indicated significant decrease ( $P < 0.05$ ) in the body weight gain of rats supplemented with shaddock juice on the 1<sup>st</sup>, 2<sup>nd</sup> and 4<sup>th</sup> weeks of this study when compared with those of the control group. Result of the study also showed no significant difference ( $P > 0.05$ ) in the bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate amino transferase concentrations of rats supplemented with shaddock juice when compared with the control. The result also showed increase in red blood cell, haemoglobin and packed cell volume levels of rats supplemented with shaddock juice when compared with the control. The result of this study suggests that shaddock citrus fruit juice is not toxic to the liver and could assist in the body weight control and in boosting the blood parameters.

**Key words:** Shaddock fruit juice • Liver function markers • Body weight • Haematology and Wistar rats

### INTRODUCTION

The plant shaddock citrus fruit (*Citrus maxima*) or pomelo are native to Southeastern United States and they are also widely cultivated in some regions of West Africa [1]. Shaddock is a hybrid of grape (*Citrus vinifera*) and orange (*Citrus sinensis*) with a smooth yellowish skin and may either be seeded or seedless. In some cases, through selection and breeding, shaddock has been crossed with tangerine *Citrus reticulata* to obtain a variant with juicy, thick skinned, easy to peel and tangelo [2]. The pulp of shaddock citrus fruit is stated to possess appetizer, antitoxic, cardiac stimulant and stomach tonic properties, as reported in ancient and medieval literature [3]. The major flavanones of shaddock citrus fruit are neohesperidin and naringin, which are higher in the seed than in unripe fruits [4] and its extracts exhibited

antioxidant activity potentials through free radical scavenging *in-vitro* and reduced reactive oxygen species (ROS) in H<sub>2</sub>O<sub>2</sub> treated Hep G2 cells [5]. Liver function tests/markers measure various chemicals in the blood made by the liver. An abnormal result indicates a problem with the liver and may help to identify the cause. Further tests may be needed to clarify the cause of the liver problems. Some of the liver function markers include alanine transferase (ALT), Aspartate amino transferase (AST), alkaline phosphatase (ALP), albumin, total protein and bilirubin [6].

While haematology is the study of blood, the blood forming organs and blood forming diseases. Haematology includes the study of etiology, diagnosis, treatment, prognosis and prevention of blood diseases that affect the production of blood and its components, such as blood cells, haemoglobin, blood proteins and the mechanism of blood coagulation.

The term body weight is used in biological and medical sciences to refer to a person's mass or weight. Body weight is most usually measured in kilograms. The body weight is one way of determining a person's health [7]. Strictly speaking, the body weight is the weight of the person without any items on, but practically body weight is taken with clothes on but often without the shoes and heavy accessories. Ideal body weight (IBW) was initially introduced by Devine in 1974 to allow estimation of drug clearances in obese patients. Researchers have since shown that the metabolism of certain drugs relate more to IBW than total body weight [8] While the Wistar rat is an out bred albino rat. These stock were developed at the Wistar Institute in 1906 for use in biological and medical research and is notably the first rat developed to serve as a model organism at a time when laboratories primarily used the common house mouse. More than half of all laboratory rat strains descended from the original colony established by physiologist Henry Donaldson (Clause, 1998). The Wistar rat is currently one of the most popular rats used for laboratory research. It is characterized by its wide head, long ears and having a tail length that is always less than its body length.

Since shaddock citrus fruits juice has a lot of nutritive and medicinal potentials, the need to unravel its daily ingestion effect on the liver, body weight and haematology of male Wistar rats was what this study aimed at.

## MATERIALS AND METHODS

**Shaddock:** The shaddock citrus fruits was purchased from the local market at Ojoto, Idemili South Local Government Area, Anambra State. The shaddock citrus fruit juice was extracted by peeling off the back of the shaddock fruit and pounded with a pestle inside a mortar, after which the cheese cloth was used to sieve the filtrate (juice) out of the residue.

**Chemicals:** All the chemicals used in the study were of analytical grades.

**Animals Used:** Mature male Wistar albino rats of average age of twelve (12) weeks, weighing 130-170 grams were procured from Faculty of Veterinary Medicine Laboratory Animal House, University of Nigeria, Nsukka Campus. The rats were acclimatized for seven (7) days before the commencement of the study. The rats were all supplied with normal standard pelleted growers mash feed and water *ad libitum*.

**Rat Feed:** The normal rat feed used in the study was pelleted growers mash of Vital<sup>®</sup> Feed, produced by Grand Cereals and Oil Mills Jos, Nigeria.

**Experimental Design:** Sixteen (16) mature male Wistar rats weighing 130-170 grams were used in the study. The rats were separated into (2) groups of eight (8) rats each and kept in two (2) different standard aluminum cages as follows: group A – Wistar rats fed on rats feed only and group B - Wistar rats fed on rats feed and supplemented with shaddock citrus fruit juice. All the protocols as approved by Institutional Animal. Ethics Committee (IAEC) were observed in this study. The study lasted for 28 days, while at the end of the 28days feeding experiment, the rats were bled from the retro-balbar plexus of the medial canthus of the eyes. The blood samples were collected into Ethylene DiamineTetraacetic Acid (EDTA) tube and plain sample bottles. EDTA acted as anticoagulant. The blood samples in EDTA bottles were used for hematological analysis, while the blood sample collected in the plain tubes without EDTA were allowed to stand for 30minutes and clotted. The clotted blood was then centrifuged at the speed of 30,000 revolutions per minutes for 10 minutes. The serum was extracted and used for liver function markers assays using standard biochemical methods.

**Administration of Shaddock Citrus Fruit Juice:** Shaddock citrus fruit juice was given to the rats by administering 12ml/kg body weight of the juice orally to the rats each morning before being fed for the day.

The body weight of the rats were measured at weekly intervals using digital electronic compact weighting balance and the weight were recorded in grams. The total white blood cell was determined using Haemocytometer method as reported by Schalim *et al.* (1975) [8], while total red blood cell was also determined using Haemocytometer. The haemoglobin concentration was determined by Cyanomethaemoglobin method as reported by Kachmar (1970) [9], while packed cell volume (PCV) was determined by the Microhaematocrit method as reported by Coles (1986).

**Statistical Analysis:** The data collected from the variables were expressed in mean and standard deviation. The data collected from the variables were subjected to student t-test using the statistical package for social sciences (SPSS) version 17. Also student t-test was used in comparing the mean differences in variables amongst the two groups involved in this study. The acceptable level of significance was  $P < 0.05$ .

## RESULTS

Results in Table 1 revealed that there was significant decrease ( $P < 0.05$ ) in the body weight gain of rats on the first, second and fourth weeks for rats supplemented with shaddock citrus fruit juice (Groups B) when compared with of those their corresponding control (group A) respectively.

The result above (Table 2) revealed that there was no significant difference ( $P > 0.05$ ) in the mean total WBC count of rats supplemented with shaddock citrus fruits juice (group B) when compared with those of the control (group A). The result in Table 2 further indicated that there were increases in the mean red blood cell, haemoglobin and packed cell volume concentrations of rats supplemented with shaddock citrus fruit juice (group B) when compared to those of the control (group A).

Table 3 showed that there was a very strong positive correlation between RBC, Hb and PCV. This suggests that PCV increases as Hb and RCB increases as well.

The result in Table 4 also indicated that there was a little positive correlation between WBC and PCV although the positive correlation was not statistically significant ( $P > 0.05$ ).

The result of liver function tests in Table 4 revealed that there were no mean significant difference ( $P > 0.05$ ) observed in bilirubin, alkaline phosphatase, alanine amino transferase and aspartate amino transferase concentrations of rats supplemented with shaddock citrus fruit juice (group B) when to those of the control (group A).

## DISCUSSION

The pulp of shaddock citrus fruit has been reported to possess some nutritive and medicinal potentials [3]. In this study, the effect of shaddock citrus fruit juice on the liver, body weight and some haematological parameters of male Wistar rats were evaluated.

The result of body weight changes at weekly intervals (Table 1) revealed that there was significant decrease ( $P < 0.05$ ) in the body weight gain of rats on the first, second and fourth weeks of the study for rats supplemented with shaddock citrus fruit (group B) when compared with those of their corresponding control (group A). The result of this study suggests that shaddock citrus fruit juice could be useful in the management of obesity, since it could assist obese

Table 1: Body weight gain in grams at weekly intervals for different groups of rats used in the study

Group	0 week	1 <sup>st</sup> week (g)	2 <sup>nd</sup> week (g)	3 <sup>rd</sup> week (g)	4 <sup>th</sup> week (g)
A	0.00 ± 0.00	38.33 ± 9.40	17.43 ± 4.91	7.20 ± 5.45	13.95 ± 6.70
B	0.00 ± 0.00	28.75 ± 15.18	15.63 ± 5.84	7.33 ± 1.53	8.88 ± 2.32

Table 2: Concentrations of total white blood cell (WBC), red blood cell (RBC), Haemoglobin (Hb) and packed cell volume (PCV) of different groups of rats used in the study

Group	Total WBC (x 10 <sup>3</sup> /μl)	RBC (x 10 <sup>6</sup> /μl)	Hb (g/dl)	PCV (%)
A	24.59 ± 3.87	7.60 ± 1.06	15.68 ± 1.31	44.75 ± 3.57
B	20.91 ± 3.85	8.68 ± 1.14	16.94 ± 0.30	45.63 ± 1.65

Table 3: Correlation existing between PCV, RBC and total WBC of the rats in the study

	RBC x 10 <sup>6</sup> /μl	Hb (g/dl)	WBC x 10 <sup>3</sup>
PCV(%)	0.65**	0.68**	0.24
p-value	0.007	0.004	0.37
N	16	16	16

Pearson correlation sig (2 tailed)

Table 4: Concentrations of some serum liver function markers of rats used in the study

Group	Bilirubin (mg/dl)	ALP (Iμ/L)	ALT/(Iμ/L)	AST (Iμ/L)
A	0.41 ± 0.13	203.39 ± 57.29	36.34 ± 3.03	70.33 ± 6.41
B	0.38 ± 0.04	198.95 ± 16.87	37.25 ± 4.15	69.23 ± 4.09

persons in their weight control. The result of the study also revealed that the longer the supplementation with shaddock juice the more the reduction in weight gain as could be easily observed along the group on movement from first week to the fourth week.

Furthermore, the result of the haematological analysis of Wistar albino rats supplemented with shaddock citrus fruit juice (Table 2) indicated that there was no significant difference ( $P > 0.05$ ) observed in total white blood cell count of rats supplemented with shaddock citrus fruit juice when compared to those of the control. This suggest that citrus fruit juice may not possibly have a direct effect on the immune system of the Wistar rats in this study. However, results in Table 2 also indicated increase in the Hb, PCV and Rbc counts of rats supplemented with shaddock citrus fruit juice when compared to those of the control. This suggests that shaddock citrus fruit juice has the capability of boosting some of the haematological parameters like Hb, PCV and red blood cells in Wistar rats. The result of this work is in agreement with the findings of Oyedepo (2012) who also observed increase in PCV, Hb and red blood cells counts of diabetic rats supplemented with *Citrus maxima* (shaddock) juice. The result of this study suggests that shaddock citrus fruit juice could be useful in the prevention and treatment of anaemia, since it could assist in boosting the levels of some of these blood parameters. Result in Table 3 also revealed that a very strong positive correlation exists between the RBC and Hb with PCV. This implies that as the concentration of packed cell volume is increasing, that the level of total red blood cell and haemoglobin are also increasing and vice versa.

The result of some liver function markers evaluated in the study (Table 4) indicated that there was no significant difference ( $P > 0.05$ ) observed in the bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase concentrations of rats supplemented with shaddock citrus fruit juice (group B) when compared with those fed with animal feed only (control group A).

The result of the liver function markers assayed for in this study suggests that daily ingestion of shaddock citrus fruit juice was not toxic to the liver of rats supplemented with this juice. The result of this study therefore suggests that shaddock citrus fruit juice intake should be encouraged, considering its good nutritive and medicinal potentials [3].

## CONCLUSION

The result of this study suggests that shaddock citrus fruit juice daily ingestion at the dose of 12ml/kg body weight was not toxic to the liver, since all the liver function parameters examined were still normal. The result of the study also showed significant decrease ( $P < 0.05$ ) in the body weight gain of rats supplemented with shaddock fruit juice when compared to those of the control. This suggests that shaddock citrus fruit juice could be useful in the weight control by obese individuals. Furthermore, the result of this study indicated increases in the concentrations of haemoglobin, packed cell volume and total red blood cell counts of rats supplemented with shaddock citrus fruit juice (group B) when compared with those of the control (group A). This suggests that shaddock citrus fruit juice could assist in protecting the body against anaemia and also in the treatment of anaemia

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