Acute and sub-chronic toxicological studies of *Citrus aurantium* fruit juice in Wistar rats

Abstract

Citrus aurantium also known as Bitter orange remains an excellent source of vitamins and phytochemicals. *C. aurantium* has been associated with lots of medicinal uses ranging from weight management to the treatment of nausea, cardiovascular diseases and cancer. This study therefore aimed at determining the acute and sub-chronic toxic effects of *C. aurantium* (Lemon) fruit juice in Wistar rats. The median lethal dose (LD_{50}) for the extract was determined by Lorke's method. A total of 20 rats of mean weight of 125 ± 5 *g* randomized across 4 groups of five rats each was used for the study. Groups B, C and D were administered 2-, 4- and 8-ml body weight of the extract for 3 months while group A served as the control. Random blood glucose levels of rats were m at monitored on monthly basis. Blood samples were collected for various biochemical assays as kidney and liver functions, serum lactate dehydrogenase enzyme activity, lipid profile, lipid peroxidation and electrolyte levels using standard diagnostic methods. Results showed a lethal dose of 70ml/kg body weight. Creatinine and urea levels were observed to decrease markedly in groups C and D against control. Significant reductions in the liver function parameters were noted for the test groups when compared to the control with a comparative significant increase in the activity of lactate dehydrogenase within the test groups. There was also a significant decrease in the levels of total cholesterol, low density lipoprotein, very low-density lipoprotein and triglycerides within the test groups as compared to the control. The findings suggest that prolonged administration of *C. aurantium* could exert some degree of chronic toxicity involving tissue and/or organ damage. It can also be inferred that the fruit juice of *C. aurantium* could be employed to remedy numerous maladies that affect the body based on the lethal dose.

Keywords: *Citrus aurantium*, acute, sub-chronic, toxicity, lethal dose, lipid profile

INTRODUCTION

Citrus aurantium also known as bitter orange, sour orange or *Oroma Inu* [1] is an easily available tree with ever-green leaves and yellow edible fruits belonging to the family *Rutaceae* [2] that is often used as a food flavoring and acidifying agent. The major active biological constituents in citrus herbs are mainly synephrine, flavonoids, hesperidin, naringin and alkaloids. These biological constituents give bitter orange its strong odor and flavor and accounts for many of its medicinal effects as they contain anti-inflammatory, antibacterial, and antifungal properties [3]. The medicinal benefits of bitter orange ranges from weight management to the treatment of nausea, cardiovascular diseases and cancer in humans [4].

Recent research shows that approximately 1.9 billion adults are overweight and 600 million are obese worldwide [5]. According to the United States Food and Drug Administration [6], five (5) weight loss drugs had been approved for long-term use in obese (body mass index [BMI] ≥30) or

overweight (BMI≥27) individuals with at least 1 weight-associated co-morbidity (type 2 diabetes, hypertension, hyperlipidemia) [7]. These drugs include orlistat, lorcaserin, naltrexone-bupropion, phentermine-topiramate, and liraglutide. However, what has made bitter orange well known and popularized is its effective use in weight management products such as diet pills due to its reputed effects on metabolic processes such as lipolysis, appetite suppression and an increase in basal metabolic rate [8].

The high usage of *C. aurantium* is also due to the prohibition of the Ephedra stimulant by the FDA in the year 2004. *Citrus aurantium* has since then been used as a replacement for Ephedra, as it contains *p*-synephrine, a phenylethanolamine type alkaloid, which is chemically similar to ephedra [9] but without the ephedra side effects, thus it is vastly used in "ephedra-free" herbal weight-loss products by dietary supplement manufacturers [3]. The effects of ephedrine and other adrenergic agonists cannot be extrapolated to p-synephrine because p-synephrine does exhibit binding to β-3 adrenergic receptors which does not result in cardiovascular stimulation but is associated with lipid metabolism thus explaining its ability to enhance fat oxidation [10,11].

In a study by Ratamess *et al* [12], subjects were given two chocolate-flavored chew daily for three days containing 100mg of p-synephrine in the form of standardized bitter orange extract (Advantra Z®), 100mg of p-synephrine with 100mg of caffeine or placebo [13]. p-synephrine in the presence or absence of caffeine resulted in no adverse effects. p-synephrine also increased lipolysis, fat oxidation, energy expenditure, oxygen consumption, and carbohydrate metabolism.

In another study by Arbo *et al*. [14], mice were treated daily with bitter orange extract (7.5% p-synephrine) at doses of 400, 2000, or 4000 mg/kg (corresponding to 30, 150, and 300 mg of p-synephrine/kg) and a reduction in body weight gain was observed at all doses with respect to the control groups. A review showing the effect of *C. aurantium* extract and its constituent *p*-synephrine ($C_9H_{13}NO_2$), on the treatment of 360 obese subjects, showed that *p*-synephrine, alone or in combination with other ingredients, helped to enhance metabolic rate, energy expenditure and promote weight loss when given for six to 12 weeks [15].

Citrus aurantium is widely used in traditional Chinese medicine to treat nausea, indigestion, and constipation, cancer and cardiovascular effect [16,17,18]. Bitter orange has also been used in South America for traditional treatment of insomnia, anxiety, and epilepsy [19]. Our earlier research revealed that *C. aurantium* fruit juice can cause weight loss if administered for a prolonged period of three months without having observable detrimental effect on the haematological parameters [20]. *Citrus aurantium* with these well-known nutritional and medicinal properties have nonetheless not been extensively evaluated for its toxicity. The present study aimed therefore to evaluate the acute and sub-chronic toxicity on organic parameters in Wistar rats.

METHODS

Sample Collection and Identification

The *C. aurantium* fruits were collected from Mgbakwu, Awka North Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka and deposited in the University's herbarium with Voucher number NAUH 197[A].

Test Animals

A total of 20 rats of both sex weighing between 150g and 170g, purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State were used for the experiment. They were allowed to acclimatize for one week and maintained in cages under standard environmental conditions (27°C ± 3°C, 12-hour light/dark cycle) according to the National Institute of Health Guide on the Use of Experimental Animals at the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. The animals were fed Vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state *ad libitum*. At the end of the one-week acclimatization period, the animals were weighed, grouped and labeled.

Comment [DSB1]: Plz. Mention approved protocol number for animal study

Study Design

The animals were divided into 4 cohorts of five rats each. The animals were designated accordingly as below:

Group A: Normal Control
Group B: 2ml/kg bodyweight of *C. aurantium* fruit juice
Group C: 4ml/kg bodyweight of *C. aurantium* fruit juice
Group D: 8ml/kg bodyweight of *C. aurantium* fruit juice

Acute toxicity (LD_{50}) evaluation

The median lethal dose (LD_{50}) for each of the extracts were determined using Lorke's method [21]. Thirteen (13) male rats were used for the determination of the median lethal dose for each extract. The thirteen (13) rats were randomized into six groups; three rats each for the first phase which was given 10, 100 and 1000mg/kg *b.w.* and one rat each for the second phase which was given 50, 100, 200 and 400 mg/kg bw. The animals were monitored for changes in behavior and mortality within 2 hours, 24 hours and 14 days after single administration of the extracts.

LD_{50} values were calculated using the formula below:

$LD_{50} = \sqrt{HNLD x\ LLD}$

Where HNLD = Highest non-lethal dose, LLD = Least lethal dose.

$LD_{50} = \sqrt{50}x\ 100$

= 70.7 ml/kg b.w.

Animal sacrifice and blood collection
The animals were anaesthetized with chloroform and blood samples collected via cardiac puncture. The samples were collected into the universal bottles and allowed to clot, after which they were centrifuged for 10 minutes at 4000 rpm. The sera obtained were transferred into another set of test tubes. The sera were used for the biochemical analysis on the same day.

Random Blood Glucose Concentration
The blood glucose levels of the rats were checked before the induction of anemia, during, and after treatment using One Touch Glucometer (Life Scan, USA) and test strips based on the method of Trinder [22].

Kidney Function Test
Urea and creatinine were analyzed using Randox test kits. The procedures were carried out according to the manufacturer's instructions.

Comment [DSB2]: Reference for process adopted ??

Electrolyte Concentration
The serum electrolyte concentration was analyzed using AFT-300 electrolyte analyzer. The whole blood sample of the animals was centrifuged at 4000 rpm for 10 mins. The serum was separated and used for the analysis. The probe of the electrolyte analyzer aspirates the serum of the wistar rat which passes through the electrodes, aspiration pump and the electronic circuits which measure and process the electromotive force to give the test ion concentration. The electrolytes that were analyzed include Potassium ion (K^+), Sodium ion (Na^+), Chloride ion (Cl^-), Bicarbonate ion (BCO_3^-), Total Calcium (T^{cal}) and Ionized Calcium (n^{cal}).

Comment [DSB3]: Reference for process adopted ??

Liver Function Test
Serum biochemical indices routinely estimated for liver functions were analyzed. They include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Comment [DSB4]: Reference for process adopted ??

Lactate Dehydrogenase
Serum lactate dehydrogenase enzyme was determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Comment [DSB5]: Reference for process adopted ??

Lipid Peroxidation
Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) assay method of Buege and Aust [23]. The reaction depends on the formation of complex between malondialdehyde and thiobarbituric acid (TBA). Exactly 0.4ml of serum was collected into the test tubes after which 1.6ml of 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% of thiobarbituric acid and then mixed thoroughly. The reaction mixture was then placed in 100^{o}C boiling water for 15 minutes, allowed to cool and centrifuged at 3000

rpm for 10 minutes. The supernatant was collected, and the optical density recorded at 532nm against reagent blank containing distilled water.

The lipid peroxidation activity was calculated using the formula:

$$\frac{\text{Optical density}}{\text{Time}} \times \frac{\text{extinction co-efficient}}{\text{amount of sample}}$$

where the extinction coefficient value is $1.56 \times 10^{-5} M^{-1} CM^{-1}$
The unit is expressed as µmol/MDA/mg of protein.

Lipid Profile

The lipid profile (Total Cholesterol, Triglycerides, High-Density Lipoprotein-cholesterol, Low-Density Lipoprotein-cholesterol and Very Low-Density Lipoprotein-cholesterol) were determined using Randox test kits [24,25]. Low-density Lipoprotein-cholesterol (LDL-c) was calculated using a standard formula [26]. The procedure used was according to the manufacturer's instructions provided in the manual.

Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). Analyzed data were expressed as Mean ± Standard Error of Mean (SEM). Analysis of Variance (ANOVA) was used to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p < 0.05$.

RESULTS

Effect of Acute toxicity study of *C. aurantium* fruit juice

During the first phase of the oral administration of *C. aurantium* fruit juice, the group that was administered 10 ml/kg did not show any signs of toxicity. The group that was administered 100 ml/kg was slightly weak with one death record. The group that was administered 1000 ml/kg was slightly weak with three death records. During the second phase of administration, the group that was administered 50 ml/kg did not show any signs of toxicity. The groups that were administered 100 ml/kg, 200 ml/kg and 400 ml/kg were very weak with one death record in each group respectively. The LD_{50} calculated gave a result of 70.7 ml/kg *b.w.*

Table 1: Acute toxicity study of *C. aurantium* fruit juice**.**

Phase	Dose (ml/kg)	Mortality	Signs of Toxicity
First	10	0/3	
	100	1/3	
	1000	3/3	
Second	50	0/1	
	100	1/1	
	200	1/1	

	400	1/1	

Effect of *C. aurantium* fruit juice on Random blood glucose concentration

Table 2 below shows the effect of *C. aurantium* fruit juice on random blood glucose concentration in both test and normal control groups. The findings indicated no significant difference on the random blood glucose concentration throughout each month of administration with *C. aurantium.* The blood glucose concentrations observed remained within normal ranges.

Table 2 Effect of administration of *C. aurantium* fruit juice on random blood glucose concentrations

Groups	**Random Blood Glucose (mg/dl)**			
	Initial	**1st Month**	**2nd Month**	**3rd Month**
Normal Control	91.42 ± 1.56	73.55 ± 2.11	80.21 ± 1.76	93.27 ± 1.03
2ml/kg *b.w* of *C. aurantium* fruit juice	82.90 ± 0.54	77.82 ± 1.82	82.03 ± 1.35	92.40 ± 1.13
4ml/kg *b.w* of *C. aurantium* fruit juice	86.39 ± 1.31	85.13 ± 1.35	79.36 ± 1.70	86.23 ± 0.92
8ml/kg *b.w* of *C. aurantium* fruit juice	93.56 ± 2.09	89.05 ± 1.31	80.55 ± 1.32	84.52 ± 1.62

Results are presented as Mean ± SEM of triplicate determinations

Effect of *C. aurantium* fruit juice on Kidney Function Parameters

The result of the kidney function test as shown in Table 3 revealed that the administration of *C. aurantium* fruit juice caused reduction of creatinine levels in the group administered with 4ml/kg *b/w* of *C. aurantium* and significant reduction in the group administered with 8ml/kg *b/w* of the juice when compared with normal control. A decrease in urea concentration was observed in all groups. However, this was not statistically significant ($p > 0.05$).

Table 3 Effect of administration of *C. aurantium* fruit juice on kidney function parameters.

Groups	**Urea (mmol/L)**	**Creatinine (µmol/L)**
Normal Control	12.53 ± 0.01	39.46 ± 0.01
2ml/kg *b/w* of *C. aurantium* fruit juice	10.42 ± 0.03	46.63 ± 0.02
4ml/kg *b/w* of *C. aurantium* fruit juice	8.40 ± 0.01	32.85 ± 0.01
8ml/kg *b/w* of *C. aurantium* fruit juice	8.65 ± 0.01	16.92 ± 0.01**

*Results are presented as Mean ± SEM of triplicate determinations; **Significant decrease with respect to normal control.*

Effect of *C. aurantium* fruit juice on Electrolyte Levels

C. aurantium fruit juice increased the sodium ion (Na^+) levels as concentration of dose administered increased as recorded in Table 4 below. However, the increase was not statistically significant ($p > 0.05$). The bicarbonate ion (HCO_3^-), potassium ion (K^+), chloride ion and pH levels showed no significant decrease or increase but remained close to the value observed for the normal control group.

Table 4 Effect of administration of *C. aurantium* fruit juice on electrolyte levels and pH.

Groups	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	pH
Normal Control	16.33 ± 0.10	139.71 ± 0.02	102.87 ± 0.02	21.63 ± 0.02	7.37 ± 0.01
2ml/kg *b/w* of *C. aurantium* fruit juice	20.54 ± 0.02	139.79 ± 0.01	105.23 ± 0.22	21.29 ± 0.01	7.33 ± 0.01
4ml/kg *b/w* of *C. aurantium* fruit juice	15.68 ± 0.01	140.30 ± 0.05	102.46 ± 0.01	17.50 ± 0.02	7.17 ± 0.02
8ml/kg *b/w* of *C. aurantium* fruit juice	17.12 ± 0.02	142.53 ± 0.03	102.71 ± 0.02	19.35 ± 0.01	7.13 ± 0.01

Results are presented as Mean ± SEM of triplicate determinations

Effect of *C. aurantium* fruit juice on liver function parameters

Administration of *C. aurantium* fruit juice on liver function parameters shown in Table 5 below decreased the level of aspartate transaminase (AST) and alkaline phosphatase (ALP) significantly in all groups when compared with the normal control. Significant decreases were also observed in the levels of alanine transaminase (ALT) in all groups except in group administered with 8 ml/kg *b.w.* There was a significant reduction in GGT in all groups except those administered with 2 ml/kg *b.w.* No significant difference was observed for albumin, total protein, total bilirubin (T. BIL) and direct bilirubin (D. BIL) within all the groups.

Table 5 Effect of administration of *C. aurantium* fruit juice on liver function parameters.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	Albumin (g/L)	Total Protein (g/L)	T. BIL (µmol/L)	D. BIL (µmol/L)
Normal Control	147.6 ± 0.88	763.3 0 ± 0.25	503.83 ± 0.44	7.26 ± 0.11	36.57 ± 0.38	77.12 ± 0.91	15.57 ± 0.04	2.90 ± 0.07
2 ml/kg *b.w.* of *C. aurantium* fruit	98.40 ± 0.14**	692.25 ± 0.89**	122.42 ± 0.94**	5.71 ± 0.24	39.08 ± 0.75	80.15 ± 0.10	16.01 ± 0.10	4.33 ± 0.08

juice								
4 ml/kg *b.w.* of *C. aurantium* fruit juice	102.1 ± 0.43**	650.52 ± 1.21**	368.30 ± 0.28**	3.53 ± 0.90**	34.64 ± 0.53	75.72 ± 0.75	16.90 ± 0.01	3.49 ± 0.04
8 ml/kg *b.w.* of *C. aurantium* fruit juice	136.8 ± 0.45	391.74 ± 0.72**	291.43 ± 0.34**	2.20 ± 0.15**	41.97 ± 0.20	78.23 ± 0.37	15.29 ± 0.01	2.87 ± 0.02

*Results are presented as Mean ± SEM of triplicate determinations; *Significant increase with respect to normal control; **Significant decrease with respect to normal control.*

Effect of *C. aurantium* fruit juice on Lactate Dehydrogenase Activity

The administration of *C. aurantium* fruit juice increased the activity of lactate dehydrogenase in all groups showing significant increase at 2 ml/kg *b.w* and 8 ml/kg *b.w*.

Table 6 Effect of administration of *C. aurantium* fruit juice on lactate dehydrogenase activity

Groups	LDH (U/L)
Normal Control	280.61 ± 3.76
2ml/kg *b/w* of *C. aurantium* fruit juice	346.75 ± 1.41*
4ml/kg *b/w* of *C. aurantium* fruit juice	294.11 ± 2.71
8ml/kg *b/w* of *C. aurantium* fruit juice	301.53 ± 5.65*

*Results are presented as Mean ± SEM of triplicate determinations; *Significant increase with respect to normal control.*

Effect of *C. aurantium* fruit juice on Lipid Peroxidation

The different concentrations of *C. aurantium* fruit juice administered showed no significant difference on the malondialdehyde level of the Wistar rats as shown in table 7.

Table 7 Effect of administration of *C. aurantium* fruit juice on malondialdehyde levels

Groups	MDA (µmol/L x 10^{-9})
Normal Control	5.78 ± 0.03
2ml/kg *b/w* of *C. aurantium* fruit juice	5.82 ± 0.07
4ml/kg *b/w* of *C. aurantium* fruit juice	5.48 ± 0.02
8ml/kg *b/w* of *C. aurantium* fruit juice	5.63 ± 0.05

Results are presented as Mean ± SEM deviation of triplicate determinations

Effect of *C. aurantium* fruit juice on Lipid Profile

The different concentrations of *C. aurantium* fruit juice administered showed significant reduction ($p < 0.05$) in total cholesterol (TCHOL), low density lipoprotein (LDL-*c*), triglycerides (TRIG) and very low density lipoprotein (VLDL) when compared with the normal control while the high density lipoprotein (HDL-*c*) concentration increased in all the groups with respect to the normal control although the increase was not statistically significant ($p > 0.05$).

Table 8 Effect of administration of *C. aurantium* fruit juice on lipid profile of Wistar rats after three months of continuous administration.

Groups	**TCHOL (mg/dl)**	**HDL-*c* (mg/dl)**	**LDL-*c* (mg/dl)**	**TRIG (mg/dl)**	**VLDL-*c* (mg/dl)**
Normal Control	127.54 ± 0.56	46.15 ± 0.37	72.51 ± 0.62	44.20 ± 0.23	8.84 ± 0.12
2 ml/kg *b.w.* of *C. aurantium* fruit juice	65.00 ± 0.76**	49.30 ± 0.32	11.29 ± 0.53**	22.05 ± 0.37**	4.41 ± 0.02**
4 ml/kg *b.w.* of *C. aurantium* fruit juice	61.18 ± 0.89**	53.25 ± 0.76	4.50 ± 0.55**	17.15 ± 0.34**	3.43 ± 0.11**
8 ml/kg *b.w.* of *C. aurantium* fruit juice	80.30 ± 0.10**	50.79 ± 0.14	25.59 ± 0.90**	19.60 ± 0.52**	3.92 ± 0.23**

*Results are presented as Mean ± Standard deviation of triplicate determinations; **Significant decrease with respect to normal control.*

DISCUSSION

Glucose is the metabolic fuel for the brain and any acute interruption of glucose supply may result in functional brain failure and eventually lead to coma and death. According to American Diabetes Association [27], blood glucose is considered normal below 100 mg/dL and higher ranges could indicate pre-diabetes or diabetes. The result from table 2 reveals no significant difference in the blood glucose profile of animals administered with the various doses of extract. The blood glucose concentrations observed remained within normal ranges.

High levels of creatinine and blood urea nitrogen could be a sign of an underlying condition affecting the kidneys [28], but no significant increase in creatinine or blood urea nitrogen was observed (table 3) which may be an indication that *C. aurantium* fruit juice have no adverse or toxic effect on kidney function. This finding agrees with the research work carried out by [29]. In their research, the safety of a pre workout dietary supplement with and without p-synephrine which showed no adverse effects on the kidney function was investigated.

C. aurantium fruit juice increased the sodium ion (Na^+) level as concentration of dose administered increased. However, the increase was not statistically significant ($p>0.05$). The bicarbonate ion (HCO_3^-), potassium ion (K^+), chloride ion and pH levels showed no significant

difference but remained close to the value observed for the normal control group. (table 4). The above result suggests no toxic effect of *C. aurantium* on electrolyte levels.

Liver function parameters of the serum level of the enzymes AST, ALT and Alkaline phosphatase and bilirubin are used to detect the presence of liver disease or potential harm to the liver and any kind of liver injury that can cause a rise in ALT [30]. The release of ALT and AST from the cytosol occurs when there is injury to hepatocytes, especially in membrane damage [31]. The results of our analysis in table 5 showed that there was a significant decrease ($p < 0.05$) in the levels of ALP, AST, and ALT when compared to the values of the normal control group and no significant difference was seen in albumin, total protein, total bilirubin (T. BIL) and direct bilirubin (D. BIL) within all the groups This could however suggest the non-toxic effect of *C. aurantium* on the liver. No adverse effect on liver function was also observed in the examination carried out by (Jung *et al.* [29] on pre-workout dietary supplement with and without p-synephrine.

Lactate dehydrogenase is an enzyme that catalyzes the conversion of lactate to pyruvate and back which is aimed at turning sugar into energy for cells. LDH is present in nearly all organs and tissues throughout the body including the liver, heart, pancreas, kidney, skeletal muscles and blood cells. A high level of LDH in the blood is a spotlight to acute or chronic cell damage. However, the increased activity of lactate dehydrogenase observed in table 6 suggests that increase administration of *C. aurantium* may result in organ or tissue damage.

Malondialdehyde commonly known as a biomarker for oxidative stress and cellular injuries [32] showed no significant increase or decrease with respect to the normal control. However, it may be safe to infer that *C. aurantium* fruit juice does not have any adverse effect on the malondialdehyde level of the Wistar rats. In another study by [14], no adverse effects were observed on biochemical parameters after the daily treatment of mice with bitter orange extract at different dose concentrations. Also, earlier studies on C. aurantifolia fruit juice used by many to control bodyweight revealed that proper doses of the juice do not cause any harmful effect to the organs and tissues in the body [33].

The levels of LDL-*c*, TRIGS and VLDL-*c* should be at lower concentrations in the body to avoid buildup of plaques such as atherosclerosis and heart attacks, while HDL which is known to be the good cholesterol is better at high level in the body because it transports cholesterol to the liver to be expelled, helping the body eradicate excess cholesterol, reducing the possibility of them blocking the arteries to form plaques and enhancing the prevention of cardiovascular risk factors [34] However, The different concentrations of *C. aurantium* fruit juice administered showed significant reduction ($p < 0.05$) in TCHOL, LDL-*c*, TRIG and VLDL when compared with the normal control while the HDL-*c* concentration increased in all the groups significantly ($p < 0.05$) with respect to the normal control this findings supports the research work carried out by Sharma *et al.* [35], which showed that administration of the ethanolic extract of *C. aurantium* led to a significant fall in the level of triglycerides, total cholesterol, LDL and VLDL, and improved the HDL levels, in normal rats.

CONCLUSION

The findings of this study have established the acute and sub-chronic effects of *C. aurantium* in Wistar rats administered with different doses of the fruit juice. The results suggested a significant increase and decrease of vital biochemical parameters indicating acute toxicity of the fruit juice. Though it has been widely used traditionally to ameliorate certain disease conditions arising from microbial infections and in weight management, this study has thus helped to profile its contributing effect on organ damage and possible toxicity. It is therefore believed that further histological studies would elucidate the pathology of different toxicities of the plant fruit juice.

CONSENT
This is not applicable here.

ETHICAL APPROVAL
All experiments were inspected and approved by the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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