Toxicological studies of *Citrus aurantifolia* fruit juice in Wistar Rats

ABSTRACT

Background: *Citrus aurantifolia* has been well known for its economic importance either as food or medicine. Previous studies have shown the antidiabetic, anti-cholesterol, hepato-protective and antioxidant properties of the fruit juice.

Purpose: This study focused on the effects of continuous oral administration of *C. aurantifolia* (lime) fruit juice in albino rats for a period of three months.

Methods: Acute toxicity test (LD_{50}) was done using Lorke's method. Twenty (20) rats of both sexes weighing between 120 g and 130 g were randomized into 4 groups of five rats each. Group A (normal control) was given feed and water only, Group B, C and D were given 2, 4 and 8 ml/kg bodyweight (b.w) of *Citrus aurantifolia* fruit juice respectively. At the end of the treatment period, fasting blood glucose level was determined and blood samples were collected by cardiac puncture from the experimental animals for the evaluation of the serum concentrations of biochemical parameters (liver function, kidney function, electrolytes, lipid profile, lactate dehydrogenase, and lipid peroxidation). These analyses were carried out using standard diagnostic techniques.

Results: From the result of the LD_{50}, no death was recorded for doses of 50 ml/kg b.w and below while at doses of 60 and 100 ml/kg b.w, there were signs of toxicity and death of the experimental animals. The mean lethal dose (LD_{50}) was 54.8 ml/kg bw. The fasting blood sugar levels were maintained within the normal range throughout the period of administration. The concentrations of ALT, AST, ALP, urea and creatinine were significantly (p<0.05) decreased while GGT, Albumin, Total protein, total bilirubin, K^+, Na^+, Cl^-, HCO^{3-} and the pH levels were not significantly (p<0.05) altered for the 3 doses tested when compared to the normal control. However, a dosage of 8 ml/kg bw significantly decreased the direct bilirubin level. The levels of total cholesterol, LDL-C, triacylgylcerol and VLDL were significantly (p<0.05) reduced with a significant (p<0.05) increase in the HDL-C when compared to the normal control. There was also a significant (p<0.05) increase in blood LDH levels. Dosages of 2 and 4ml/kg bw did not significantly alter the levels of MDA in relation to the normal control but 8 ml/kg bw significantly (p<0.05) decreased the MDA levels of the rats.

Conclusion: The biochemical parameters assayed suggests that proper doses of the *C. aurantifolia* fruit juice do not cause any harmful or adverse effect to the organs and tissues in the body.

Keywords: *Citrus aurantifolia, fruit, juice, toxicity, biochemical,*

INTRODUCTION

Due to the distinct aroma and delicious taste, citrus may be called a miracle fruit that is cultivated worldwide, especially in tropical and subtropical regions [1]. There are many natural metabolites in citrus fruit that potentially provide advantage and good for health [2]. Products of citrus fruit such as essential oil and pectin of fruit peel are used in the cosmetic and pharmaceutical industries.

Until recently, health-promoting properties of citrus had always been associated with their content of Vitamin C. However, studies within the last decade have focused on identifying the bioactive compounds [3]. Some of the major classes of compounds in citrus include: flavonoids, limonoids, coumarins and phytosterols [4]. Preliminary screening of *C. aurantifolia* fruit and other parts showed the presence of alkaloids, flavanoids, tannins, saponins, steroids, cardiac glycosides, carbohydrates, phenols and reducing sugars [5,6,7,4,8]. Other bioactive compounds present in citrus include: pectin, furocoumarins, coumarins, lycopene (in grape fruit), pyranocoumarins, sitosterol, monoterpenes and sesquiterpenes.

There is an increasing interest in citrus fruits consumption across the world because of their rich sources of vitamin C, folate, dietary fibre, and minerals as well as many phytochemicals, including flavonoids, amino acids, triterpenes, phenolic acids and carotenoids. There are about 37 limonoid aglycones and 19 glycosides in *C. aurantifolia* and their hybrids [3]. Limonoids are principally found in citrus fruit peels where they produce the bitter taste and the zest aroma. They are also found in large amount in the citrus fruit juice, tissue and seeds [9]. Limonoids possess the ability to inhibit tumor formation by stimulating the enzyme glutathione S-transferase (GST) which is a detoxifying enzyme that catalyzes the reaction of glutathione to form less toxic and more importantly water-soluble compounds that can be easily excreted from the body [10]. Citrus is rich in flavonoids and the most abundant flavonoids in *C. aurantifolia* extracts include: apigenin, rutin, quercetin, kaempferol, nobiletin, hesperidin, hesperitin, and neohesperidin. The flavonoids have strong inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activity [11]. It has been demonstrated that quercetin, one of the most active flavonoids possess significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation. Quercetin also showed remarkable anti-tumor properties and may have positive effects in combating or helping to prevent cancer, prostatitis, heart diseases, cataracts, allergies/inflammations and respiratory diseases such as bronchitis and asthma [12]. Carotenoids are also found in citrus and are believed to reduce the incidence of age-related macular degeneration, the leading cause of blindness in human after the age of sixty-five [13]. They play essential roles as sources of Vitamin A. The most active role is protection against serious disorders such as cancer, heart diseases and degenerative eye diseases. It is an antioxidant and acts as regulator of the immune system. Carotenoids commonly found in citrus are βcarotene, lutein, zeaxanthin and cryptoxanthin [14]. Citrus is one of the main sources of Vitamin C [15]. Ascorbic acid in the body aids in iron absorption from the intestines. It is required for connective tissue metabolism especially the scar tissue, bones and teeth. It is necessary as an anti-stress and protector against cold, chills and damp. It prevents muscle fatigue and scurvy. It is needed for normal wound healing [16]. The production of collagens is also dependent on Vitamin C. It helps in the promotion and restoration of skin [17].

METHODS

Sample Collection and Identification

The *Citrus aurantifolia* fruits *were* purchased from Eke Market, Awka, Awka South Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAUH 196[A].

Test Animals

A total of 20 wistar albino rats of both sexes weighing between 120–130g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State and used for the experiment. They were maintained and housed in cages under standard environmental conditions (27°C±3°C, 12-hour light/dark cycle) in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment for one week before use. The animals were fed Vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state and fed *ad libitum*. At the end of the one-week acclimatization period, the animals were weighed, grouped and labeled.

Study Design for Acute toxicity (LD_{50}) evaluation

The median lethal dose (LD_{50}) for *Citrus aurantifolia* fruit juice was determined using modified Lorke's method [18]. Sixteen (16) rats were used for the determination of the median lethal dose. The Sixteen (16) rats were randomized into eight groups; four groups of three rats each were used

for the first phase and were given 5, 10, 50 and 100 ml/kg bodyweight respectively. Subsequently, four groups of one rat each were used for the second phase and given 15, 25, 40 and 60 ml/kg bodyweight. The animals were monitored for changes in behaviour and mortality within 2 hrs, 24 hrs and 14 days after a single administration of the extracts.

Study Design for Sub-chronic Toxicity Study

A total of 20 rats of both weighing between 120g and 130g were randomized into 4 groups of five rats each. The *Citrus aurantifolia* fruit was freshly squeezed and the juice administered to the test groups daily for a period of three (3) months.

Group A: Normal Control (fed with feed and water only)
Group B: 2 ml/kg bodyweight of *Citrus aurantifolia* fruit juice
Group C: 4 ml/kg bodyweight of *Citrus aurantifolia* fruit juice
Group D: 8 ml/kg bodyweight of *Citrus aurantifolia* fruit juice

Random Blood Glucose Concentration

The blood glucose levels of the rats were checked before the induction of diabetes, during, and after treatment using One Touch Glucometer (Life Scan, USA) and test strips based on the method of Trinder [19].

Liver Function Test

Serum biochemical indices routinely estimated for liver functions were analysed. 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.

Kidney Function Test

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

Electrolyte Concentration

The serum electrolyte concentration was analysed using AFT-300 electrolyte analyzer. The whole blood sample of the wistar rat was centrifuged at 4000 rpm for 10 mins. The serum was separated and used for the analysis. The probe of the electrolyte analyser 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}).

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 [20,21]. Low-density Lipoprotein-cholesterol (LDL-c) was calculated using a standard formula [22]. The procedure used was according to the manufacturer's instructions provided in the manual.

Lactate Dehydrogenase

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

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 malondialehyde and thiobarbituric acid (TBA). 0.4ml of serum was collected into the test tubes; 1.6ml of 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% of thiobarbituricacid 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 umol/MDA/mg of protein.

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). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests 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

Acute toxicity studies of Citrus aurantifolia fruit juice

This test was carried out to determine the dose of *C. aurantifolia* fruit juice which can be lethal to experimental animals. There was no mortality recorded for doses of 50 ml/kg b.w and below while a dose of 60 and 100 ml/kg b.w indicated signs of toxicity which eventually led to the death of the experimental animal. From the calculations below, the mean lethal dose LD_{50} is 54.8 ml/kg b.w.

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

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

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

$LD_{50} = \sqrt{50x\ 60}$

$= 54.8$ *ml/kg bw.*

Table 1: Acute toxicity studies of *Citrus aurantifolia* fruit juice.

Phase	Dose (ml/kg)	Signs of Toxicity	Mortality
First	5	None	0/3
	10	None	0/3
	50	Slightly weak	0/3
	100	Very weak	3/3
Second	15	None	0/1
	25	None	0/1
	40	Reduced movement	0/1
	60	Very weak	1/1

Effect of continuous administration of C. aurantifolia fruit juice on fasting blood glucose concentrations.

The fasting blood glucose levels were checked to know the effect of continuous administration of *C, aurantifolia* fruit juice on fasting blood sugar level. Dosages of 2ml/kg bw, 4ml/kg bw and 8ml/kg bw were administered to different groups of the wistar rats and their fasting blood glucose levels measured after intervals of 1,2 and 3 months and recorded (Table 2). The fasting blood sugar levels

were maintained within the normal range for the period of continuous administration.

Table 2: Effect of continuous administration of *C. aurantifolia* fruit juice on fasting blood glucose concentrations.

Groups	Fasting Blood Glucose (mg/dl)			
	Initial	**1st Month**	**2nd Month**	**3rd Month**
Normal Control	74.61±1.35	76.91±1.29	88.54±1.94	84.32±1.77
Group A (2ml/kg bw).	70.33±1.29	75.47±0.25	85.51±1.24	78.43±1.29
Group B (4ml/kg bw)	81.45±0.76	79.02±0.56	82.40±2.17	75.52±1.52
Group C (8ml/kg bw).	92.03±1.61	80.22±1.76	86.25±0.32	82.64±1.45

Effect of Citrus aurantifolia fruit juice on Liver function

The result of the effect of continuous administration of *C. aurantifolia* fruit juice on liver function parameters is represented in Table 3. The concentrations of ALT, AST and ALP were significantly (p<0.05) decreased for the 3 dosages of 2ml/kg bw, 4ml/kg bw and 8ml/kg bw administered continuously for 3 months, when compared to the normal control. GGT, Albumin, Total protein, and total bilirubin levels were not significantly altered. However, a dosage of 8ml/kg bw significantly decreased the direct bilirubin level.

Table 3: Effect of continuous administration of *C. aurantifolia* 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	223.13±0.21	1020.82±1.26	396.24±0.62	4.41±0.24	40.31±0.09	70.30±0.34	16.35±0.02	3.62±0.01
Group A (2ml/kg bw).	137.51±0.63[b]	481.36±0.85[b]	321.63±0.46[b]	3.46±0.69	38.01±0.24	72.63±0.61	14.53±0.01	5.41±0.01
Group B (4ml/kg bw)	103.01±0.14[b]	496.14±0.84[b]	128.90±0.92[b]	3.92±0.69	41.85±0.30	85.11±0.12	15.16±0.03	2.63±0.01
Group C (8ml/kg bw).	107.31±0.25[b]	539.62±0.54[b]	326.53±0.77[b]	6.60±0.65	35.83±0.80	62.31±0.11	14.06±0.03	1.36±0.02[b]

[a]Significant increase with respect to normal control; [b]Significant decrease with respect to normal control.

Effect of Citrus aurantifolia fruit on Kidney Function Parameters

The result of the effect of continuous administration of *C. aurantifolia* fruit juice on kidney function parameters is represented in Table 4. In comparison with the normal control, the concentrations of urea and creatinine were significantly (p<0.05) reduced.

Table 4: Effect of continuous administration of *C. aurantifolia* fruit juice on kidney function parameters.

Groups	**Urea (mmol/L)**	**Creatinine (µmol/L)**
Normal Control	10.73±0.02	41.62±0.01

Group A (2ml/kg bw)	7.42±0.01[b]	36.26±0.01
Group B (4ml/kg bw).	8.35±0.01	44.87±0.05
Group C (8ml/kg bw).	8.45±0.01	27.43±0.01[b]

[a]Significant increase with respect to normal control; [b]Significant decrease with respect to normal control.

Effect of Citrus aurantifolia fruit juice on Electrolyte Levels

The levels of K^+, Na^+, Cl^-, HCO^{3-} and the pH were not significantly ($p>0.05$) altered on continuous administration of the *C. aurantifolia* when compared with the normal control (Table 5).

Table 5: Effect of continuous administration of *C. aurantifolia* fruit juice on electrolyte levels.

Groups	**K^+ (mmol/L)**	**Na^+ (mmol/L)**	**Cl^- (mmol/L)**	**HCO_3^- (mmol/L)**	**pH**
Normal Control	14.10±0.01	140.31±0.01	102.59±0.01	20.34±0.13	7.26±0.01
Group A (2ml/kg bw)	19.57±0.01	142.26±0.11	102.91±0.04	16.70±0.01	7.14±0.01
Group B (4ml/kg bw).	18.73±0.01	141.71±0.01	102.92±0.01	21.58±0.01	7.27±0.01
Group C (8ml/kg bw).	11.85±0.01	141.03±0.20	102.71±0.25	21.15±0.01	7.20±0.01

Effect of Citrus aurantifolia fruit juice on Lipid Profile

The lipid profile test was carried out to investigate the effects of continuous administration of *C. aurantifolia* fruit juice on wistar rats. Treatment with *Citrus aurantifolia* fruit juice for a period of 3 months significantly ($p<0.05$) reduced the levels of TCHOL, LDL-C, TRIG and VLDL with a significant ($p<0.05$) increase of the HDL-C when compared to the normal control.

Table 6: Effect of treatment with *Citrus aurantifolia* 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 (mg/dl)**
Normal Control	133.82±0.67	54.05±0.50	67.93±0.17	59.12±0.81	11.82±0.13
Group A (2ml/kg bw)	76.47±0.50[b]	57.56±0.81	14.61±0.24[b]	21.50±0.62[b]	4.30±0.15[b]
Group B (4ml/kg bw).	103.25±0.44[b]	79.80±0.18[a]	20.45±0.72[b]	14.76±0.67[b]	2.95±0.05[b]
Group C (8ml/kg bw).	110.95±0.23	76.65±0.54	28.88±0.31[b]	26.85±0.64[b]	5.37±0.02[b]

[a]Significant increase with respect to normal control; [b]Significant decrease with respect to normal control.

Effect of Citrus aurantifolia fruit juice on Lactate Dehydrogenase Activity

This test was carried out to know whether continuous administration of *C. aurantifolia* fruit juice results to tissue damage thereby leading to increase in blood LDH levels. With respect to the normal

control, the three different dosages administered to three different groups of rat for 3 months resulted to a significant ($p<0.05$) increase in blood LDH levels.

Table 7: Effect of continuous administration of *Citrus aurantifolia* fruit juice on lactate dehydrogenase activity.

Groups	**LDH (U/L)**
Normal Control	231.14±2.87
Group A (2ml/kg bw)	260.57±1.93[a]
Group B (4ml/kg bw).	283.72±2.51[a]
Group C (8ml/kg bw).	264.45±2.34[a]

[a]Significant increase with respect to normal control.

Effect of Citrus aurantifolia fruit juice on Lipid Peroxidation (Malondialdehyde)

This test was carried out to determine the effect of *C. aurantifolia* fruit juice on lipid peroxidation. MDA levels were tested as an indicator of lipid peroxidation. Dosages of 2ml/kg bw. and 4ml/kg bw. didn't significantly alter the levels of MDA in relation to the normal control. 8ml/kg bw. significantly ($p<0.05$) decreased the MDA levels of the rats.

Table 8: Effect of continuous administration of *C. aurantifolia* fruit juice on malondialdehyde level of wistar rats.

Groups	**MDA (µmol/L x 10^{-8})**
Normal Control	5.41±0.03
Group A (2ml/kg bw)	5.13±0.11
Group B (4ml/kg bw).	5.62±0.02
Group C (8ml/kg bw).	4.85±0.12

DISCUSSION

Some essential biochemical indices which serve as toxicity markers were assayed to investigate the safety of continuous administration of *C. aurantifolia* fruit juice. The acute toxicity studies show that taking in high concentrations of the *C. aurantifolia* fruit juice can be lethal with the mean lethal dose as 54.8 ml/kg bw. Akhtar, [5] also showed that doses above 3.5g/kg were toxic to rats. This can be attributed to the presence of coumarins and furocoumarins which is hepatotoxic and phototoxic when ingested in large quantities. Therefore, intake of large doses of this fruit juice can be toxic to the body.

The fasting blood glucose levels of the rats were maintained at a normal level with no significant alteration throughout the three months of continuous administration of *C. aurantifolia* fruit juice. The normal blood glucose level for Wistar rat is given to be 50-135mg/dl [24]. High phenolic content and flavonoids present in *C. aurantifolia* fruit juice acts as an antihyperglycemic agent in addition to other phytochemicals present.

Liver function parameters is used to detect the presence of liver damage which a substance or a plant extract whether crude or fractionated can cause to the liver [25]. This is determined by assaying the

serum level of these enzymes: ALT, AST, ALP, GGT, Albumin, Total protein, Total bilirubn and Direct bilirubin. ALT is more specific for liver damage because it is localized solely in the cellular cytoplasm, whereas AST is both cytosolic and mitochondrial. The release of ALT and AST from the cytosol occurs when there is injury to hepatocytes, especially in membrane damage [26]. The levels of AST, ALT and ALP were significantly ($p<0.05$) decreased for the three groups of rat administered different doses. Flavonoids present in *C. aurantifolia* fruit such as apigenin, naringenin and genistein are responsible for this effect [27]. From the results of our study, it can be said that *C. aurantifolia* fruit juice is hepatoprotective and also has restorative potential in cases of liver damage or injury.

High levels of urea and creatinine in the blood can be an indication of underlying condition affecting the kidney. However, the levels of urea and creatinine assayed for indicated no nephrotoxicity as there was a noticeable significant decrease ($p<0.05$) in the values of these markers as against that of the normal control. Hence, the nephroprotectivity of *C. aurantifolia* fruit juice. Also some study indicates that citrus fruits like lime are high in citric acid which may prevent kidney stones by raising levels of citrate and binding stone-forming minerals in the urine [28].

Electrolytes play an important role in the body; they regulate the osmotic pressure in cells and help maintain the function of muscle and nerve cells. Levels of serum electrolytes like potassium, sodium, bicarbonate and chloride which are too high or too low are suggestive of tubular dysfunction. With respect to the normal control, the concentrations of Na^+, Cl^-, $HCO3^-$ and the pH were not significantly altered on continuous administration of the *Citrus aurantifolia* fruit juice. K^+ levels were slightly increased for 2ml/kg bw. and 4ml/kg bw. but 8ml/kg bw. slightly reduced the concentration. This implies that *C. aurantifolia* fruit juice does not induce electrolyte imbalance in experimental animals.

In the analysis of the effect of *C. aurantifolia* fruit juice on the lipid profile of wistar rats, all groups showed a significant decrease in TCHOL, LDL-C, TRIG and VLDL-C, and a significant increase in HDL-C levels in comparison with the normal control. This is an indicator of the safety and effectiveness of administering *C. aurantifolia* fruit juice. HDL is known to be the good cholesterol in the body because it transports cholesterol to the liver to be expelled, helping the body get rid of excess cholesterol so it is less likely to end up in the arteries to form plaques, facilitating the prevention of cardiovascular risk factors [29]. However, LDL-C, TRIGS and VLDL-C are better at lower levels in the body and our study showed the effectiveness of *C. aurantifolia* fruit juice in achieving this.
There was a significant increase in the levels of LDH activity for the three different study groups compared to the normal control (Table 7). However, the values were still within the normal range of 280U/L [30]. Increased levels of LDH activity in the blood can be an indicator for liver disease, anemia, heart attack, bone fractures, muscle trauma, cancers, and infections such as encephalitis, meningitis and encephalitis [31]. From this study, the levels of LDH activity were still within the normal range on administering the *C. aurantifolia* fruit juice for three months.

Malondialdehyde is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Lipid peroxidation predisposes one to several disease conditions [32]. MDA level is commonly known as a marker of oxidative stress [33]. The levels of MDA were not significantly altered after a continuous administration of doses of 2ml/kg bw, 4ml/kg bw and 8ml/kg bw for three months. This suggests that *C. aurantifolia* fruit juice in the doses used for this study does not induce lipid peroxidation.

CONCLUSION

The therapeutic potentials of *C. aurantifolia* fruit juice cannot be overemphasized. Previous studies showed that it has antidiabetic, antilipidemia, and antioxidant effects. This is due to the presence of phytochemicals and vitamins in the fruit. This study indicates that continuous administeration of *C. aurantifolia* fruit juice in doses above the LD_{50} (54.8ml/kg bw.) can be lethal. The biochemical parameters assayed suggests that proper doses of the *C. aurantifolia* fruit juice do not cause any harmful or adverse effect to the organs and tissues in the body. Our findings also showed the therapeutic effects of *C. aurantifolia* fruit juice such as the antihyperlipidemia, hepatoprotectivity and nephroprotectivity.

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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