

## Research Article

## Acute and Sub-Acute Toxicity Study of *Citrus limon* (L) Juice in Sprague Dawley Rats

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**Abstract:** Great usefulness has been attributed to *Citrus limon* but little is known in the toxicity of the juice, this work was carry out to evaluate possible Acute and sub-acute Toxicity of the *Citru limon* juice, 50 Sprague dawley rats of 25 days old, weighing 100- 150g were used, For acute toxicity study, *Citrus limon* juice was administered to the 5 experimental animal per grouped, I,II,III and IV at doses 0%, 25%, 50%, 75% and 100% via the oral galvage route respectively and observed for mortality and other symptoms of toxicity and general behavioural changes over a period of 24 hours, while in sub – acute testing, new batch of 20 Sprague dawley were divided into four concentrations based on the LD50 value from the Acute toxicity using Lorke;s method and the animals were grouped into Group 1; control (water and feed alone), group 2; *Citrus limon*, 8.88%, group 3; *Citrus imon*, 17.32% and group 4; *Citrus limon*, 25.98% and they were treated for 28 days, At the end of the administration of the *Citrus limon* juice, animal were sacrificed, fresh blood were immediately obtained for Activated Partial Thromboplastin, Prothrombin and clotting time, while the remaining blood obtained from each animals were transferred into heparinized containers for heamatological anlysis, serum was collected by centrifugation for liver and kenal function assay, Liver and kidney tissues were dissected out, weighed, fixed immediately in Neutral buffered formalin for 48 hours, processed to paraffin, cut at 5µm, stained with Heamatoxylin and Eosin method and analysed histopathologically using digital microscope. Result, LD50 value of 86.60 % of *Citrus limon* was considered to be safe for consumption, while the value administered for sub-toxicity study were 8.88%, 17.32% and 25.98% respectively however there was no behavioral or locomotor activities changes, no ataxia and sign of mortality, Clothing time, Activated Partial Thromboplastin, Prothrombin, clothing time, Renal and Liver function profile were not significantly different in all the groups when compared to control group at p<0.05, Kidney and Liver histopathology revealed normal cellular profile without any area of cellular abnormalities when compared to control group. In conclusion, *Citrus Limon* juice do not exhibit potential hazard that is injurious to the experimental animal, however it could be considered non –toxic and extremely safe for consumption even at above 80 % concentration.

**Keywords:** Acute Toxicity, Sub-toxicity, *Citrus limon* Juice, Sprague Dawley Rats.

### INTRODUCTION

Medicinal plants are the pre-entire source of drugs for most of the world's population, and possess an important position in the drug discovery and development. Medicinal plants remain an important source of new drugs (WHO, 2008) despite the advantages of the synthetic chemicals and molecular modeling and many modern drugs started from traditional medicine from different culture backgrounds. Herbs and plant products were used in medicine in treating many diseases since thousands of years (Trease and Evans 1983). Moreover, the combination between traditional medicine and other new biotechnological

tools have to be established in order to make new drug development (Ahmed *et al.*, 2012).

*Citrus limon* (L) are small green trees commonly found in Asia, producing yellow fruit that produce 5% citric acid juice, they are found usually round the year, the trees of *Citrus limon* are usually between 12 to 22 feet tall with their twigs containing sharp thorns, the leaves are reddish at young stage and become green at adult stage their wings are usually slender on the petiols, *Citrus limon* contain fruit that is oval in shape with nipple like protuberance at the apex and they are 6-13cm in length, containing light yellow

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peel sometime could be greenish or white in colour, the pulp has about 6-12 segments, they are juicy and slightly acidic, they are aromatic and possess oil gland that are dotted, the fruit may possess seeds which some citrus limon fruit have no seed ([www.missouribotanicalgarden.org](http://www.missouribotanicalgarden.org)).

Calomme *et al.*, 1996, have attributed *Citrus limon* fruit as juice to possess antioxidant and anti-cancer properties, this may be due to its fruit constituent such as essential oil or d-limonene, citric acid which could be nonamal, decamal, dodecal, yarcuyl, linanyl, citronelyl flavonoids, neohesperidine, rutin, erioatin anthronil acid, limonins and methyl ester. (OECD 2008)

In alternative and ayurvedic medicine, it is considered as liver tonifier and purifier, digestive, immune and intergumentary system tonic, while lemon essential contain vapour, which has been experimented to ameliorate effect of stress in mice study. Other pharmacological usage properties include the insecticide, repellent, fungicide and termacide. (Yoo *et al.*, 2008)

Little or no experimental study has been carried out on the safety of Citrus limon, despite the huge benefit acclaimed to possess, however there is need for documentation evidence for Citrus limon, this study was carried out to investigate possible acute and sub-acute toxicity effect of Citrus limon in sprague dawley rats

## MATERIALS AND METHOD

### Chemicals, Reagents and Equipments

All the chemicals, reagents and equipments used in this study are of international standard organization grade, stardandardised by ISO and of analytical grade without any form of impurities.

### *Citrus Limon* fruit Collection

The *Citrus limon* fruit samples of the same species and varieties were collected from a the local farm in Uyo, Akwa Ibom, Nigeria within the month of October and December 2016 in sterilized conditions from the same set of trees in sterilized polythene bags, stored at 4°C in a refrigerator until use, they were authenticated by Botanist at the Plant Biotechnology unit, Derindam Research Institute of Biotechnology, Voucher specimen number **DRIB-ABU-005.11** was created for the fruit and deposited in the herbarium.

### Animals and Management

The experimental procedure was approved by the institutional Animal Care and Use Committee (IAUC) of Derindam Research Institute of Biotechnology, Nigeria and research was conducted according to the guidelines for the care and use of laboratory animals of the Institute. 40 virgin-female Sprague-Dawley (SD) rats (25 days old) weighing 180-

220g were obtained from the DRIB, the animals were divided into Two stages of 20 Sprague dawley rats in each stage that was subdivided into 4 groups of 5 rats per group. Animals are housed two rats per plastic cages and allowed to acclimatize in standard conditions under a 12 hours light and dark reaction, free access to distilled water and commercialized food throughout the experiment.

### Extraction of *Citrus limon* (L) Juice

The fruits were washed thoroughly in water, rinsed in distilled water, the juice was extracted manually, by cutting the fruits in halves and carefully squeezing to extract juice. The collected juice was filtered through 4-fold muslin cloth and the pulp free juice was collected in clean container and use for the experiment (Ishiwu and Oluka, 2004).

### Acute Toxicity Study of *Citrus limon* (L) juice

Acute toxicity study of *Citrus limon* (L) juice was carried using modified Lorke's method 1983. 50 Sprague Dawley rats were made to fast overnight prior to the experiment. Twenty five rats were divided into four (5) groups with each group consisting of five (5) animals, *Citrus limon* was administered to them at doses 0%, 25%, 50%, 75% and 100% via the oral galvage route respectively, they were observed for mortality, symptoms of toxicity and general behavioural changes over a period of 24 hours. Based on the finding in the first in stage 1, the remaining Twenty five animals were also divided into four (5) groups, each group consisting of five (5) animals, and *Citrus limon* juice was administered to them at doses 0%, 25%, 50%, 75% and 100% via the oral galvage route respectively, they were further observed for 24 hours for mortality and other signs of toxicity. The Median Lethal dose (LD<sub>50</sub>) for each drug was calculated using the formular below:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

D<sub>0</sub> = Highest dose that gave no mortality

D<sub>100</sub> = Lowest dose that produced mortality

### Sub- Acute Toxicity Study of *Citrus limon* (L) juice

Following acute toxicity testing, new batch of 20 Sprague dawley were divided into four concentrations based on the findings obtained from the LD50 value from the Acute toxicity. Groups were named as follow: Group 1; control (water and feed alone), group 2; *Citrus limon*, 8.88%, group 3; *Citrus imon*, 17.32% and group 4; *Citrus limon*, 25.98% and they were treated for 28 days.

### Heamatological Analysis

On the last day of the administration, the animals were anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture into EDTA bottles and used for assay of haematological parameters; Heamoglobin, Red Blood Cell, Packed Cell Volume Mean Corpuscular Haemoglobin, Mean

Corpuscular Haemoglobin Concentration, White Blood Cell (WBC), Platelet and white Blood cell differentials were determined according to Jain 1993, Boron *et al.*, 2005, (Ochei and Kolhakaar 2008)

#### Determination of clotting time

At sacrifice, 200µl of blood was taken directly from the heart into a prewarm test tube at 37°C, incubated in the water bath. Immediately, the blood was placed in the tubes, the tubes were filled at angle 90° and observed for clotting at 10 seconds interval, readings were taken as the blood clot and the flow of blood stopped in the tubes using automatic stop watch to take the time of clotting in line with procedure of Abdullah *et al.*, 2010.

#### Determination of Prothrombin time (PT)

Using the method of Dacie, J.V. and Lewis, S.M. (2001), prothrombin time was determined within 3 hours of blood collection using Prothrombin time test kits at ratio of 1 to 9 with the blood samples from the experimental animals; plasma was obtained from the blood by centrifugation at 1000 resolution per minute. Simultaneously, Thromboplastin and 0.1 ml of plasma were placed differently in the water bath to prewarm at 37°C, after prewarming, 0.2ml of prethrombin was forcefully added to the plasma test tube, mixed gently, stopwatch was started as the tubes was tilted reportedly at of range of 90 degree till the formation of clot and the time interval were recorded.

#### Determination of Activated Partial Thromboplastin Time (APTT)

APTT was done by mixing equal volume (0.1ml) of plasma and APTT reagent obtained from Operon Biotech were incubated for 5 minutes at 37°C, followed by addition of 100µl of 0.025ml solution of calcium chloride to observe the time of fibrin formation, APTT was considered and the value was recorded in the interval between the addition of CaCl<sub>2</sub> to the incubated solution based on the method of Dacie, J.V. and Lewis, S.M. (2001).

#### Biochemical Analysis of Renal Function Profile

Blood was also collected into plain bottles centrifuged at 3000 revolution per minute using centrifuge machine, the supernatant were taken using pipette and stored in well labeled plain bottles for biochemical and immunoassay parameters analysis. Kidney function assay were carried out on the the parameter such as Glucose, Potassium Sodium, Chloride using the methof Tietz, N.W., 1974, Bicarbonates estimation according to Young DS, 1990, urea estimation using the method of Wybenga, D.R. *et al.*, 1971 and creatinine estimation by the methods Henry JB, 1974. These parameters were were analysed by using UV-VIS Spectrophometer, absorbances were recorded and concentration were intrapolated for the absorbance readings.

#### Biochemical Analysis of Liver Function Profile

Blood was also collected into plain bottles centrifuged at 3000 revolution per minute using centrifuge machine, the supernatant were taken using pipette and stored in well labeled plain bottles for biochemical and immunoassay parameters analysis. Serum Albumin Assay, Gamma-Glutamate Transferase (γ-GT), Total Bilirubin, Direct Bilirubin, Aspartate Transferase (AST) Alanine Aminotransferase (ALT) Alkaline Phosphatase (ALP) Total Protein and Globulin were analysed by the method of Wybenga *et al.*, 1971. These parameters were analysed by using UV-VIS Spectrophometer, absorbances were recorded and concentration were intrapolated for the absorbance readings.

#### Histopathological Anaysis of the vital organs (Liver and Kidney)

24 Hours after the completion of administration of Citus limon juice, animals were sacrificed after final weighing by anasthesis using chloroform soaked in cotton in the desicator, Kidney, Liver and Lungs were also extracted out and weighed using sensitive weighing balance sensitive to 0.0001g in unit, tissues extracted out were fixed in the the Buffered formalin to prevent autolysis and putrefaction and stored in the laboratory, they were processed to paraffin wax, cut at 5um using rotary microtome and stained with Heamatoxylin and Eosin, mounted with DPX, coverslipped and observed under digital microscope for pathological changes (Hummason, 1962).

#### RESULT

##### Acute toxicity study of *Citrus limon* (l) juice

**Phase 1:** No Mortality and clinical toxicity was observed at 75% with Citrus limon, even at over 100% concentration.

**Phase 2:** The doses used in the phase 2 were based on the results obtained in phase 1.

It was observed that the highest dose that gave no mortality was 75% while the lowest dose that produced mortality was 100% revealed non- clinical signs of toxicity. The median lethal dose (LD<sub>50</sub>) was calculated using Lorke's method as follows:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D<sub>0</sub> = Highest dose that gave no mortality

D<sub>100</sub> = Lowest dose that produced mortality

$$LD_{50} \text{ of } Citrus \text{ limon juice} = \sqrt{75\% \times 100\%} = 86.60 \%$$

Therefore, 10%, 20% and 30% concentration of *Citrus limon* juice (CLJ) at LD<sub>50</sub> values 8.88%, 17.32% and 25.98% were used as Low, Middle and High dose respectively.

**Sub-acute toxicity study of *Citrus limon* (L) juice**

The oral administration of 10%, 20%, and 30% of LD<sub>50</sub>*Citrus limon* juice (CLJ) did not produce any unusual changes in behaviour or in locomotor activity in the animals, also no ataxia and no signs of toxicity or mortality during the 28 days of study except for slight agitation at the time of drug administration. No changes in fur coating, eyes and neurobehavioral and respiratory functions were observed.

Clotting time evaluated in the sub-acute studies reveal normal clotting time interval in the control group administered with only water and rat feed, it was not significantly different from group 2, treated with *Citrus limon* at 8.88%, group 3, *Citrus limon* at 17.32% and group 4, treated with *Citrus limon* at 25.98%. The clotting time occurred in the entire group were within normal time taken for clotting to occur in Sprague dawley rat, meanwhile, non-significant difference in all the group including the control group.

Sub-acute toxicity study of the Prothrombin Time Test showed normal time for prothrombin to be converted to thrombin, the treated group of *Citrus limon* at 8.88%, 17.32% and 25.98% revealed non-significant value less than 0.05 as compared to the group without.

Activated Prothrombin Time Test also revealed normal time interval in seconds, no significant difference observed in the control group when compared to the groups administered with *Citrus limon* at 8.88%, 17.32% and 25.98% though traces of slight increase was observed in the *Citrus limon* group at 8.88% when compared to control, they were not significant.

Biochemical profile of the control group was slightly increase, within the treatment group but was not significant when compared to *Citrus limon* juice groups administered with 8.88%, 17.32% and 25.98%, the Alanine transferase exhibit slight increase but not significant in all the group when compared to control group, Total protein, albumin, globulin and gamma glutamyl transferases were not significantly increased or reduced, the concentration are uniform within the

normal range when compared the control group at  $p>0.05$

Renal biochemical profile revealed normal range in concentration of urea, creatinine, bicarbonate, glucose, sodium, potassium and urea, there was reduction in the concentration of the control group against *Citrus limon* groups at 8.88%, 17.32% and 25.98% were insignificantly difference with control on comparison mode at  $p>0.05$ .

Heamatological profile revealed normal cellular distribution of red cell indices, the RBC, MCV, MCHC, PCV and hemoglobin concentration of the control group was not significantly different from the *Citrus limon* treated group at 8.88%, 17.32% and 25.98% at  $p>0.05$ .

Similarly the white blood cell profile revealed normal distribution range of leucocyte, neutrophil, monocytes, basophil and eosinophil within the normal limited cellular profile, the control group administered with water only without juice was non-significantly reduced in the count when compared to the treated groups administered with 8.88%, 17.32% and 25.98% at  $p>0.05$ .

Toxicopathology of the kidney also revealed normal cellular profile, no abnormality effect, there was evidence of enhance normal renal profile of the cortex containing renal corpuscle lined with simple squamous epithelium, the convulated tubules, proximal and distal are well displayed with no evidence of abnormality, collecting ducts and loop of henles revealed normal cellular content as compared to the *Citrus limon* juice administered groups at 8.88%, 17.32% and 25.98%.

Toxicopathological study of the Liver revealed normal cellular profile of portal triad, sinusoidal lining, parenchyma cavity, hepatocyte distribution with slight area of pyknotic index, all within normal cellular profile while in the group 2, 3, 4 treated with 8.88%, 17.32% and 25.98% of *Citrus limon* juice, there was no evidence of cellular abnormality, the hepatic profile were within normal cellular limit with enhance nucleocytoplasmic ratio.

**Table 1: Showing Sub – Toxicity study of the effect of *Citrus limon* juice on the Clotting time, Prothrombin Time and Activated Prothrombin Time intervals of Sprague Dawley Rats.**

| Groups | Activated Prothrombin Time(s) | Clotting Time (s)         | Prothrombin Time (s)      |
|--------|-------------------------------|---------------------------|---------------------------|
| 1      | 34.40±3.385                   | 55.60±2.768               | 15.80±0.663               |
| 2      | 35.20±3.652 <sup>ns</sup>     | 57.40±2.713 <sup>ns</sup> | 15.60±0.678 <sup>ns</sup> |
| 3      | 32.20±1.960 <sup>ns</sup>     | 58.00±2.345 <sup>ns</sup> | 15.40±1.030 <sup>ns</sup> |
| 4      | 36.00±2.302 <sup>ns</sup>     | 58.40±1.661 <sup>ns</sup> | 15.60±0.872 <sup>ns</sup> |

Values are Mean ± SEM of 5 rats in a group. <sup>ns</sup>*Citrus limon* juice groups were not significant different compared to to control group ( $p<0.05$ ).

**Table 2: Showing Sub – Toxicity study of the effect of *Citrus limon* (L) juice on the Heamatological Profile of Sprague Dawley Rats**

| Groups | Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> ) | Erythrocytes (10 <sup>6</sup> /mm <sup>3</sup> ) | Heamoglobin (g/dl)        | Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> ) | MCH (p/g)                 | MCHC (g/dl)               | Monocytes (10 <sup>3</sup> /mm <sup>3</sup> ) | Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> ) | PCV (%)                   | Platelets (10 <sup>3</sup> /ml) | WBC (10 <sup>3</sup> /mm <sup>3</sup> ) |
|--------|---|--|---------------------------|---|---------------------------|---------------------------|---|---|---------------------------|---------------------------------|---|
| 1      | 0.054±0.002                                     | 5.500±0.241                                      | 12.58±0.707               | 12.02±0.710                                     | 17.34±0.216               | 21.80±1.744               | 0.028±0.006                                   | 0.728±0.050                                     | 41.60±1.208               | 508.4±28.47                     | 3.600±0.221                             |
| 2      | 0.048±0.002 <sup>ns</sup>                       | 5.600±0.245 <sup>ns</sup>                        | 13.56±0.390 <sup>ns</sup> | 9.700±0.398 <sup>ns</sup>                       | 14.02±0.263 <sup>a</sup>  | 24.20±1.068 <sup>ns</sup> | 0.048±0.005 <sup>b</sup>                      | 0.720±0.046 <sup>ns</sup>                       | 43.40±1.208 <sup>ns</sup> | 646.6±27.98 <sup>ns</sup>       | 4.380±0.227 <sup>c</sup>                |
| 3      | 0.042±0.006 <sup>ns</sup>                       | 6.600±0.192 <sup>c</sup>                         | 14.14±0.398 <sup>ns</sup> | 8.040±0.368 <sup>c</sup>                        | 13.94±0.197 <sup>a</sup>  | 27.00±2.387 <sup>ns</sup> | 0.054±0.005 <sup>ns</sup>                     | 0.612±0.034 <sup>ns</sup>                       | 44.20±0.735 <sup>ns</sup> | 640.2±17.89 <sup>ns</sup>       | 5.120±0.159 <sup>c</sup>                |
| 4      | 0.036±0.004 <sup>c</sup>                        | 7.260±0.242 <sup>c</sup>                         | 14.63±0.164 <sup>ns</sup> | 6.880±0.204 <sup>b</sup>                        | 17.28±0.252 <sup>ns</sup> | 31.00±0.316 <sup>c</sup>  | 0.072±0.007 <sup>c</sup>                      | 0.682±0.034 <sup>ns</sup>                       | 41.80±0.735 <sup>ns</sup> | 610.0±22.52 <sup>ns</sup>       | 5.060±0.334 <sup>ns</sup>               |

Values are Mean ± SEM of 5 rats in a group. <sup>ns</sup>*Citrus limon* juice groups were not significant different compared to control group (p<0.05)

**Table 3: Showing Sub – Toxicity study of the effect of *Citrus limon* (L) juice on the Liver functional profile of Sprague Dawley Rats**

| Groups | Albumin (g/l)             | ALP (U/L)                 | ALT (U/L)                 | AST (U/L)                 | D.Bilirubin (mg/dl)       | GGT (g/dl)                | Globulin (g/dl)           | T.Bilirubin (mg/dl)       | T. Protein (mg/dl)        |
|--------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 1      | 3.480±0.231               | 68.80±5.843               | 35.40±2.015               | 72.80±9.074               | 0.040±0.003               | 0.544±0.039               | 1.700±0.100               | 0.058±0.004               | 5.640±0.246               |
| 2      | 3.560±0.129 <sup>ns</sup> | 67.80±8.114 <sup>ns</sup> | 36.60±1.470 <sup>ns</sup> | 82.20±3.904 <sup>ns</sup> | 0.480±0.037 <sup>b</sup>  | 0.440±0.051 <sup>ns</sup> | 1.660±0.087 <sup>ns</sup> | 0.044±0.005 <sup>ns</sup> | 5.300±0.138 <sup>ns</sup> |
| 3      | 3.420±0.128 <sup>ns</sup> | 74.20±5.398 <sup>ns</sup> | 40.40±1.327 <sup>ns</sup> | 76.60±9.341 <sup>ns</sup> | 0.116±0.071 <sup>ns</sup> | 0.480±0.035 <sup>ns</sup> | 1.720±0.107 <sup>ns</sup> | 0.054±0.006 <sup>ns</sup> | 5.340±0.163 <sup>ns</sup> |
| 4      | 3.560±0.103 <sup>ns</sup> | 81.40±4.155 <sup>ns</sup> | 49.40±2.874 <sup>ns</sup> | 82.40±3.868 <sup>ns</sup> | 0.046±0.005 <sup>ns</sup> | 0.464±0.041 <sup>ns</sup> | 1.160±0.068 <sup>ns</sup> | 0.060±0.003 <sup>ns</sup> | 5.560±0.103 <sup>ns</sup> |

Values are Mean ± SEM of 5 rats in a group. <sup>ns</sup>*Citrus limon* juice groups were not significant different compared to control group (p<0.05).

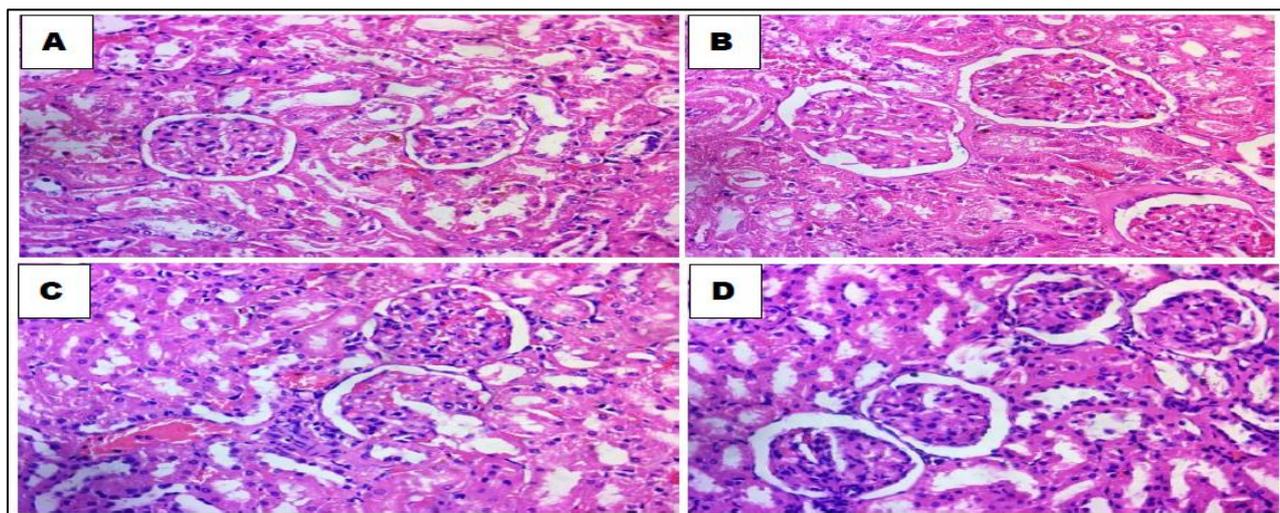
**KEYS:** Albumin (Alb), Gamma glutmmate Transferases (GGT), Total Biiirubin (TB), Direct Birubilin (DB), Alanine Ttransaminase (ALT), amino transferases (AST), Alkalin Phospahatases (ALP), Total Protein (TP) and Globulin (GLO)

**Table 4: Showing Sub – Toxicity study of the effect of *Citrus limon* (L) juice on the Renal functional profile of Sprague Dawley Rats**

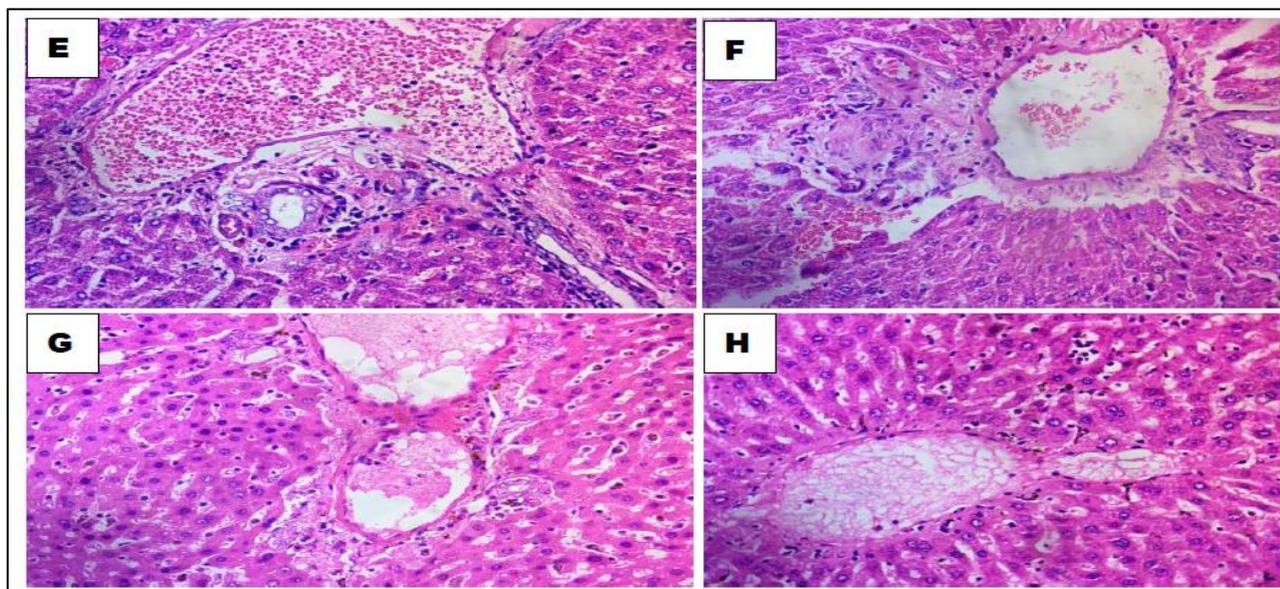
| Groups | Bicarbonate (mmol/L)      | Chloride (mmol)          | Creatinine (mg/dl)        | Glucose (mmol)            | Potassium (mmol)         | Sodium (mmol)             | Urea (mg/dl)              |
|--------|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|
| 1      | 25.80±1.200               | 93.20±2.596              | 0.310±0.029               | 93.80±3.693               | 3.760±0.093              | 137.6±2.804               | 13.88±0.683               |
| 2      | 27.20±1.158 <sup>ns</sup> | 105.8±2.672 <sup>c</sup> | 0.234±0.029 <sup>ns</sup> | 90.60±8.140 <sup>ns</sup> | 4.684±0.160 <sup>b</sup> | 129.8±5.598 <sup>ns</sup> | 12.72±0.475 <sup>ns</sup> |
| 3      | 27.00±1.378 <sup>ns</sup> | 99.60±2.249 <sup>n</sup> | 0.208±0.020 <sup>ns</sup> | 87.80±6.445 <sup>ns</sup> | 4.830±0.312 <sup>n</sup> | 133.0±4.909 <sup>ns</sup> | 12.81±0.202 <sup>ns</sup> |
| 4      | 25.80±2.035 <sup>ns</sup> | 101.0±2.881 <sup>n</sup> | 0.264±0.010 <sup>ns</sup> | 97.40±4.567 <sup>ns</sup> | 4.250±0.043 <sup>n</sup> | 125.0±2.983 <sup>ns</sup> | 12.20±0.411 <sup>c</sup>  |

Values are Mean ± SEM of 5 rats in a group. <sup>ns</sup>*Citrus limon* juice groups were not significant different compared to control group (p<0.05).

### Histopathology of Liver and Kidney



**Photomicrographs:** Control Kidney (A), Treated rats administered with *Citrus Limon*,8.88% (B) *Citrus Limon*,17.32% (C) and *Citrus Limon*,25.98% (D) in sub-acute toxicity study revealed normal renal profile with conspicuous renal tubules centered with glomerulus and lined with distinct squamous epithelium, distinct collecting ducts, convulated tubules equally present within normal limit as compared to control group following treatment for 28 days. Mag. X400 and H&E Method.



**Photomicrographs:** Control Liver (A), and treated rats administered with *Citrus Limon*,8.88% (B) *Citrus Limon*,17.32% (C) and *Citrus Limon*,25.98% (D) in sub-acute toxicity study revealed area of portal triad,hepatic vein, hepatic artery, Bile duct and hepatocytes with normal cellular limit,no abnormalities as compared to control after treatment for 28 days. Mag. X400 and H&E Method.

### DISCUSSION.

Acute toxicity of *Citrus limon* was carried out to establish concentration LD50 that is safe for human consumption, it is either carried once singularly within 24 hours of exposure or with multiple exposure with shortest period of time in 24 hours in line with MSDS hyglossary 2006 and IUPAC, compendium of clinical technology, 2006, which help to determine the safe dose range in which the juice drug can be used without any harmful effect to animals and in the computing of

therapeutic index of drug and chemicals (Rang and Dale, 2001)

LD<sub>50</sub> of *Citrus limon* juice used for the study were at 8.88%, 17.32% and 25.98% respectively as low, middle and high dose respectively, though there was slight mortality at over 100% concentration while at 75% or lower concentration, there was no mortality, this may occur as a result of the acidic content of citric acid present in *Citrus limon* due to high acidity at over 100% within 24 hours of exposure of the experimental

animals. Subacute toxicity study of the juice given to the experimental animal at low, middle and high dose based on the LD<sub>50</sub> value did not produce any unusual changes in behavior, locomotor activity in the animals, also there is no ataxia and any sign of toxicity during the duration of the study except for slight reaction at the time of administration, no changes in fur coating eyes, neurobehavioural respiratory function were observed to be normal (Gandhere *et al.*, 2013)

Sub-acute toxicity testing has been known to be one of the toxicological evaluating measures to determine the hazardous or detrimental activities of plant or agent in term of consumption if given to living organs (Aschwanden C. 2001). Sub-toxicity study of activities of *Citrus limon* juice at various concentration was verified, it was found that *Citrus limon* juice could not inflict any toxicity on the experimental animals, the *Citrus limon* juice contain high levels of flavonoid, which is known to possess strong anti-oxidative capacity thereby protective and enhance the cellular profile of the animals based on its inhibit action on free radicals, enabling cell to thrive and survive successfully without any disturbance.

The clotting time has been used as a tool to measure platelet disorder, deficient of platelets activating factor could result into clotting disorder, from this study, experimental animals administered with *Citrus limon* juice showed no sign of abnormality or induce clotting disorder or impair any of the clotting factor abnormalities, the presence of factor I to XII had shown their ability resist any bleeding disorder, Prothrombin Time Test in this study exhibited enhanced functional ability, active enzymatic conversion of prothrombin to thrombin as been found to be active, no under or over activities of enzyme observed, the intrinsic and extrinsic pathway were actively normal with the PTT in normal range due non-toxicity of *Citrus limon* juice may have been responsible for preservation of intrinsic and extrinsic pathway (Abdulla *et al.*, 2010), APTT assay also revealed anticoagulant activities of *Citrus limon* juice with its non-toxicity activities, the use of APTT in investigation of bleeding and clotting disorder and charismas diseases which associate with deficiency of factor XII, APTT restored the lost of fibrin content and bring about coagulation, *Citrus limon* juice has been found to be anticoagulant and enhancer for intrinsic and extrinsic pathway associated with clotting formation. Bleeding disorder are likely to occur, when circulating blood is deficient of clotting time factor, prothombin, thrombiplastin factor XII and deficiency of thromboxane and throbokinase, *Citrus limon* may contain active ingredients which may spread of the reaction thereby converting clotting factors within the blood to prevent bleeding disorder.

Injury to the liver are usually exposed by estimation of Alanine transferase, Alkaline phosphatase, Aspetate transaminase, the concentration of these liver enzyme were normal in all the groups in proportion to the enzymatic reaction, no injury was observed, the *Citrus limon* juice contain antioxidant agent, flavonoid moping off the effect of radicals which are likely to produce oxidative damage to the cells, the presence of these flavonoid and ascorbic acid may be responsible for the regulatin of the liver enzymes, the activities of these enzymes were regulated. (Young, D. S, 1990). *Citrus limon* juice were to preserve liver cells thereby preventing liver degeneration or liver damage. Albumin content were normal in all the group including control alone normal production of albumin within the cell, total and direct bilirubin were normal revealing no damage to the liver bile duct and bile canaliculi, *Citrus limon* juice have provided cellular oxidative protection, enhancement and tonification (Young, D. S, 1990).

In renal profile of all the groups, similar findings were observed in the urea, creatine, bicarbonate, sodium, potassium and *Citrus limon* juice displayed renal protective effect with no trace of renal damages was observed.

The use of histopathological approach in toxicity study cannot be overemphasized, cellular content of the Liver was observed under the microscope revealed none area of cellular damages in the *Citrus limon* juice at 8.88%, 17.32% and 25.98%. Ability of any plant, drugs or agent to inflict injury on the liver and kidney have shown that the efficiency of these drugs or agent is usually revealed the impotency in such plants. Liver is an hematopoietic and detoxifying organ, it is the most valuable and reliable metabolic organ with the body, drug conserved or taking during ailment directly go into the liver for metabolism before radiating for the other target organ Further toxicity study popularly known as sub-acute toxicity revealed the protective, enhancing and tolerating capacity of the liver to the *Citrus limon* juice, which contain, lineonic acid, citric acid, flavonoid, ascorbic acid and other active components that made the activities and morphological studies of the liver to be in good shape despite the increase in the juice administration, no damages was observed throught the period of administration, in similar manner, kidney as the excretory organ in the body, during detoxification the waste product are normally excreted via the kidney, glomerular infiltration and selective reabsorption are the role of kidney which have been documented, ability of the kidney to remove the waste product during administration of *Citrus limon* juice at different concentration without any deleterious effect on the kidney have revealed the potential efficiency of the juice. Renal profile hismorphology were used couple with the glomerular filtration rate activity, the electrolyte balance were kept intact with their regular activity and the loop of henle cuboidal and the

squamous epithelial lining were not distorted or affected, the higher the concentration of *Citrus limon* juice on the renal histomorphology have indicated less and insignificant toxicity level action display by the juice, no obvious cellular ability rather, the enhancement of the levels, and functional activity of the liver increase displayed and remarkably display (Elston and Ellis, 1991)

## CONCLUSION

In the course of looking for possible, less effect, toxic and safe drugs for the cure of diseases, many studies have been carried out to find possible solution to various disorders and it was on this purpose this research was conducted to find the capacity and the activities of *Citrus limon* juice.

From this study, it was discovered that in search for safe and effective drugs for the treatment or cure of diseases, there is need for documentation on the safety of the use of plant such as *Citrus limon* juice that are obtained from the fruit of the *Citrus limon* which proved to be considered safe due to his active improvement of the heamatological, histological and biochemical functions, meanwhile Citrus Limon does not show any toxic or deleterious effect on nthe experimental study thereby considered to be safe in the treatment of ailments and disorders.

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