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# Histomorphological Effects Of Citrus Aurantifolia (LIME) Leaf Extract On Acetaminophen-Induced Hepatotoxicity In Wistar Rats

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***Abstract:*** ***In medical practice, citrus aurantifolia (Lime) leaf are used for various ailment treatments. The aim of the study was to determine the histomorphological effects of citrus aurantifolia on acetaminophen induced hepatotoxicity in Wistar rats. The objectives were to (a) determine the LD50 of citrus aurantifolia leaf in Wistar rat (b) evaluate the histomorphological effects both protective and ameliorative effects of citrus aurantifolia leaf extracts on the liver of Wistar rats. Forty-four adult Wistar rats of both sex with weight ranged of 170-250g were recruited for the experiment. Nine were used to determine the lethal and the convenient dose of extracts while thirty-five were used for the experiment proper. After 3 weeks of acclimatization, the health conditions of the rats were screened via urinalysis using combi 10. The Wistar rats were assigned into seven groups of five rats of similar body weight per group. Experimental group 1 and 2 (positive controls) group 3 (negative) were given single dose 200mg/kg acetaminophen orally and sacrificed at day 4 and 22 respectively. Group 4,5,6,7 were given single dose of 200mg/kg acetaminophen +500mg/kg and 1000mg/kg body weight of the extract for 72hours and 21days. At the end of the treatment, rats were sacrificed at day 4 and 22. The liver organs were histologically processed and stained using Heamatoxyline and Eosin. Mean and SD were used while the significant level was determined at 0.05 (5%) using T-test statistical tool. The findings: The LD50 of the extract was found to be higher than 5000mg/kg. There was significant weight gain on the body weight of the animal while no significant weight gains on the organs (liver). The histological features showed evidence of hepatotoxicity by acetaminophen Furthermore, citrus aurantifolia exhibited anti-hepatotoxic activity showing both protective and ameliorative effects, this provide a sight into the management of hepatotoxicity using natural agent. Additionally, from the present investigation, it was established that acetaminophen can induce liver hepatotoxicity and that lime leaf is effective in its management. The effects are both time and dose dependent.***

***Keyword: HistomorPhological effects, citrus aurantifolia, Hepatotoxicity, Liver, Wistar Rats.***

## I. INTRODUCTION

Lime leaf is chew by individuals to prevent Nausea, stomach upset and vomiting. Lime fruits in the other hand are used to disinfect wound, cuts and bruises, in the case of diarrhea. The lime leaf is utilized in cooking due to its flavor; this strong flavor is due to the high concentration of alkaloids, citronellol, limonene, nerol and other organic compounds (Kuntal, 2008). This leaf can be directly rubbed onto the gums to promote good oral health and eliminate harmful bacteria. Its oil can be used in the treatment of blood-borne and chronic blood related diseases by eliminating pathogens or foreign agents in the blood, while helping the liver and lymphatic system strain out dangerous substance (Kasuan, 2013). It has an anti-inflammatory effect like lemon grass and cashew stem bark due to some of its organic constituents. Lime leaf boost the body immunity because it acts as an antibacterial, antioxidant, thereby neutralizing free radical, the dangerous

by-products of cellular respiration that can cause cell mutation or apoptosis as well as cancer, it also helps to prevent serious gastrointestinal issues such as colorectal cancer, hemorrhoids or gastric ulcers. The liver is a vital organ of vertebrates and some other animals. In human, it is located in the upper right quadrant of the abdomen, below the diaphragm (Skandalakis *et al.*, 2004). The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion (Kogure *et al.*, 2006).

It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The gall bladder is a small pouch that sits just under the liver, stores bile produced by the liver (Sutherland *et al.*, 2002). The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Jamieson, 2006).

Anatomically, the liver is a reddish brown wedge-shaped organ with four lobes of unequal size and shape. A human liver normally weighs 1.44-1.66kg (3.2-3.7 1b). It is both the heaviest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlaps the gallbladder (Jamieson, 2006). The liver is connected to two large blood vessels: the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. Lobules are the functional units of the liver. Each lobule is made up of millions of hepatic cells (hepatocytes) which are the basic metabolic cells. The lobules are held together by fine areolar tissue which extends into the structure of the liver, by accompanying the vessels (veins and arteries) ducts and nerves through the hepatic portal, as a fibrous capsule called Glisson's capsule (Kogure *et al.*, 2007). There are no reports in the literature on the histomorphological effects of ethanolic extract of *Citrus aurantifolia* leaf on acetaminophen induced hepatotoxicity on liver of Waster rats and based on the recent clamoring for alternative medicine in the treatment of ailment in the country claimed by the trado-medical practitioners, seen to make alternative medicine more potent, efficacious and most common than orthodox medicine in the treatment of liver related induced hepatotoxicity; it is against this background that this work or researched aimed at knowing the histoprotective and ameliorative effects of *Citrus aurantifolia* (lime leaf) extracts in treatment of acetaminophen induced hepatotoxicity in wistar rats.

## II. MATERIALS AND METHODS

### PLANT MATERIALS

Sample of *Citrus aurantifolia* (lime) leaf were plucked at Umuorji Community in Apani Ikwerre Local Government Area, Rivers State and were identified and authenticated in department of Plant Science and Biotechnology, Rivers State University science and technology.

### EXTRACT PREPARATION

The leaves of *Citrus aurantifolia* were washed, Air dried at room temperature and pulverized with mortar and pestle. 408.5g of coarse powder of *Citrus aurantifolia* were macerated with 70% ethanol, stirred and incubated for 24hrs, then filtered. This process was repeated three times. The extract solution was separated from the pulp by filtration using white handkerchief, then finally with 185mm size Whatman filter paper and after third extraction (72hours), the filtrates were mixed together. The filtrates were then concentrated using rotary vacuum evaporator in water bath at temperature of 40.5C. This process produced a viscous extract of *Citrus aurantifolia* which were poured in a crucible dish and heated in water bath to obtain dry extract. The extracts were stored in refrigerator until used for the experiment.

### EXPERIMENTAL ANIMALS

Forty-four healthy Wistar rats were purchased from the Animal House of the Department of pharmaceutical sciences, University of Port Harcourt with their weight ranging between 170-250g. The rats were housed and maintained under standard condition (12h light/dark cycles). The rats were fed with pellet Food, distilled water was given *ad libitum* throughout the study period. The animals were cared for in accordance with the recommendation provided in "Guide for Care and Use of Laboratory Animals" prepared by the National Academy of Science (National Institutes of Health, 1985).

### EXPERIMENTAL DESIGN

Total of forty-four Wistar rats were used for the work. Pre-analytic tests were conducted via urine of the rats before and after the experiment to determine the organ function using combi 10 and their weight were obtained using weighing scale. The rats were grouped for acute toxicity study and experimental main study. The acute study, nine rats of similar body weight were grouped into three A, B, C with three rats each, group A, B, C were respectively given 500mg/kg, 2000mg/kg, and 5000mg/kg body weight of *citrus aurantifolia* leaf extract, the were observed for 30mint, 1 hour, 4 hours, 6hours and 24 hours for mortality and other signs of toxicity. In the main experimental study, thirty-five animals were randomly divided into seven group of five rats each, (group 1and2 positive control, group 3 negative control, group 4,5,6,7 treatment groups). Group 1 and 2 received single dose of acetaminophen 200mg/kg body weight orally to induced hepatotoxicity while group 1 were allowed for 72hours to cause hepatotoxicity and group 2 were left for 21days to observed for reversibility. Group 4, 6 were treated with single dose of acetaminophen +500mg/kg body weight of extract for 72hours then weighed and sacrificed at day 4 while group 5,7 were treated with single dose of acetaminophen 200mg/kg +1000mg/kg body weight of the extract from 4 to 21days, weighed and sacrificed at day 22.

The extract was administered orally by carefully inserting a cannula attached to syringe into the oral cavity of the rats.

*HISTOLOGICAL TECHNIQUE*

The excised liver was fixed by immersion in 10% buffered formal saline fixative for 24hours, dehydrate through series of graded alcohol, cleared in xylene, infiltrated and embedded in molten paraffin wax. The tissue blocks were sectioned at 3um thickness deparaffinized and stained with Heamatoxyline and Eosin. The sections were examined with light microscope and photomicrographs of the sections were taken for further analysis.

*DATA ANALYSIS AND INTERPRETATION*

The results are reported as mean and S.D. The T- test statistical tool via SPSS version 21computer package was used to analyzed and compared the results at 0.05 (5%) significant level.

## III. RESULTS

### PHYSICAL AND BEHAVIOURAL OBSERVATION

From observation made, there was little difference in behavioural changes noticed between the three groups for the acute toxicity study when compared to the control. There was no mortality. Overt signs of toxicity such as sleeping, calmness, stretching out and resting in the corner of the cage, closing of eyes, weight loss were noticed as shown in table 1.1 and 1.2

| Cage | Dosage mg/kg | Skin fur | Dullness | Sleeping | Mortality | Reduced actives | Eye close | Diarrheal | Weight gain/loss |
|---|---|---|---|---|---|---|---|---|---|
| B1 Lime leaf | 500 | No skin fur | Agile | No sleeping | No Mortality | Reduced actives | No Eye close | No diarrheal | Weight loss |
| B2 Lime leaf | 2000 | No skin fur | Calmness | Sleeping | No Mortality | Reduced actives | Eye close | No diarrheal | Weight loss |
| B3 Lime | 5000 | No skin fur | Calmness | Sleeping | No Mortality | Reduced actives | Eye close | No diarrheal | Weight loss |
| Control | Water/feed | No skin fur | Very Agile | No sleeping | No mortality | Very active | No eye close | No diarrheal | No weight loss |

*Table 1.1: The behavioral and toxic symptoms exhibited by the animal for toxicity study lime leaf extract*

| Cages | Dose in mg/kg weight | Mean average weight before administration of extract(g) | Mean average weight after administration of extract (g) | S D Before | S D After | Physical weight loss or gain | Behavioral toxic symptoms |
|---|---|---|---|---|---|---|---|
| B1 Lime | 500 | 213.2 | 195.0 | 7.80 | 4.30 | ↓↓ | + |
| B2 Lime | 2000 | 175.66 | 162.00 | 3.39 | 6.38 | ↓↓ | + |
| B3 Lime | 5000 | 217.46 | 198.66 | 0.65 | 0.48 | ↓↓ | ++ |

*Table 1.2: Summary of empirical and physical measurement for acute toxicity study for the three extracts*

### BODY WEIGHT AND LIVER ORGAN WEIGHT

From the statistical analysis there was significant weight differences in the weight of the animal before and after in the main experimental groups and no significant difference in the organ weight (liver) of the test groups when compared with the control group in table 2.1,2.2 and 3.1,3.2

| | Mean | Std. Deviation | t-Test | df | p-value | Remark |
|---|---|---|---|---|---|---|
| Group1 Weight before | 170.000 | .0000 | -5.363 | 4 | .006 | Sig |
| Group1 Weight after | 175.740 | 2.3933 | | | | |
| Group2 Weight before | 170.000 | .0000 | -6.396 | 4 | .003 | Sig |
| Group2 Weight after | 228.400 | 20.4157 | | | | |
| Group3 Weight before | 170.000 | .0000 | -9.552 | 4 | .001 | Sig |
| Group3 Weight after | 233.000 | 14.7479 | | | | |
| Group4 Weight before | 172.200 | .4472 | -4.312 | 4 | .013 | Sig |
| Group4 Weight after | 175.580 | 2.1638 | | | | |
| Group5 Weight before | 170.000 | .0000 | -13.984 | 4 | .000 | Sig |
| Group5 Weight after | 235.200 | 10.4259 | | | | |
| Group6 Weight before | 173.800 | .8367 | -4.885 | 4 | .008 | Sig |
| Group6 Weight after | 176.360 | 1.0015 | | | | |
| Group7 Weight before | 181.200 | .4472 | -5.001 | 4 | .007 | Sig |
| Group7 Weight after | 221.600 | 17.8690 | | | | |

*Table 2.1: Mean comparism of experimental weight before and after and the control group*

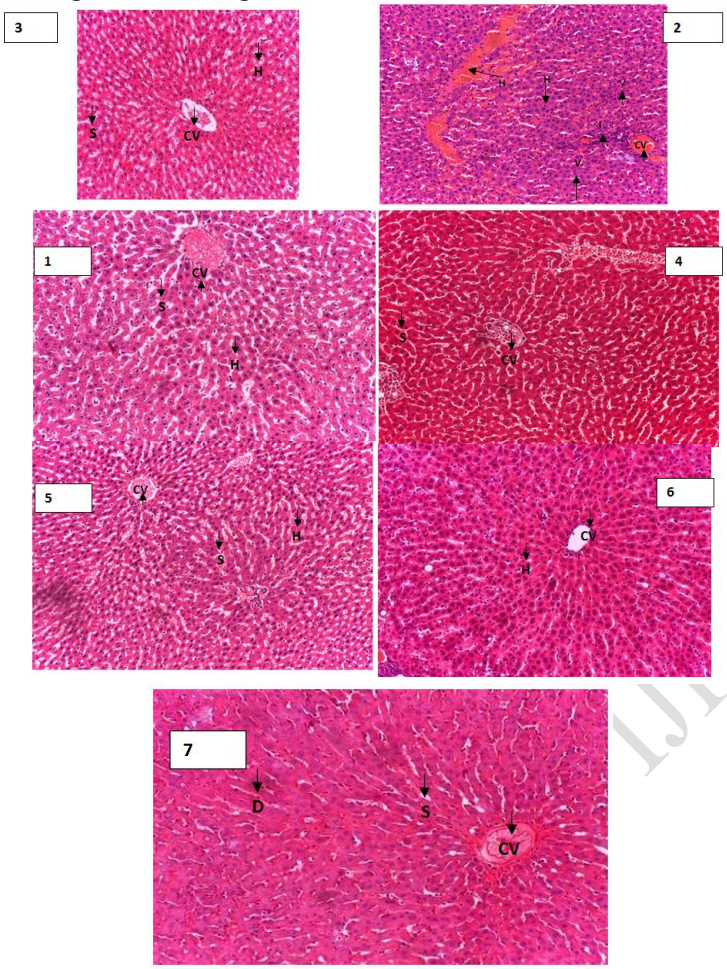
| Group | Drug extract | Dosage mg/kg body weight | Mode of determination | Total body weight(g) | Mean weight(g) | Standard deviation |
|---|---|---|---|---|---|---|
| Group 1 | PCM | 200 | E.W Scale | 31.5 | 6.30 | 0.72 |
| Group 2 | PCM | 200 | E.W Scale | 37.0 | 6.84 | 0.53 |
| Group 3 | Water/ feed | Water/feed | E.W Scale | 28.5 | 6.50 | 1.002 |
| Group 4 | PCM + lime leaf | 200+500 | E.W Scale | 29.6 | 5.92 | 0.57 |
| Group 5 | PCM + lime leaf | 200+500 | E.W Scale | 33.5 | 6.36 | 0.35 |
| Group 6 | PCM + lime leaf | 200+1000 | E.W Scale | 31.8 | 6.36 | 0.56 |
| Group 7 | PCM + lime leaf | 200+1000 | E.W Scale | 29.9 | 5.98 | 0.62 |

*The mean weight and standard deviation of liver organ*

### HISTOLOGICAL OBSERVATION

The histological observation of the liver showed distortion in cell architectures of the acetaminophen induced at

72 hours plate, while rats induced with acetaminophen and left for 21 day for reversibility appears normal. The rats induced with acetaminophen and treated alongside with the 500mg/kg and 1000mg/kg Citrus *aurantifolia* leaf extracts for 72 hours showed no evidence of hepatotoxicity and the rats induced and treated from 4-21days with 500mg/kg of *Citrus aurantifolia* also showed no evidence of distortion in the cell architectures while those induced and treated with 1000mg/kg of *Citrus aurantifolia* leaf extract from 4-21days showed some degenerative changes.




*Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 500mg/kg, 1000mg/kg of lime leaf extract daily for 4 days and 21 days (H&E X 100)*

- ✓ Rat orally given 200mg/kg acetaminophen showing extensive heamorrage, marked cellularity due to stromal proliferation, vascularization infiltration by inflammatory cells and alteration in central vein, lamina and sinusoid (72hrs).
- ✓ Positive control liver section of rat orally given (200mg/kg) acetaminophen showing hepatocytes (H), sinusoids (S), nucleus (N), central vein appearing normal (21 days).
- ✓ Negative control liver section of rat with central vein (cv), surrounding hepatocytes (H), sinusoids (S), and nucleus (N) intact.
- ✓ Rat administered single dose of acetaminophen 200mg/kg plus 500mg/kg *C. aurantifolia* leaf extract showing nuclei and other features appearing normal (72hrs).
- ✓ 5. The histological section of liver shows no obvious pathological change at 21days after administration of single dose 200mg/kg acetaminophen plus 500mg/kg *C. aurantifolia* leaf extract.
- ✓ 6. The histological section of liver showing no obvious pathological change at 72hrs after administration of single dose of 200mg/kg acetaminophen plus 1000mg/kg *C. aurantifolia* leaf extract.
- ✓ The histological section of liver shows stromal degeneration after administration of 200mg/kg acetaminophen plus 1000mg/kg *C. aurantifolia* leaf extract for 21 days.

## IV. DISCUSION

The result of the acclimatization/ health screening used for the study revealed activeness of the rats before and after acclimatization. The increased in size and weight of the rats subjected to acclimatization during this period as observed in this present study suggests minimal pathologic condition with a good physiologic process in addition to good nutrition resulting from proper feeding. Based on the qualitative observation for toxic symptoms on the Wistar rats after the administration of *Citrus aurantifolia* leaf extracts, Group A, did not show much toxic and behavioural symptoms compared to group B and C as recorded in this study; is not in consistent with research done by Mir *et al*., (2013). Furthermore, rats in group B and C demonstrated more reduced activities expressed in form of sleeping, calmness, stretching out and resting in the corner of the cage, closing of eyes illustrate the adverse toxic effects (post exposure effects) of the extracts on the rat's study. The findings suggest that the extract of *Citrus aurantifolia* leaf was relatively toxic to rats at oral dose of 2000mg/kg and 5000mg/kg because the symptoms of pronounced behavior were noted only after oral administration of relatively high dose of 2000mg/kg and 5000mg/kg. The result of the body weight and the organ weight as revealed in this study showed no dissimilarity in both the treatment and the control groups In additionally to the weight gains as observed in all animal administered with both acetaminophen alone and acetaminophen +*Citrus aurantifolia* leaf extracts point that acetaminophen +*Citrus aurantifolia* leaf extract present no evidence of interference in term of weight. Besides, the extract and the drug acetaminophen presumably did not interfere with the normal metabolism of the animal contradicts the work of Harlita *et al*., (2016) which examined the toxicity of cashew nut shell extracts in Albino rats.

Investigation of the vital organ (liver) macroscopically which demonstrated no variation between groups as observed from gross section through the result of the gross parameters; is in agreement with a study performed in 2013 by Ping and Colleagues in their study which evaluated the oral administration of methanolic extract of Euphorbia hirta at a dose of 5000mg/kg which had no adverse effect in the relative organ weight macroscopically. Notably, organ weight is an important index of physiological and pathological status in animals besides the relative organ weight is fundamental to diagnose whether it was exposed to injury or not.

*LIGHT MICROSCOPIC EXAMINATION:* APAP administration 200mg/kg causes acute liver injury in rats. In high doses of APAP, the oxidation pathway is initiated by the formation of the reactive metabolite NAPQ1 which is generated mainly by the cytochrome P450 enzymes Cypzel in rats and human. Excessive NAPQ1 formation after APAP over dose depletes cellular gluthathione induced mitochondrial oxidant stress and dysfunction which then result in nuclear DNA fragmentation and necrotic cell death. APAP overdose results in destruction of hepatocytes. The histomorphological observation of the liver section showed severe architectural distortion within 72 hours of administration of single dose Acetaminophen of 200mg/kg, Acetaminophen + *citrates aurantifolia* leaf extract. The histological results of the present investigation in plate 1 revealed that single dose of 200ng/kg acetaminophen alone led to severe liver distortion ranging from extensive haemorrhage, marked cellularity due to stromal proliferation, vascularization, infiltration by inflammatory cells and alteration in central vein, lamina, and sinusoid within 72 hours of administration of acetaminophen while there was reversibility of this distortion in plate 2 of 21 days acetaminophen alone perhaps as a result of the tissue regeneration in the liver which is in line with (Davidson and Easthan, 1966; Boyer and Rouff 1971). The liver section of the negative control group showed well differentiated hepatocytes, central vein with sinusoids and lamina well aligned. Plate 4 and 6 which is the protective group revealed that within 72 hours of single dose acetaminophen alongside with *Citrus aurantifolia* leaf extract showed no obvious pathological change within 72 hours. plate 6 that is the curative group also showed no pathological distortion at 21 days mean that *Citrus aurantifolia* leaf extract have both protective and curative effects while plate 8B of 21 days which caused damage could be due to presence of toxic substances in the extract which is expected to be highly concentrated in the high doses or the long time administration of this extract at high dosage. Further studies are necessary to determine whether the compound is responsible for the damage shown.

## V. CONCLUSION

In conclusion, this study has established that: (a) The LD50 of the extract was found to be higher than 5000mg/kg. (b) That there were significant weight gain on the body weight of the rats before and after the administration of the acetaminophen and the extract *C. aurantifolia* ($P < 0.05$) while there were no significant weight again in the organ (liver) which could be that the acetaminophen and the extract did not interfere with the normal metabolism of the animal. (c) That *Citrus aurantifolia* leaf extract tested against acetaminophen induced hepatotoxicity, exhibited antihepatotoxic activity and may therefore serves as potential sources of safe effective and affordable acetaminophen induced hepatotoxic drug. The displayed high property on the liver is both time and dose dependent. This rendered *Citrus aurantifolia* leaf a candidate that could be developed into new lead structures and candidate for drug development program against human acetaminophen hepatotoxicity.

## VI. RECOMMENDATION

From the findings of the study, the following recommendations were made:

- ✓ Individuals and Nigerian Governments, through their respective concerned organs such as NAFDAC, NDLEA etc should regulate rampant and the indiscriminate peddling of herbal medicine as well as uncontrolled use of acetaminophen.
- ✓ That lime can be use in prevention of liver toxicity since it was revealed to be effective in the recent experiment.

## VII. CONTRIBUTION TO KNOWLEDGE

In the course of the study, it was deduced that *Citrates aurantifolia* (lime) leaf has a protective and ameliorative effects against liver damage from acetaminophen toxicity. The LD50 of *Citrus aurantifolia* leaf extract was higher than 5000mg/kg, lastly Acetaminophen induced hepatoxicity can be reversed within three weeks.

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