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Histomorphological Effects of Ethanolic Extract of Cymbopogoncitratus Leaf Anacardium occidentale Stem Bark and Citrus Aurantifolia Leaf on Acetaminophen Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Inmedical practice, *Anacardiumoccidentale* (Cashew) stem bark, *Cymbopogoncitratus* (Lemon Grass) and *Citrus aurantifolia* (Lime) leaf are used for various ailment treatments. The aim of the study was to determine the histoprotective and ameliorative effects of *A. occidentale* stem bark *Cymbopogoncitratus* and *citrus aurantifolia*on acetaminophen induced hepatotoxicity in Wistar rats. The objectives were to (a) determine the LD_{50} of *Cymbopogon citrates* leaf, *A. Occidentale*stem bark and *citrus aurantifolia* leaf in Wistar rat (b) evaluate the protective and ameliorative effects of *A. occidentale* stem bark, *Citrus aurantifolia* and *Cymbopogon citrates* leaf extracts on the liver of Wistar rats histomorphologically. One hundred and twenty two adult Wistar rats of both sexes with weight range of 170-250g were recruited for the experiment. Twenty seven were used to determine the lethal and the convenient dose of extracts while ninety five were used for the experiment. The Wistar rats were assigned into nineteen groups of five rats per group. Experimental groups 1 and 2 assigned positive controls, were given 200mg/kg acetaminophen orally and sacrificed at Day 4 and 22 respectively. Group A's and B's received PCM 200mg/kg +500mg/kg and 1000mg/kg of the extracts for 72 hrs, and 21days. Group D rats represented the negative control and were given water and pellet food only. The liver organs were histologically processed and stained using Heamatoxylin and Eosin. Mean and Standard Deviation were statistically used while the significant levels were determined at 0.05 (5%) using T-test and F-test statistical tool. The findings showed that: The LD_{50} of the extracts studied was found to be higher than 5000mg/kg. There was significant weight again on the body weight of the animal while no significant weight gain on the organ (liver). The histological features showed evidence of hepatotoxicity by acetaminophen. Furthermore, among the three extracts tested *Citrus aurantifolia* exhibited the most potent anti-hepatotoxic activity showing both protective and healing effect. This provided an insight into the management of hepatotoxicity using natural agent. Additionally, from the present investigation, it was recorded that acetaminophen can induce liver hepatotoxicity and that lime leaf is effective in its treatment while cashew stem bark and lemon grass leaf are not. The effects were both time and dose dependent.

KEY WORD: **Histomorphological, Extract, *Cymbopogoncitratus, Anacardiumoccidentale, Citrus aurantifolia* Acetaminophen, Hepatotoxicity, Wistar Rats**

1. INTRODUCTION

In the last 20years, the interest in medicinal plants has increased together with the number of investigation into their biological effects on humans and animals [1]. Medicinal plants are believed to be an important source of new chemical substance with potential therapeutic effects. Although, poisonous plants are ubiquitous, herbal medicine is used by up to 80% of the population in the developing countries [2]. 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*Cymbopogoncitratus* (lemon grass) leaf, *Anacardiumoccidentale* (cashew) stem bark and *Citrus aurantifolia* (lime) leaf extracts in treatment of acetaminophen induced hepatotoxicity in Wistar rats.

1.2 Aim and Objectives

Aim

To investigate the histomorphological (protective and healing) effect *Cymbopogoncitratus* (Lemon grass) leaf,*Anacardium occidentale* (Cashew) stem bark and *Citrus aurantifolian*(lime) leaf on Acetaminophen-induced hepatotoxicity in Wistar rats.

Objectives

1. To determine the LD_{50} of *Cymbopogoncitratus* (Lemon grass) leaf *Anacardiumoccidentale* (Cashew) stem bark and *Citrus aurantifolia* (Lime) leafin Wistar rats
2. To determine the histoprotective and healing effects of *Cymbopogon citratus*(lemon grass) leaf, *Anacardiumoccidentale* (cashew) stem bark and *Citrus aurantifolia* (lime) leaf extracts on the liver of Acetaminophen-induced Wistar rats.

2. Materials and Methods

2.1 Area of Study

This study was carried out in University of Port Harcourt, Department of Pharmaceutical Science and Histopathology Laboratory of University of Port Harcourt Teaching Hospital all in Rivers State, Nigeria.

2.2 Plant Materials

Anacardiumoccidentale (Cashew) Stem Bark, *Cymbopogoncitratus*(Lemon grass) leafand *Citrus aurantifolia*(lime) leaf were collected from Umuorji Community in ApaniIkwerre Local Government Area, Rivers State and were identified and authenticated by DrMrs Mercy Ajuru in department of Plant Science and Biotechnology, Rivers State University of science and technology and parts were kept at the herbarium.

2.3 Extract Preparation

Fresh stem barks of *A. occidentale, Cymbopogoncitratus* leaf, *Citrusaurantifolia* leaf were washed in water to remove impurities and air dried at room temperature for three weeks (21 days). The dried plant materials were pulverized with mortar and pestle separately. 408.5g of coarse powder of *A. occidentale*, 342.4g of *Citrus arantifolia* and 376.7g of *Cymbopogoncitratus* were macerated with 70% ethanol, stirred and incubated for 24hrs, then filtered. This process was repeated three times for each plant. On each extraction, the extract solution was separated from the pulp by filtration using 185mm size Whatman No 1 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 *A.occidentale, Citrus arantifolia*and *Cymbopogoncitratus* 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.

2.4 Experimental Design

The experiment was divided in two stages: Acute toxicity study and Main experimental study. The acute toxicity study were divided into three groups A, B, and C which were subdivided into three groups each A1, A2, A3, (cashew stem back) B1, B2, B3, (lime leaf) C1, C2, C3, (lemon grass) each containing three rats. Groups received 500mg/kg, 2000mg/kg and 5000 mg/kg body weight respectively per day orally. All the animals were observed after 30 minutes, 1hour, 4hours, 6 hours and 24hours of extract administration for mortality and other signs of toxicity. This toxicological study was to determine the lethal doses of cashew stem bark, lime leaf, and lemon grass leaf extract and eventually determine convenient doses of the extracts for the main experimental study [3].

Main Experimental Study: Ninety five Wistar rats were divided into ninteen groups containing five rats of similar body weights. GROUP D: served as the negative control group for the experiment (received normal feed and water only). GROUP 1 and 2: served as the positive control group for the experiment. The rats were administered single dose of acetaminophen 200 mg/kg body weight per day orally to induce hepatoxicity [4]. Group 1 was allowed for 3days to cause hepatotoxity and killed. Group 2 was left for 21 days after acetaminophen administration before sacrificed for reversibility [4]. GROUP 3A and 3B: received single dose of acetaminophen (200mg/kg), and oral administration of 500mg/kg cashew extract daily for 3days and 21days respectively. Group 3A received acetaminophen 200mg/kg followed by 500mg/kg cashew extract for 3days while Group 3B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 500mg/kg cashew extract for 21days. GROUP 4A and 4B: received single dose of acetaminophen (200mg/kg), and oral administration of 1000mg/kg cashew extract daily for 3days and 21days respectively. Group 4A received acetaminophen 200mg/kg followed by 500mg/kg cashew extract for 3days while Group 4B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 1000mg/kg cashew extract for 21days. GROUP 5A and 5B: received single dose of acetaminophen (200mg/kg), and oral administration of 500mg/kg lemon grass extract daily for 3days and 21days respectively. Group 5A received acetaminophen 200mg/kg followed by 500mg/kg lemon grass extract for 3days while Group 5B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 500mg/kg lemon grass extract for 21days. GROUP 6A and 6B: received single dose of acetaminophen (200mg/kg), and oral administration of 1000mg/kg lemon grass extract daily for 3days and 21days respectively. Group 6A received acetaminophen 200mg/kg followed by 1000mg/kg lemon grass extract for 3days while Group 6B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered

1000mg/kg lemon grass extract for 21days. GROUP 7A and 7B: received single dose of acetaminophen (200mg/kg), and oral administration of 500mg/kg lime extract daily for 3days and 21days respectively. Group 7A received acetaminophen 200mg/kg followed by 500mg/kg lime extract for 3days while Group 7B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 500mg/kg lime extract for 21days. GROUP 8A and 8B: received single dose of acetaminophen (200mg/kg), and oral administration of 1000mg/kg lime extract daily for 3days and 21days respectively. Group 8A received acetaminophen 200mg/kg followed by 1000mg/kg lime extract for 3days while Group 8B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 1000mg/kg lime extract for 21days. GROUP 9A and 9B: received single dose of acetaminophen (200mg/kg), and oral administration of 500mg/kg cashew, lemon grass, and lime extracts daily for 3days and 21days respectively. Group 9A received acetaminophen 200mg/kg followed by 500mg/kg cashew, lemon grass, and lime extracts for 3days while Group 9B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 500mg/kg cashew, lemon grass, and lime extracts for 21days. GROUP 10A and 10B: received single dose of acetaminophen (200mg/kg), and oral administration of 1000mg/kg cashew, lemon grass and lime extracts daily for 3days and 21days respectively. Group 10A received acetaminophen 200mg/kg followed by 1000mg/kg lime extract for 3days while Group 10B received 200mg/kg acetaminophen and were left for 72hours to cause hepatotoxicity, after the 3days were administered 1000mg/kg cashew, lemon grass and lime extracts for 21days.

2.5 Administration of the Extracts

Extracts were administered orally to the rats by carefully inserting the cannula attached to syringe into the oral cavity of the rats and the extract administered slowly to ensure that the volume measured was delivered completely. The dosage was determined using milligram per kg body weight as a standard.

2.6 Animal Sacrifice

The animal body weights were taken before and at the end of the extracts administration and their mean body weight were determined. Equally urine samples were collected and all the animals were sacrificed under diethyl ether anesthesia, the liver organ were harvested, weighed and fixed in buffered formal saline for 48 hours for histological studies.

2.7 Histological Analysis

The liver tissue samples were analyzed using standard histological techniques. The specimen were weighed and transferred immediately into 10% buffered formal saline fixative for 24 hours to avoid post mortem changes (autolysis and putrefaction). The tissues were dehydrated in ascending concentrations of alcohol in separate beaker of 70%, 95%, and three changes of absolute alcohol at 2 hours intervals each. The tissues were cleared in two changes of xylene at 2 hours interval to remove alcohol and to improve the optical differentiation of the tissue. The tissues were then impregnated in two changes of molten paraffin wax in an oven at 58^{o}C at 2 hours each, and were embedded in molten paraffin wax using embedding mould to harden the tissue for easy microtomy. The tissues were finally sectioned serially using a Rotary microtome at 3 micrometer thickness. The sections were first floated in grease free slide with 20% alcohol to stretch, then transferred to warmed water-bath at a temperature of 40^{O}C to flattened, with a microscopic slide, sections tissue were floated out and then place on a hot plate to de-wax and fixed on the slide. The tissue sections were stained with Haematoxylin and Eosin (H and E) staining techniques. this techniques is based on the principle: Haematoxylin is a basic dye and thus has affinity for the acidic part of the cellular components which is the nucleus Hence it therefore stains the nucleus blue while Eosin on the hand is an acidic dye which has affinity for the basic component of the cell which is the cytoplasm. It therefore stains the cytoplasm pink. This staining procedure is facilitated with a mordant that links the stain to the tissue and a differentiator (acid alcohol) which will differentiate the nuclear stain from the cytoplasmic stain. The slides were de-wax in two changes of xylene for10 minutes each, and hydrated in descending grade of alcohol; absolute, 95%, 70% and 50% for 10 minutes each and finally in tap water. The slides were then stained in Ehrlich's Haematoxyline for 20 minutes, rinsed in tap water for 30 seconds. The tissue slides were differentiated in 1% acid alcohol for a deep then washed immediately in a tap water for 1minute. Blued in warm water for 5 minutes after wards, the slides were counter stained with eosin for 2 minutes, and finally washed in tap water. The tissue slides were dehydrated in ascending grades of alcohol; 70%, 90% and two change of absolute for 5 minutes each. The slides were cleared with two changes of xylene for 5 minutes each then mounted with D.P.X and cover slip.

3.Data Analysis and Interpretation

The results are reported as Mean and S.D. The F and T test statistical tool via SPSS version 21 Computer package were used to analyse and compare the results at 0.05 (5%) significant level.

4. RESULT

Table 4.1AT shows the summary of empirical and physical measurement for acute toxicity study for the three extracts.

Cages	Dose in mg/kg weight	Mean avenge weight before administration of extract(g)	Mean avenge weight after administration of extract (g)	Physical weight loss or gain	Behavioral toxic symptoms
A1 cashew	500mg	187.53	175.37	↓↓	±
A2 cashew	2000	247.86	218.33	↓↓	+
A3 cashew	5000	171.03	151.36	↓↓	++
B1 Lime	500	213.2	195.0	↓↓	+
B2 Lime	2000	175.66	162.00	↓↓	+
B3 Lime	5000	217.46	198.66	↓↓	++
C1Lemon grass	500	172.0	157.66	↓↓	+
C2 Lemon grass	2000	185.66	174.66	↓↓	+
C3 Lemon grass	5000	178.66	167.66	↓↓	++

Average weight of rats

Key to toxicity scoring: ↑-slight increase in weight; ↑↑ -marked increase in weight; ↓-slight decrease in weight loss; ↓↓ -severe decrease in weight loss; + -presence of feature; ++ - intermediate feature; +++ Marked presence of feature; - Absence of feature; ± Little or absence of feature

Table E 4:1 shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of 200mg/kg acetaminophen for three days (positive control).

The mean weight of 175.74g after administration of PCM against the mean weight of 170.00g before the administration of PCM showed that there was slight gain in weight of the animal three days after the administration of PCM. Also the standard deviation of 2.39 after administration to that of 0.00 before administration showed a high variability or dispersion between the mean weights before and after the administration of substance (table E4.1).

Table E4.1: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of 200mg/kg acetaminophen for three days (positive control) for experimental study.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale Weight before administration	5	850.0	170.00	0.00
Weight after administration	5	878.7	175.74	2.39

TABLE E4:2 shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of 200mg/kg acetaminophen for 21days (Revesibility group).

The mean weight of 228.40g after the administration of PCM against the mean weight of 170.00 before the administration of PCM showed that they were a marked weight again in the animal at 21days. Also the standard deviation of 20.42 at 21days against the standard deviation of 0.00 before the administration of PCM showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance.

Table E4.2: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of 200mg/kg acetaminophen for 21days (Revesibility group) for experimental study

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale Weight before administration	5	850	170.00	0.00
Weight after administration	5	1142	228.40	20.42

Table E4:3A shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg cashew stem bark extract for three days. The mean weight of 175.68g after administration of the substance against the mean weight of 170.40g before the administration of substance showed that they were a slight weight again in the animal at three days. Also the standard deviation of 4.07 at 4days against the standard deviation of 0.55 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.3A).

Table E4.3A: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *A.occidentale* Stembarkextract (3days) for experimental study

Mode of determination	Sample size	Total weight (g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	852.0	170.40	0.55
Weight after administration	5	878.4	175.68	4.07

Table 4:3B shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg cashew stem bark extract for 21days. The mean weight of 232.20g after administration of the substance against the mean weight of 188.000g before the administration of substance showed that they were a marked weight again in the animal at 21days. Also the standard deviation of 21.48 at 21days against the standard deviation of 4.30 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.3B).

Table E4.3B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *A.occidentale* stem bark extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	940	188.00	4.30
Weight after administration	5	1161	232.20	21.48

Table E4:4A showmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg cashew stem bark extract for 3days. The mean weight of 175.28g after administration of the substance against the mean weight of 170.00g before the administration of substance showed that there was a slight weight again in the animal at three days. Also the standard deviation of 2.22 at three days against the standard deviation of 0.00 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.4A).

Table E4.4A: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *A.occidentale* stem bark extract for 3days.

Mode of determination	Sample size	Total weight (g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	850.0	170.00	0.00
Weight after administration	5	876.4	175.28	2.22

Table E4:4Bshowsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg cashew stem bark extract for 21days. The mean weight of 207.20g after administration of the substance against the mean weight of 170.00g before the administration of substance showed that there was a marked weight again in the animal at 21day. Also the standard deviation of 13.98 at 21days against the standard deviation of 0.00 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance

Table E4.4B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *A. occidentale* stem bark extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	850	170.00	0.00
Weight after administration	5	1036	207.20	13.98

TableE4:5A showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after theadministration of acetaminophen 200mg/kg + 500mg/kg lemon grass leaf extract three days.

The mean weight of 175.72g after administration of the substance against the mean weight of 170.02g before the administration of substance showed that there was a slight weight again in the animal at three days. Also the standard deviation of 1.59 at three days against the standard deviation of 0.45 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.5A).

Table E4.5A: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *Cympongoncitrates* leaf extract three days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	850	170.02	0.45
Weight after administration	5	879	175.72	1.59

Table E4.5B showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg lemon grass leaf extract for 21days. The mean weight of 234.60g after administration of the substance against the mean weight of 172.80g before the administration of substance showed that there was a marked weight again in the animal at 21days. Also the standard deviation of 20.44 at 21days against the standard deviation of 0.45 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.5B).

Table E4.5B:Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *Cympongoncitrates* leaf extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	864	172.80	0.45
Weight after administration	5	1172	234.60	20.44

Table E4:6Ashows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *Cympongoncitrates* leaf extract for 3days.

The mean weight of 186.00g after administration of the substance against the mean weight of 180.80g before the administration of substance showed that there was a slight weight again in the animal at three days. Also the standard deviation of 2.25 at three days against the standard deviation of 0.84 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.6A).

Table E4.6A: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *Cympongoncitrates* leaf extract for 3days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	904	180.80	0.84
Weight after administration	5	930	186.00	2.25

Table E4.6B shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg lemon grass leaf extract for 21days.

The mean weight of 229.80g after administration of the substance against the mean weight of 170.80g before the administration of substance showed that there was a marked weight again in the animal at 21days. Also the standard deviation of 2.16 at 21days against the standard deviation of 0.44 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.6B).

Table E4.6B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *Cympongoncitrates* leaf extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	854	170.80	0.84
Weight after administration	5	1149	229.80	15.30

Table E4.7A showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg lime leaf extract for three days.

The mean weight of 175.58g after administration of the substance against the mean weight of 172.20g before the administration of substance showed that there was a slight weight again in the animal at three days. Also the standard deviation of 2.16 at three days against the standard deviation of 0.45 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean

weight before and after the administration of substance (table E4.7A).

Table E4.7A:Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *Citrus aurantifolia* extract for three days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	861	172.20	0.45
Weight after administration	5	877.9	175.58	2.16

Table E4.7B showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg lime leaf extract for 21days. The mean weight of 235.2g after administration of the substance against the mean weight of 170.0g before the administration of substance showed that there was a marked weight again in the animal at 21days. Also the standard deviation of 9.33 at 21days against the standard deviation of 0.0 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.7B).

Table E4.7B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *Citrus aurantifolia* leaf extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	850	170.0	0.00
Weight after administration	5	1176	235.20	10.43

Table E4.8A showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg lime leaf extract for three days.

The mean weight of 176.4g after administration of the substance against the mean weight of 173.8g before the administration of substance showed that there was a slight weight again in the animal at three days. Also the standard deviation of 1.00 at three days against the standard deviation of 0.84 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance

Table E4.8A: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *Citrus aurantifolia* leaf extract for three day.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	869	173.8	0.84
Weight after administration	5	88.8	176.4	1.00

Table E4. 8B showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg lime leaf extract for 21days. The mean weight of 221.60g after administration of the substance against the mean weight of 181.2g before the administration of substance showed that there was a marked weight again in the animal at 21days. Also the standard deviation of 17.87 at 21days against the standard deviation of 0.44 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.8B).

Table E4.8B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 1000mg/kg *Citrus aurantifolia* leaf extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	906	181.2	0.44
Weight after administration	5	1108	221.60	17.87

Table E4.9A shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg combination of cashew stem bark, lime leaf and lemon grass extract for three days.

The mean weight of 177.14g after administration of thesubstance against the mean weight of 170.6g before the administration of substance showed that there was a weight again in the animal at three days. Also the standard deviation of 1.75 at three days against the standard deviation of 0.8 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.9A).

Table E4.9A: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *Citrus aurantifolia, Cympongoncitrates* leafand *A.occidentale* stem bark extract for three days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	853	170.60	0.89
Weight after administration	5	885.7	177.14	1.93

Table E4.9B shows mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg combination for 21days. The mean weight of 211.20g after administration of the substance against the mean weight of 172.00g before the administration of substance showed that there was a marked weight again in the animal at 21days. Also the standard deviation of 8.64 at 21days against the standard deviation of 0.71 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.9B).

Table E4.9B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg + 500mg/kg *Citrus aurantifolia, Cympongoncitrates* leafand *A.occidentale* stem bark extract for 21days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	860	172.00	0.71
Weight after administration	5	1056	211.20	8.64

Table E4.10A showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg +1000mg/kg combination of cashew stem bark ,lime leaf and lemon grass extract for three days.

The mean weight of 175.68g after administration of the substance against the mean weight of 171.0g before the administration of substance showed that there was a slight weight again in the animal at three days. Also the standard deviation of 1.81 at three days against the standard deviation of 0.00 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.10A).

Table E4.10A:Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg +1000mg/kg *Citrus aurantifolia, Cympongoncitrates* leafand *A.occidentale* stem bark extract for three days.

Mode of determination	Sample size	Total weight(g)	Mean weight(g)	Standard deviation
Weight scale				
Weight before administration	5	855	171.00	0.00
Weight after administration	5	878.4	175.68	1.81

Table E4.10B showsmean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg +1000mg/kg combination of cashew stem bark, lime leaf, and lemon grass extract for 21days.

The mean weight of 234.40g after administration of the substance against the mean weight of 172.20g before the administration of substance showed that there was a marked weight again in the animal at 21days. Also the standard deviation of 4.34 at 21days againstthe standard deviation of 0.45 before the administration of substance showed a wide spread or variability among the individual weight of the animal or their mean weight before and after the administration of substance (table E4.10B).

Table E4.10B: Mean and standard deviation analysis of the effect on weight of the Wistar rats before and after the administration of acetaminophen 200mg/kg +1000mg/kg *Citrus aurantifolia, Cympongoncitrates* leafand *A.occidentale* stem bark extract for 21days.

Mode of determination	Sample size	Total weight (g)	Mean weight (g)	Standard deviation
Weight scale				
Weight before administration	5	861	172.20	0.45
Weight after administration	5	1172	234.40	4.34

Table E4.11 showsmean and standard deviation analysis of the effect on weight of the Wistar feed with water and pellet food before and after the experimental (negative control). The mean weight of 233.0 of the Wistar rats after the experiment, against the mean weight of 170.0 before the experiment showed that there was again in weight of the animal 21days after the experiment and standard deviation of 14.73 after experiment showed a high spread or variability between the mean weight of 0.00 before the experimental period.

Table E4.11: Mean and standard deviation analysis of the effect on weight of the Wistar feed with water and pellet food before and after the experimental (negative control).

Mode of determination	Sample size	Total weight (g)	Mean weight (g)	Standard deviation
Weight scale				
Weight before administration	5	850	170.00	0.00
Weight after administration	5	1165	233.00	14.73

Table E4.12 analyses the P-value of weight before and weight after administration of acetaminophen, acetaminophen + extracts for 3 and 21 days. From the table E4.12 below, the P-values implies that there were significant difference (effect) in the body weight of the both treatment and control animal (P<0.05).

Table E4.:12 shows Paired Samples t-Test for Experimental groups

		Paired Differences					T	df	p-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Group1Weightbefore - Group1Weightafter	-5.7400	2.3933	1.0703	-8.7117	-2.7683	-5.363	4	.006
Pair 2	Group2Weightbefore - Group2Weightafter	-58.400	20.4157	9.1302	-83.7494	-33.0506	-6.396	4	.003
Pair 3	Group3AWeightbefore - Group3AWeightafter	-5.4600	4.3085	1.9268	-10.8097	-.1103	-2.834	4	.047
Pair 4	Group3BWeightbefore - Group3BWeightafter	-44.200	19.4859	8.7144	-68.3949	-20.0051	-5.072	4	.007
Pair 5	Group4AWeightbefore - Group4AWeightafter	-5.2800	2.2197	.9927	-8.0361	-2.5239	-5.319	4	.006
Pair 6	Group4BWeightbefore - Group4BWeightafter	-37.2000	13.9714	6.2482	-54.5478	-19.8522	-5.954	4	.004
Pair 7	Group5AWeightbefore - Group5AWeightafter	-5.7000	1.5843	.7085	-7.6672	-3.7328	-8.045	4	.001
Pair 8	Group5BWeightbefore - Group5BWeightafter	-61.800	20.5110	9.1728	-87.2677	-36.3323	-6.737	4	.003
Pair 9	Group6AWeightbefore - Group6AWeightafter	-5.2000	2.4083	1.0770	-8.1903	-2.2097	-4.828	4	.008
Pair 10	Group6BWeightbefore - Group6BWeightafter	-59.000	14.6799	6.5651	-77.2275	-40.7725	-8.987	4	.001
Pair 11	Group7AWeightbefore - Group7AWeightafter	-3.3800	1.7527	.7838	-5.5563	-1.2037	-4.312	4	.013
Pair 12	Group7BWeightbefore - Group7BWeightafter	-65.200	10.4259	4.6626	-78.1455	-52.2545	-13.984	4	.000
Pair 13	Group8AWeightbefore - Group8AWeightafter	-2.5600	1.1718	.5240	-4.0149	-1.1051	-4.885	4	.008
Pair 14	Group8BWeightbefore - Group8BWeightafter	-40.400	18.0638	8.0784	-62.8291	-17.9709	-5.001	4	.007
Pair 15	Group9AWeightbefore - Group9AWeightafter	-6.5400	1.8770	.8394	-8.8706	-4.2094	-7.791	4	.001
Pair 16	Group9BWeightbefore - Group9BWeightafter	-39.2000	8.7579	3.9166	-50.0743	-28.3257	-10.009	4	.001
Pair 17	Group10AWeightbefore - Group10AWeightafter	-4.6800	1.8089	.8089	-6.9260	-2.4340	-5.785	4	.004
Pair 18	Group10BWeightbefore - Group10BWeightafter	-62.200	4.3243	1.9339	-67.5694	-56.8306	-32.163	4	.000
Pair 19	Control Weightbefore Control Weightafter	-63.0000	14.7479	6.5955	-81.3119	-44.6881	-9.552	4	0.001

Table 4.13: ANOVA of Control of Weight Before and Experiment Groups of Weight Before

	SS	Df	MS	F-value	p-value	Remark
Between	2,334.706	9	259.412			
Within	101.809	40	2.545	101.921	0.00	Sig
Total	2,436.515	49				
Between	33,856.139	9	3,761.793			
Within	3,943.484	40	98.587	38.157	0.00	
Total	37,799.624	49				

ANOVA comparing the Control of Weight After and Experiment Groups of Weight After

	SS	Df	MS	F-value	p-value	Remark
Between	33,458.420	9	3,717.602			
Within	6,973.236	40	174.331	21.325	0.00	Sig
Total	40,431.656	49				
Between	494.580	9	54.953			
Within	13.200	40	0.330	166.523	0.00	Sig
Total	507.780	49				

There is a significant weight difference in the body weight of control and treated animal before and after experiment.

Table 4.14 shows the mean weight and standard deviation of liver organ

1

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 3A	PCM +cashew	200+500	E.W Scale	30.3	6.06	0.38
Group 3B	PCM +cashew	200+500	E.W Scale	37.5	7.50	0.61
Group 4A	PCM +cashew	200+1000	E.W Scale	30.9	6.18	1.29
Group 4B	PCM +cashew	200+1000	E.W Scale	30.8	6.16	0.62
Group 5A	PCM + lemon G	200+500	E.W Scale	28.5	5.90	0.74
Group 5B	PCM + lemon G	200+500	E.W Scale	33.6	6.72	1.12
Group 6A	PCM + lemon G	200+1000	E.W Scale	28.5	5.76	0.83
Group 6B	PCM + lemon G	200+1000	E.W Scale	34.6	6.92	0.95
Group 7A	PCM + lime leaf	200+500	E.W Scale	29.6	5.92	0.57
Group 7B	PCM + lime leaf	200+500	E.W Scale	33.5	6.36	0.35
Group 8A	PCM + lime leaf	200+1000	E.W Scale	31.8	6.36	0.56
Group 8B	PCM + lime leaf	200+1000	E.W Scale	29.9	5.98	0.62
Group 9A	PCM + combination	200+500	E.W Scale	31.9	6.38	1.92
Group 9B	PCM + combination	200+500	E.W Scale	28.0	5.60	0.58
Group 10A	PCM + combination	200+1000	E.W Scale	33.1	6.62	0.83
Group 10B	PCM + combination	200+1000	E.W Scale	28.4	5.68	0.33
Group D	Water/ feed	Water/feed	E.W Scale	28.5	6.50	1.002

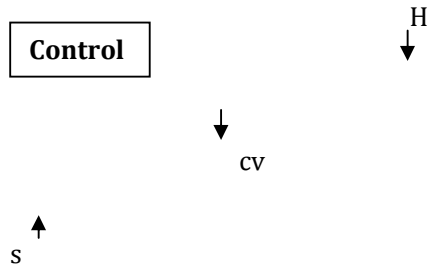
Despite the few variations in the organ weight generally, there were no different in liver organ weight when compared with the control group as it were expressed stastistically using Mean ± SD.

Table 4.15: ANOVA showing Organ Weights of Control and Experimental Groups

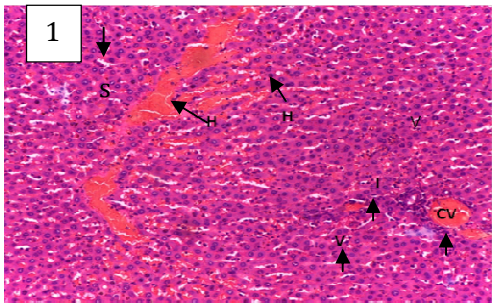
	SS	Df	MS	F	P	Remark
Between	12.033	9	1.337			
Within	27.564	40	0.689	1.940	0.074	N/Sig
Total	39.597	49				
Between	9.032	9	1.004			
Within	31.521	40	0.788	1.274	0.281	N/Sig
Total	40.553	49				

From table 4.14, there is no significant weight difference in the test organ when compare to the control group.

CONTROL GROUP: Shows normal histological liver section.



GROUP 1 (Acetaminophen, sacrificed on day 4): Histological section shows hepatic tissue with extensive haemorhage (H) and marked cellularity due to stromal proliferation. There is stromal infiltration by inflammatory cells (I). Also the stromal is vascularized (V). The central vein (CV) appears unremarkable. The lamina and sinusoids are not clearly identifiable.



GROUP 2 (Acetaminophen, sacrificed on day 21): The histological section shows no obvious pathological changes.the hepatocyte, sinusoids and limina are intact.

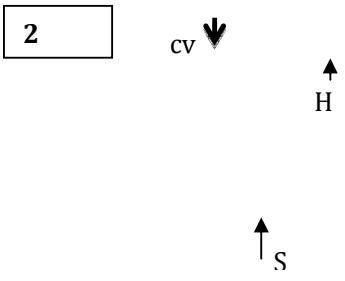
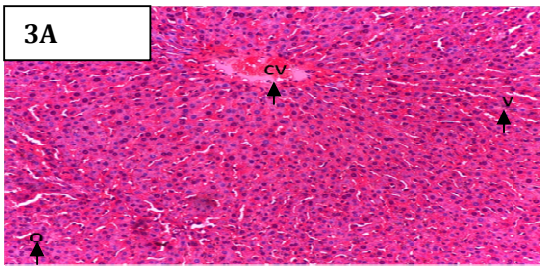


Figure 4.1:Photomicrographs of the rat liver section given single dose 200mg/kg acetaminophen (200 mg/kg po) and sacrificed on day 4 and day 21 (H EX100)

Group 3A (Cashew extract x 4 days): Revealed histopathological section with stromal proliferation and vascularity (V), the central vein (CV) appear dilated with degenerative changes. There is stromal congestion and polymorph infiltrate's. The lamina and the sinuisoids are not properly arranged.



Group 3B (Cashew extract x 21 days): Histopathological section shows heptatic tissue with normal stromal arrangement and central vein (CV), there is moderate polymph (P) infiltration in areas

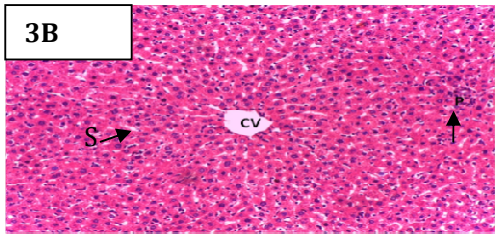
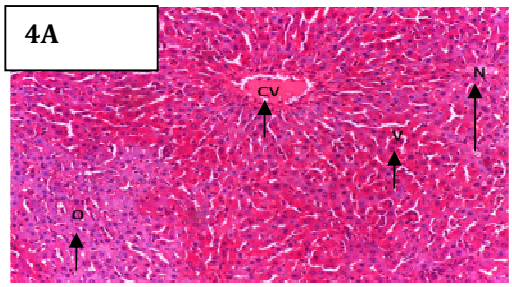


Figure 4.2:Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 500mg/kg of cashew stem bark extract daily for 4 days or 21 days (H&E X 100).

.Group 4A (Cashew extract x 4 days): Histological section shows hepatic tissue with stromal proliferation and marked vascularity with odematic (O) areas. The central vein (CV) appears slightly dilated. There is marked cellularity in areas. The sinusoids and lamina appear unidentifiable. Also observed is a necrotic area (N).



Group 4B (Cashew extract x 21 days): Histological section shows hepatic tissue with slightly dilated central vein (CV) and few cells with hyperchromatic nuclei. Other stromal elements appear unremarkable

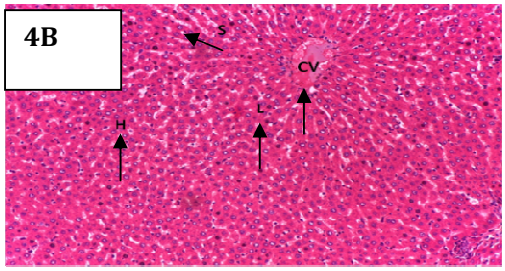
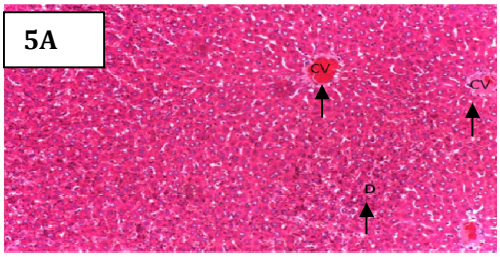


Figure 4.3:Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 1000mg/kg of cashew stem bark extract daily for 4 days and 21 days (H&E X 100).

Group 5A (Lemon grass extract x 4 days): Histological section shows hepatic tissue with degeneration and congestion stromal. The central vein (CV) contains blood clot and copious cellular debris (D) seen in the tissue stromal. The sinusoids and lamina arrangement are unidentifiable and the hepatocytes appear pyknotic and degenerated.



Group 5B (Lemon grass extract x 21 days): There are no obvious pathological changes.

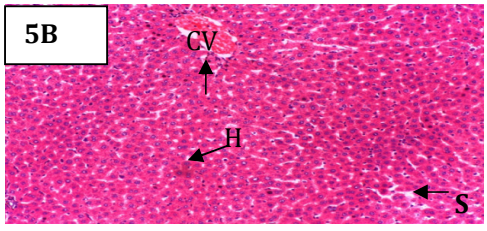
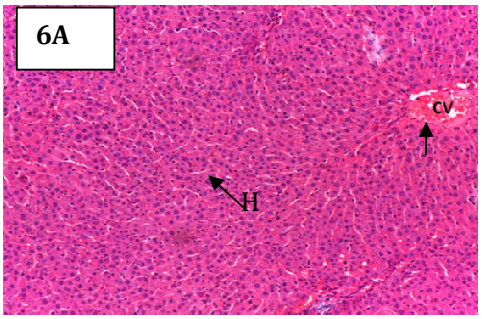


Figure 4.4:Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 500mg/kg of lemon grass leaf extract daily for 4 days and 21 days (H&E X 100).

Group 6A (Lemon grass extract x 4 days): Histological section shows hepatic tissue with congested stromal, severe proliferation and marked cellularity.There is infiltration of the stroma by polymophs. The central vein (CV) appears slightly enlarged. The simisoid and the lamina appear unidentifiable.



Group 6B (Lemon grass extract x 21 days): Histological section shows hepatic tissue with few cells with hyperchromatic nuclei, other stromal elements appear normal.

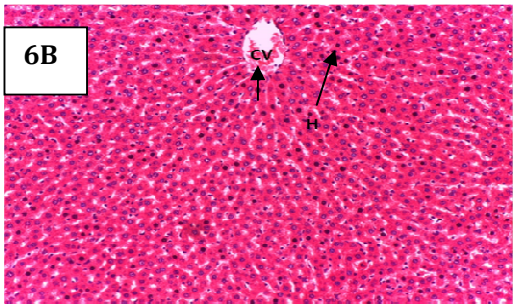
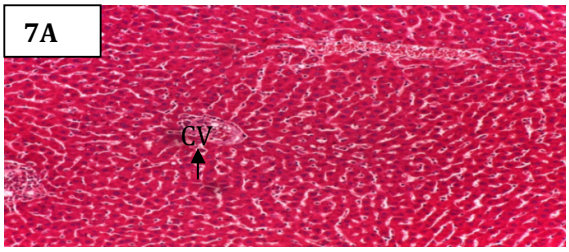


Figure 4.5:Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 1000mg/kg of lemon grass leaf extract daily for 4 days and 21 days (H&E X 100).

Group 7A (Lime leaf extract x 4 days): The histological section shows there are no obvious pathological changes



Group 7B (Lime leaf extract x 21 days): The histological section shows no obvious pathological changes.

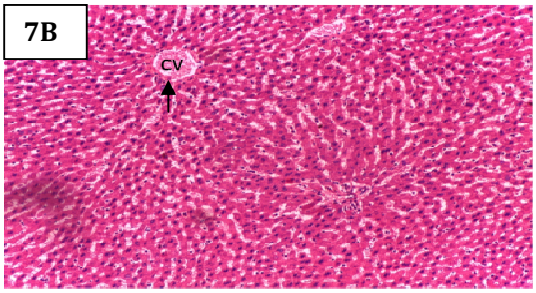
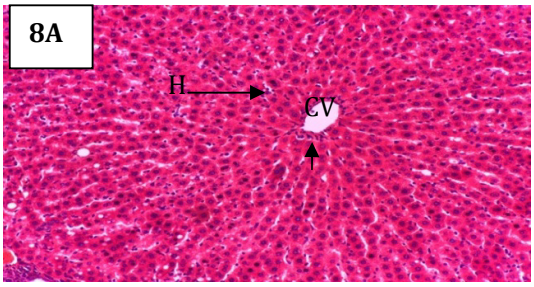


Figure 4.6:Photomicrographs of the rat liver section given acetaminophen 200mg/kg single dose plus 500mg/kg of lime leaf extract daily for 4 days and 21 days (H&E X 100).

Group 8A (Lime leaf extract x 4 days): The histological section shows no obvious pathological changes.



Group 8B (Lime leaf extract x 21 days): Histological section shows hepatic tissue with severe stromal degeneration. The entire tissue architecture is distorted.

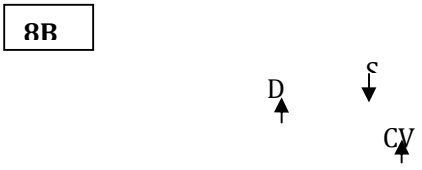
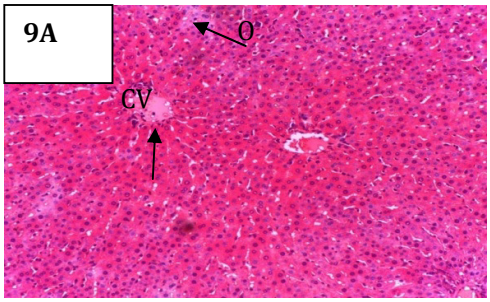


Figure 4.7:Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 1000mg/kg of lime leaf extract daily for 4 days and 21 days (H&E X 100).

Group 9A (Combination x 4 day): Histological section shows hepatic tissue with proliferation and marked cellularity, the stromal appear edematous (O) and hyalinized. Thre are polymorph infiltrates (P). The central -vein appears unremarkable but contain blood clot.



Group 9B (Combination x 21 day): The histological sections shows no obvious pathological changes

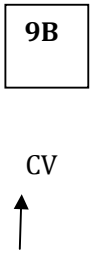
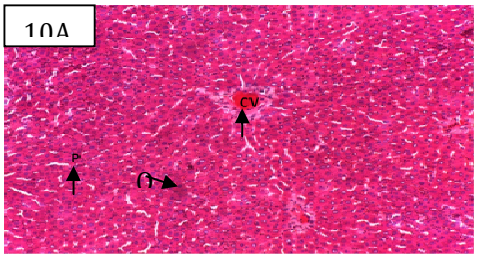


Figure 4.8:Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 500mg/kg lemon grass, lime leaf and cashew stem bark extracts daily for 4 days and 21 days (H&E X 100).

Group 10A (Combination x 4 day): Histological section shows hepatic tissue with proliferation and marked cellularity the stromal appear edematous (O) and hyalinized. There are polymorph infiltrates (P). The central-vein appears unremarkable and contains blood clot.



Group 10B (Combination x 21 day): The histological section shows no obvious pathological changes.

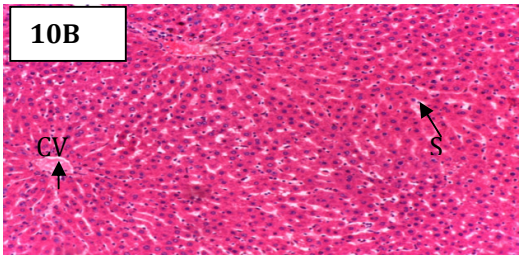


Figure 4.9: Photomicrographs of the rat liver section given acetaminophen 200 mg/kg single dose plus 1000mg/kg lemon grass, lime leaf and cashew stem bark extracts daily for 4 days and 21 days (H&E X 100).

5.1 DISCUSSION

The result of the acclimatization/ physical activities 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 *A.occidentale* stem bark *Cymbopogoncitrates* leaf and *Citrusaurantifolia* leaf extracts, Group A1, B1, C1 did not show much toxic and behavioural symptoms compared to group A2, B2, C2 and A3, B3, C3 as recorded in this study; is not in consistent with research done by [5]. Furthermore, rats in group A2, B2, C2 and A3, B3, C3 demonstrated more reduced activities expressed in form of sleeping, calmness, stretching out and resting in the corner of the cage, closing of eyes which illustrated the adverse toxic effects (post exposure effects) of the extracts on the rats study. The findings suggest that the extracts of *A.occidentale* stem bark, *Cymbopogoncitrates* leaf and *Citrusaurantifolia* leaf were 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. At least up to maximum of dose 2000mg/kg of *A.occidentale* acquous extracts has previously been reported to have no toxic effects via orall [6]. 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+ *A.occidentale* stem bark, *Cymbopogoncitrates* leaf and *Citrusaurantifolia* leaf extracts point that acetaminophen+ *A.occidentale* stem bark, *Cymbopogoncitrates* leaf and *Citrusaurantifolia* leaf extracts present no evidence of interference in term of weight. Besides, the extracts and the drug

acetaminophen presumably did not interfere with the normal metabolism of the animal contradicted the work of [7] 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 [8]. Notebly, organ weight is an important index of physiological and pathological status in animals besides the relative organ weight could be 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 pathways 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 hepatocytes showed severe architectural distortion of the liver within 72 hours of administration of single dose Acetaminophen of 200mg/kg, Acetaminophen and *A. occidentale* stem bark extract,*Cympongoncitrates* leaf extract and *citratesaurantifolia* 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 [4],[9]. The liver section of the negative control group showed well differentiated hepatocytes, central vein with sinusoids and lamina well alined. Plate 3A and 4A which is the protective group revealed that within 72 hours of single dose acetaminophen alongside with *A.occidentale* stem bark extract showed hepatic with stroma proliferation and vascularisation of central vein appearing dilated with degenerative changes.the stromal is congested and infiltrated with polymorpho nuclear cells, eodematic and necrotic cells. Also lamina and sinusoids are not properly alined. This implies that *A.occidentale* stem bark extract could not prevent the hepatotoxicity effects of acetaminophen on the test animal. Plate 3B showed normal liver section with moderate polymph infiltration while plate 4B showed hepatic tissue with slight dilated CV with few hyperchromatic nuclei cell which could be as aresult of the high dose of *A.occidentale* stem bark extract for a long period of three weeks.this is in consistent with [10],[11] which state that high dose of the cashew stem bark may have side effects. Plate 5A and 6A showed hepatic tissue with significant stromal degeneration and congestion, severe proliferation with polymorphs nuclei cells along side with marked cellularity, the central vein contain blood cloth and cellular debris in 5A while the 6A, the central vein appear slightly dilated. There is presence of pyknotic. The sinusoids and lamina arrangement are unidentifiable [9],[12],[13]. From plate 5A and 6A the extract was unable to protect the hepatotoxicity effect of acetaminophen on test animal. In Plate 5B and 6B there were no obvious pathological changes apart from few hyperchromatic nuclei present. Plate 7A, 7B and 8A showed no obvious pathological change within 72 hours and 21 days which mean that *Citrusaurantifolia* leaf extract have both protective and curative effects as shown in the plates while plate 8B of 21 days which caused demage could be due to presence of toxic substances in the extracts which is expected to be highly concentrated in the higher doses or the long time administration of this extract at high dosage. Further studies are necessary to dertermine whether the compound was responsible for the demage shown. Plate 9A and 10A showed edamtous and sign of hydropic degeneracy resulting from loss of membrane permeability hence an influx of sodium and water into the hepatocyte causing swelling of the cells.therefore the lamina and sinusoids appeared distorted which means that effect of lime leaf as showed in plate 7A and 8A were inhibited by the action of either the drug or the other extracts. Plate 9B and 10B showed no pathological changes which is in consistent with [4].

5.2 CONCLUSION

In conclusion, this study has recorded that: (a) The LD_{50} of the extracts was found to be higher than 5000mg/kg. (b) The post results of the urine screening on the animal showed presence of hematuria in most of the rats, this could be due acetaminophen toxicity. (c) That there were significant weight gain on the body weight of the rats before and after the administration of the acetaminophen and the extracts ($P < 0.05$) 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. (d) Among the three extracts tested against acetaminophen induced hepatoxicity,(lime) *Citrusaurantifolia* leaf exhibited the most potent 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 *Citrusaurantifolia* leaf a candidate that could be developed into new lead structures and candidate for drug development programs against human acetaminophen hepatotoxicity.

5.3 RECOMMENDATION

From the findings of the study, the following recommendations could be made:

1. Individuals and Nigerian Governments, through their respective concerned organs such as NAFDAC, NDLEA should regulate rampant and the indiscriminate peddling of herbal medicine as well as uncontrolled use of acetaminophen.

2. That cashew and lemon grass are not protective to acetaminophen toxicity and therefore should not be recommended as an alternative therapy for the treatment of liver demage due to acetaminophen toxicity.

3. That lime could be used in prevention of liver toxicity as was revealed in the experiment.

4. Further study could be carried out to dertermine the amount of treatment variable (independent) that has the highest or illicit the highest effect on the dependent variable.

5.4 CONTRIBUTION TO KNOWLEDGE

In the course of the study, it was deduced that *Citratesaurantifolia* (lime) leaf has a protective and ameliorative effects againt liver demage from acetaminophen toxicity.The LD_{50} of *A.occidentale* stem bark, *Cymbopogoncitrates* leaf and *Citrusaurantifolia* leaf extracts were higher than 5000mg/kg, lastly, Acetaminophen induced hepatoxicity can be reversed within three week

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