Sub-chronic Hepatotoxicity Assessment of Ghana Cleanser® in Exposed Wistar Rats

Abstract

This study evaluated the toxicity of a polyherbal Formulation (Ghana Cleanser®) on liver function markers of exposed albino rats. Thirty (30) male and female rats of the Wistar strain were randomly divided into six (6) groups with n=5. 10.0 mL/kg distilled water was given to control groups 1 and 4. Polyherbal formulation doses of 374.0 mg/kg and 187.0 mg/kg were administered to groups 2-3 and 5-6, respectively. A modified Lorke's approach was used to compute acute toxicity. Animals were slaughtered after 60 days under diethyl ether. Blood was collected for biochemical analyses through cardiac puncture. The liver was excised from each animal and was fixed in 10% buffered formaldehyde and prepared for histological assessment. LD_{50} of the polyherbal preparation was calculated as 3740 mg/kg (oral). The results indicated an appreciable increase ($p<0.05$) in ALT activity at 374.0 mg/kg in female rats; while there was no increase recorded at 187.0mg/kg in male rats. A significant increase in ALT activity was recorded at 374mg/kg in male rats as well and increased AST activities were recorded at 187mg/kg in female rats. In the treated animals of both sexes, ALP activities were significantly elevated. Histopathology assessment of the hepatocytes showed no significant damage at 187mg/kg in rats of both sexes when compared with their respective controls while some degrees of pathologies such as hepatocyte inflammation, hyperplasia, and congestion were recorded at 374mg/kg in rats of both sexes. Results suggest caution on the long-term use of the polyherbal mixture due to its hepatotoxic potential.

Key Words: Ghana Cleanser®, Polyherbal formulation, acute toxicity, Hepatotoxicity.

Introduction

The use of plants for medicinal purposes predates human civilization. Traditional medicine practice forms a considerable percentage of the healthcare system in many developing countries in Asia and Sub-Saharan Africa[1]. The World Health Organisation estimates that about 75 percent of the world population consumes herbal remedies for health and recreative purposes[2,3]. However, the claim that herbal formulations are potent against an array of ailments and safe for human consumption with no adverse effects due to their ‘’natural’’ origin has not been scientifically elucidated. Moreover, the production process of these herbal formulations (HF) lacks standardization, lack of necessary machinery, and hygiene in some cases, and may contain impurities and contaminants such as heavy metals and microorganisms (bacteria and viruses) which may be harmful to consumers. Nonetheless, these inherent risks have not affected the consumption of herbal preparations due to their availability and folkloric use in the treatment of many ailments. In addition, the cost of conventional healthcare in many countries has also contributed to the increased use of herbal remedies which are more affordable. Interestingly, concomitant use of orthodox medication and herbal formulations is a common practice among the population of rural communities[4,5].

Ghana Cleanser® is a polyherbal mixture made up of five different types of medicinal plant extracts. They include *Zingiber officinale* (ginger), *Aloe barbadensis* (Aloe vera), *Cymbopogon citratus* (lemon grass), *Mangifera indica* (mango), and *Panax ginseng* (Asian ginseng). The product registration number A7-9528 L has been obtained by the National Agency for Food and Drug Administration and Control (NAFDAC). The product is widely used and consumed in communities throughout North-Central Nigeria, and it is known to be effective in the treatment of a variety of disorders such as inflammation, fever, haemorrhoids, diabetes, osteoarthritis, body pain, male infertility and so on. However, scientific data on the toxicological evaluation of

products with herbal origin distributed and consumed in North-Central Nigeria (Nasarawa State) are lacking. Therefore, this study was carried out to assess the sub-chronic toxicity effects of Ghana Cleanser® in albino rats. It is hoped that the data and findings of this study will provide a justification for the consumption of this herbal formulation and also protect public health against the various adverse effects that may arise from their consumption.

Materials and Method

Preparation of Stock solution

The herbal formulation (Batch number G43221, NAFDAC registration number A7-9528 L) was bought from a Pharmacy outlet in Mararaba, Nasarawa State, and the stock concentration was obtained according to the method of [5]

10 mL of the herbal preparation was poured into four clean 50 ml beakers and placed in an electric water bath (Sanyo, Japan). The temperature was set at 40 °C and the mixture was allowed to evaporate. This was done to determine the marc of the herbal mixture. A laboratory weighing balance was used to calculate the initial weights of the 50 mL beakers. Using the mathematical formula below, the stock concentration of the herbal product was estimated by taking the average of the variations in weight between the beakers and marc in 1 mL of solution:

Weight of beaker= X (g)

Weight of beaker + marc = Y (g)

Weight of Marc = Y – X (g)

The mL doses to be administered to the animals were calculated using the following formula:

Drug concentration = $\sum Y - X$ (g) / N (mL) = Z g/mL

Where N= the number of beakers used.

Experimental Animals

Both sexes of Wistar rats and Swiss albino mice were obtained from the Animal House of the Department of Pharmacology and Toxicology at Bingham University in Nasarawa State, Nigeria. The animals were kept and fed in ideal conditions (ULTIMA Feeds, Nigeria Ltd). To provide adequate lighting for the animals, a 12 h light/ 12 h dark period was maintained. Before the study began, the animals were allowed to acclimate to laboratory surroundings for seven days. All animals were provided food and water as needed.

Acute Toxicity Determination

The polyherbal product's LD_{50} was determined using the Lorke method[6], with certain modifications. Twenty (20) Swiss albino mice weighing 17-20 g were acclimatized and starved for 12 hours. Animals were allotted into five groups with n=3. Various doses of the polyherbal product were administered to the animals via the oral route as shown in Table 1. Within the first 24 hours after oral delivery, the animals were examined for visual symptoms of toxicity and mortality. The calculated LD_{50} was used to determine the optimal doses to be given for the sub-chronic toxicity experiments. The LD_{50} was determined using the following mathematical formula:

$LD_{50} = \sqrt{xy}$

Where x represents the maximal dose with 0% mortality and

y represents the minimum dose with first mortality.

Experimental Design

30 mature albino rats of both sexes were weighed and divided into six groups of five animals each (n=5) at random and treated as described in Table 2. For the duration of the test period of 60 days, the animals were given daily oral treatments[7,8]. Each group of animals was closely observed for changes in body weight, behavioral changes, drinking and feeding habits, and overall health during the entire period of study. At the end of the study, the animals were starved overnight and slaughtered under anesthesia using diethyl ether. Blood was collected for biochemical analyses via cardiac puncture into plain bottles. The liver was removed from each animal for histopathological analyses. The Laboratory Animal Care and Use Manual,[9] was strictly adhered to for the handling and care of all experimental animals used in this study.

Table 1 Experimental Design

Serial number	Group	Treatments	Dose(mg/kg)	Period (days)
1	CM	Control male	10ml/kg water	60
2	HDM	male	374.0	60
3	HDF	Female	374.0	60
4	CF	Control female	10ml/kg water	60
5	LDM	Male	187.0	60
6	LDF	female	187.0	60

CM=Control Males; HDM=High Dose Males, HDF=High Dose Females, CF=Control Females, LDM=Low Dose Males, LDF=Low Dose Females.

Biochemical Analysis

Serum transaminases: Alanine aminotransferase (ALT) and aspartate transaminase (AST) activities were determined at 340 nm using IFCC techniques[10]. equally calculated at 405 nm by the methods of Tietz[11], was Alkaline phosphatase (ALP).

ALP determination: 500 µl of the reagent was combined with 10 µl of the sample in a test tube. The absorbance was first measured at 405 nm and then again after 3 minutes. The following formula was used to compute per minute, the mean absorbance:

ALP activity (IU/l) = 2742 × ΔA 405 nm/min;

Where: ΔA 405 nm/ sec represents a change in absorbance per minute for the homogenate sample; 2742 represents the Extinction coefficient.

ALT determination: In a test tube, 50 µl of sample and 500 µl of ALT reagent were mixed together, and the initial absorbance at 340 nm was measured after 60 seconds. Simultaneously, the timer was started, and absorbance values were collected after 60, 120, and 180 seconds.

ALT activity (nm/sec) = 1746 ΔA 340 nm/min, where ΔA 340 nm/min represents homogenate sample absorbance changes per minute and 1746 represents the Extinction coefficient.[13].

AST determination: The same test procedure described for ALT was employed. The only distinction is that the ALT reagent has been replaced by the AST reagent.

AST activity (nm/sec) = 1746 ΔA 340 nm/min; where 1746 represents the Extinction coefficient and ΔA 340 nm/min represents homogenate sample absorbance changes per second.

Total and direct bilirubin determination: This was done using a colorimetric (DCA) method[14]. Briefly, into a test tube, 100 µL (0.1 mL) of the serum and 1000 µL of the working reagent (prepared by mixing DCA reagent and nitrite reagent in a ratio of 50:1 by volume) was added, mixed thoroughly, and incubated for 5 min at 37 ºC and read at 546 nm against the sample blank (prepared by adding 1000 µL of DCA reagent to 100 µL of the sample). Bilirubin reacts with 2, 4-dichloroanilline to yield azobilirubin, while the albumin-bound bilirubin is released by an ionic

detergent. The amount of total bilirubin concentration in the serum is directly proportional to the the color intensity of the mixture produced.

Histopathological Examination

Liver tissues were processed according to the method of [14]. Immediately after fixation, thin slices of liver tissue were dehydrated simultaneously through progressive concentrations of alcohol and cleared using xylene. After cleaning, the tissues were fixed in paraffin wax, and thin sections of around 5 microns were cut with a microtome. Each piece was stained with Haematoxylin and Eosin and placed on a clean glass slide. Following that, a mounting media (Canada balsam) was applied to each tissue section, followed by a cover slip and left to dry. The tissues were examined under a light microscope after drying. Mounted to the microscope was a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd.) used in taking the photomicrographs. To minimize bias, the experimental design was not given to the pathologist while interpreting the results[15].

Statistical Analysis

This study's data was analyzed using SPSS version 21. And to evaluate statistical significance between groups, a one-way analysis of variance (ANOVA) was utilized. The data were reported as mean plus standard error of mean S.E.M., with a $p<0.05$ threshold considered significant.

Results and Discussion

Acute Toxicity

The polyherbal formulation's LD_{50} value was calculated to be 3740 mg/kg (oral).

Effect of Herbal Formulation on Body Weight

Figure 1 depicts the effect of the polyherbal formulation at different doses on the body weights of rats. During the trial, animals given 187mg/kg experienced a progressive gain in body weight from the beginning to the end of the investigation, whereas groups given 374.0 mg/kg of the PF experienced a decrease in body weight during the last two weeks of the study. However, this difference in weight was not statistically significant ($p \leq 0.05$) between the treatment groups and the controls.

Biochemical results

ALT levels increased considerably in animals given 187.0 mg/kg of the polyherbal formulation compared to control values. There was also a substantial rise in the weight of rats given 374 mg/kg polyherbal formulation compared to rats given 187 mg/kg. The female group given 374.0 mg/kg (HDF) showed relatively higher ALT values when compared with both the 187.0 mg/kg-dosed male and the 374.0 mg/kg-dosed male groups respectively. (Figure 2a-e).

GPT values reported in male groups given 187.0 mg/kg were substantially ($p<0.05$) higher than in control male rats. GPT levels differed significantly between animals given 374.0 mg/kg and animals given 187.0 mg/kg. The female rats given 374.0 mg/kg had significantly higher serum GPT levels than the male rats given 374 mg/kg of the formulation and their respective controls. However, no significant ($p>0.05$) difference in GPT values was seen between male mice given 187.0 mg/kg and female animals given the same dose of the formulation.

GOT levels were significantly lower ($p<0.05$) in male mice given 187.0 mg/kg compared to controls. GOT activity was significantly reduced in female rats given 187.0 mg/kg of the polyherbal formulation compared to the control group. However, there was a significant increase

in GOT activity in the 374.0 mg/kg-dosed male group and female rats equally given with 374.0 mg/kg of the formulation, when compared to the control.

Total bilirubin levels in male groups given 374.0 mg/kg were not substantially higher than in controls. When male rats were given 187.0 mg/kg and female groups were given the same dose, there was no significant increase ($p>0.05$) in total bilirubin values when compared to their respective controls. The female group given 374 mg/kg of the formulation had significantly higher total bilirubin levels than the female groups given 187.0 mg/kg.

Histopathological Results

Hepatocytes showed well-preserved cellular architecture with functional blood arteries, well-preserved hepatocytes, and the presence of buffer cells in the liver tissue of rats from the control groups (Figure 3.a). Male and female rats administered with 187 mg/kg of the formulation equally showed normal cellular architecture of hepatocytes and the well-preserved orientation of blood vessels, and no significant pathologies were detected. However, animals of both sexes, given 374 mg/kg of the formulation revealed the presence of inflammatory cells congestion of widened blood vessels, and hyperplasia of connective tissue. These indicated various degrees of tissue degeneration (Figure 3.e).

Presently, pharmaceutical formulations (PF) are chief contributors to liver disease. Drug-induced liver injury (DILI) is responsible for acute hepatotoxicity as well as frequent withdrawal of pharmaceutical preparations from the market as well and pharmacovigilance decisions[3,16,17]. The Ghana cleanser® was administered orally in divided doses to Wistar rats of both sexes for 60 days. The polyherbal preparation's oral LD50 was determined to be 3740.0 mg/kg, indicating a wide margin of safety. However, liver biomarkers such as ALT/SGPT and AST/SGOT were

considerably increased in the male rats dosed at 187.0 mg/kg and female rats dosed with 187.0 mg/kg of the herbal formulation compared with their respective controls. AST is found predominantly in hepatocytes and released into circulation after hepatocyte injury or death. Serum alkaline phosphatase is also produced in significantly higher amounts during an acute liver injury but may not always be an indication of hepatocytic death. Enzymes such as AST and ALT catalyze the transfer of -amino groups from aspartate and alanine to the -keto group of ketoglutaric acid to produce oxaloacetate and pyruvic acids, both of contribute significantly to the citric acid cycle[18]. Bilirubin remains in cells until they are rendered water-soluble through conjugation by a specific transferase enzyme which is primarily located in the endoplasmic reticulum and the conjugated bilirubin is readily excreted in the bile. Serum levels of bilirubin could be a result of overproduction due to excessive degradation of the heme portion of hemoglobin or decreased conjugation, resulting in excretion failure due to acute liver injury or disease conditions[19]. There were no significant changes in the total bilirubin of animals treated with the varied doses of Ghana cleanser® compared with their controls respectively. There was also no difference between male-treated groups and female-treated groups at the same doses. However, the insignificant increase in total and direct bilirubin content did not hinder the liver from performing its functions. The phytochemicals such as tannins, terpenoids, alkaloids, and polyphenols which are present in varied amounts and constitute the polyherbal mixture may be responsible for the observation in this study. Furthermore, the study results study demonstrated that there was no significant difference in body weights of rats given the polyherbal formulation vs control groups, indicating that the herbal product did not interfere with their normal growth and development. All animals appeared to gain weight in steady amounts throughout the duration of the experiment.

Histopathological findings of the liver revealed that there was no inflammation in the 187.0 mg/kg-dosed groups compared with normal hepatocytes from the control group. (Figures 3.a-3.f). Photomicrographs of the liver from rats of both sexes administered with 374.0 mg/kg of the formulation revealed varied degrees of pathology. These include inflammation of hepatocytes, degenerated layers of bile ducts, hyperplasia, necrosis of connective tissue cells, abnormally spaced and vacuolated hepatocytes, widened and disoriented sinusoids, ruptured bile duct, and significant tissue degeneration. Since the polyherbal mixture has been shown to have an LD_{50} value of 3740 mg/kg body weight, the effects observed in some of the parameters may probably not wholly have resulted from the herbal formulation[20].

Conclusion

The findings from this study show that Ghana Cleanser® when consumed in moderation with small quantities is relatively safe with negligible hepatic toxicity. However, the 374 mg/kg-exposed animals presented with significant hepatotoxicity when consumed for a prolonged period of time.

References

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Table 2. Acute toxicity test of Ghana Cleanser®

Test	Dose (mg/Kg)	Fraction of death	% Mortality
1	100	0/3	0
2	1000	0/3	0
3	2000	0/3	0
4	3000	0/3	0
5	4000	1/3	33.3
6	5000	2/3	67.0

Route of administration: orally, n=18

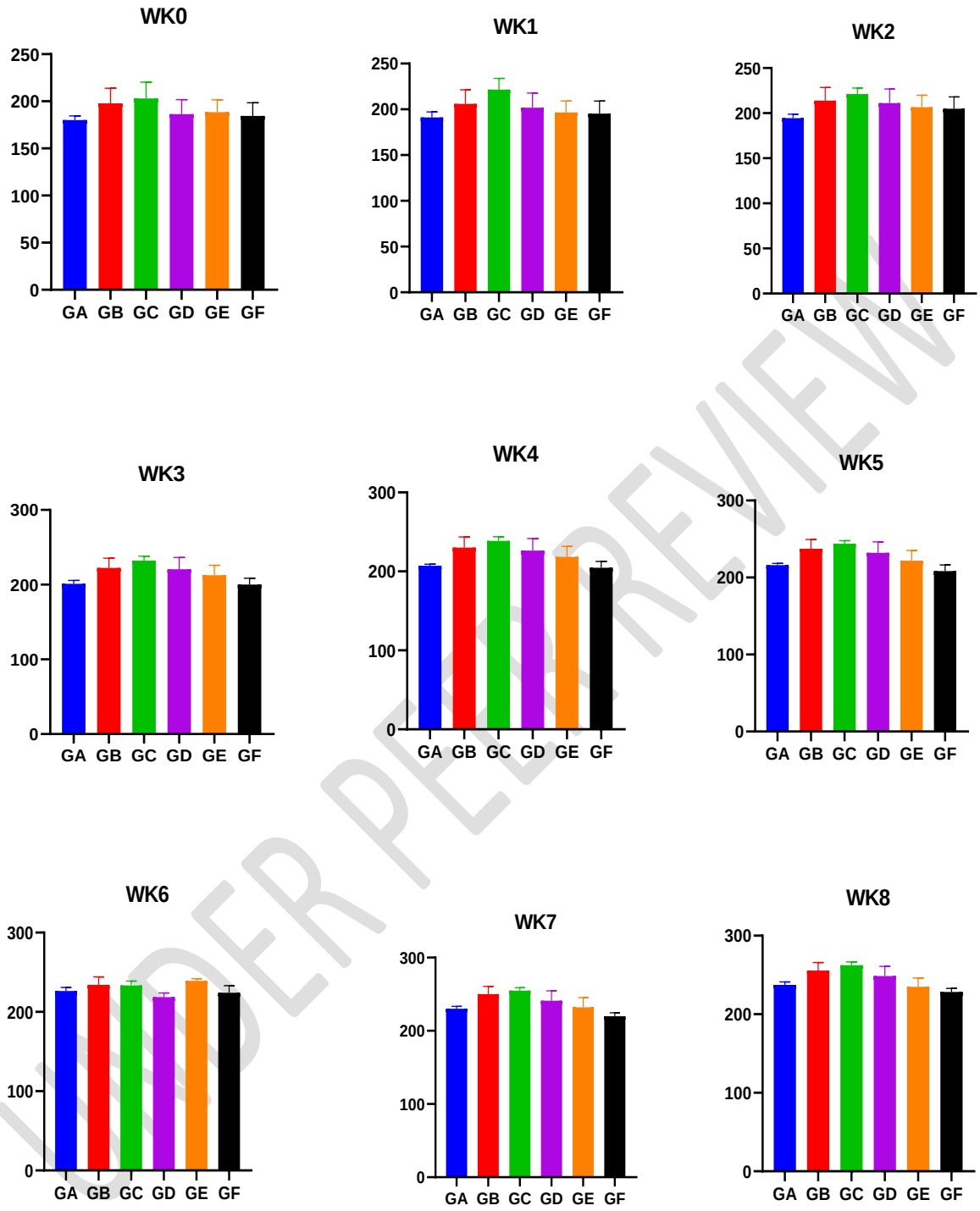
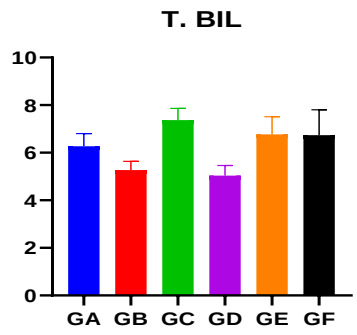
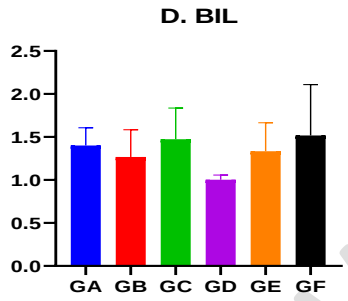


Figure 1; KEY: ***GA=Control males (CM), GB= Males rats given 374 mg/kg, GC= Female rats given 374 mg/kg, GD= Control Females (CF), GE= Male rats given 187.0 mg/kg, GF= Female rats given 187.0 mg/kg.***



A



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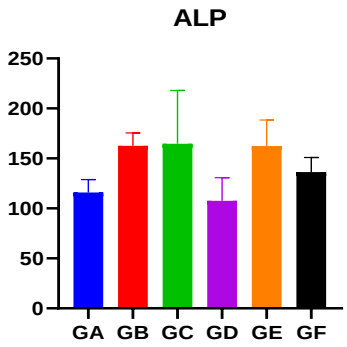

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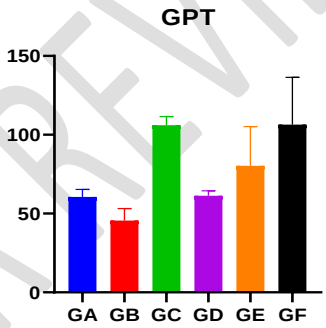

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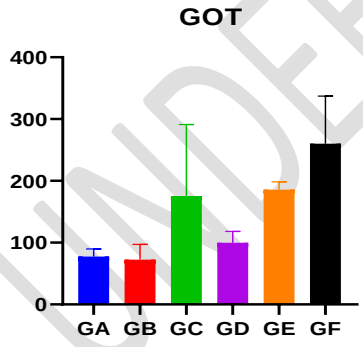

E

Figure 2: (A - E) KEY: ***GA=Control males (CM), GB= Male rats given 374 mg/kg, GC= Female rats given 187.0 mg/kg, GD=Control Females (CF), GE= Male rats given 187.0 mg/kg, GF= Female rats given 187.0 mg/kg.***

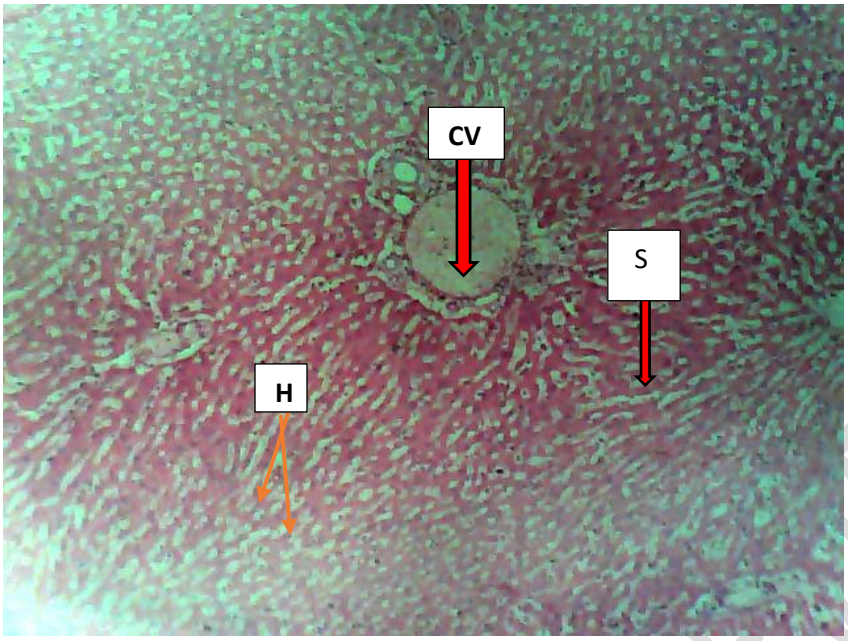

Figure 3.a: Photomicrograph of the liver of Wistar rat from Group Male (Control). H&E stain x100. Showing normal Central Vein (CV), Hepatocytes (H), and Sinusoids (S)

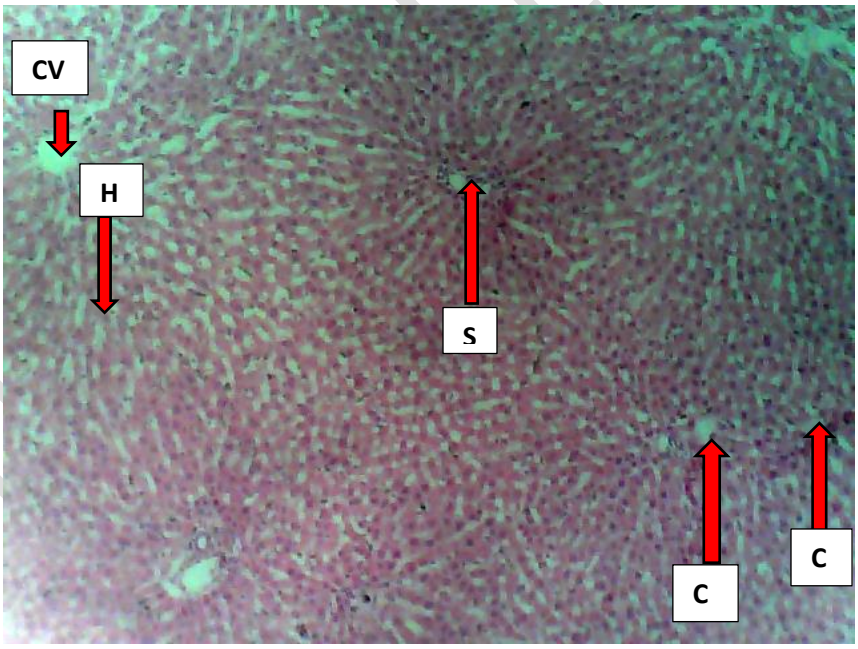

Figure 3.b: Photomicrograph of the liver of Wistar rat from female group (Control) showing normal Central Vein (CV), Hepatocytes (H), and Sinusoids (S) at different stages, H&E stain x100.

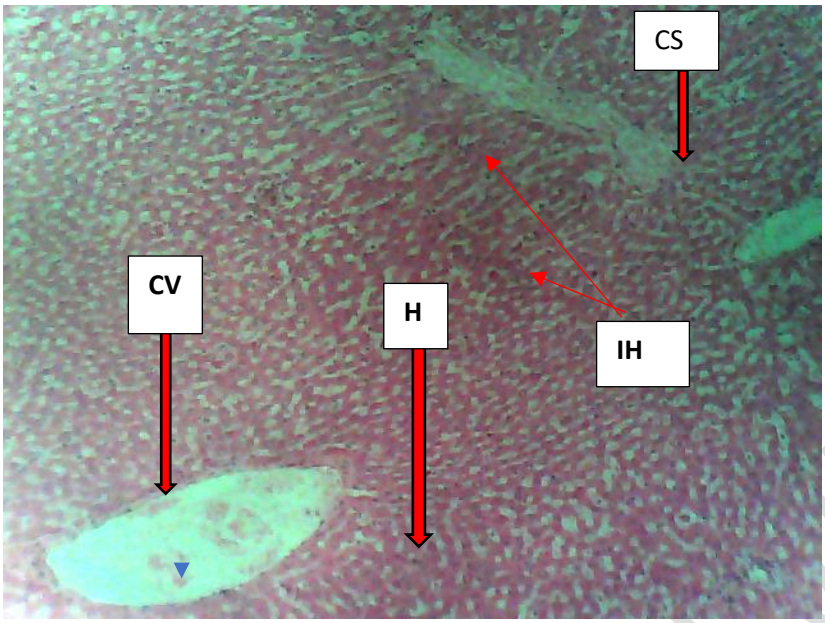

Figure 3.c: Photomicrograph of the liver of Wistar rat Administered with 374.0 mg/kg of herbal extract from Group of Male showing distended Central Vein (CV), Inflamed Hepatocytes (IH), and congested Sinusoids (CS), H&E stain x100.

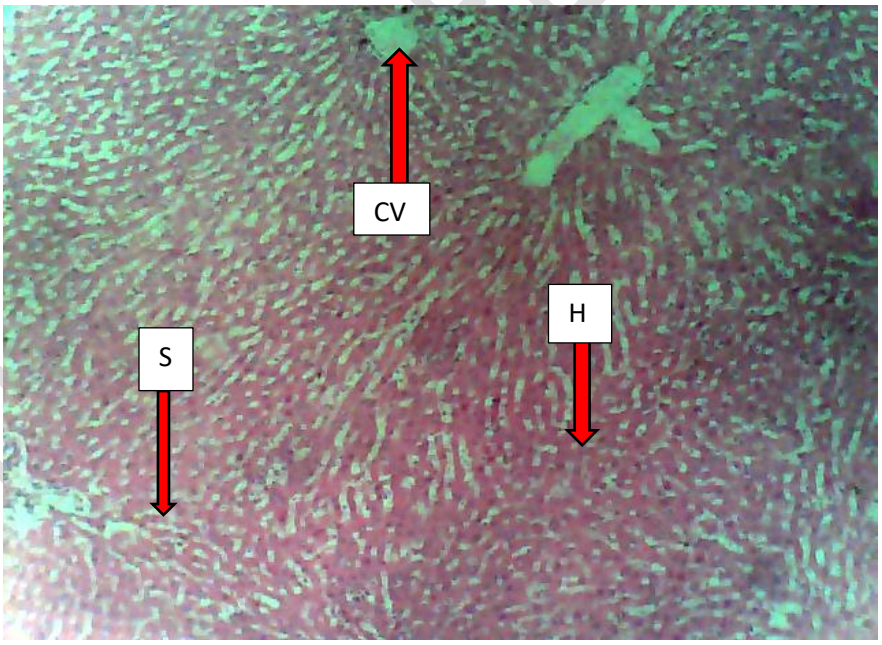

Figure 3.d: Photomicrograph of the liver of Wistar rat from male group given 187.0 mg/kg showing normal Central Vein (CV), Hepatocytes (H), and Sinusoids (S), H&E stain x100.

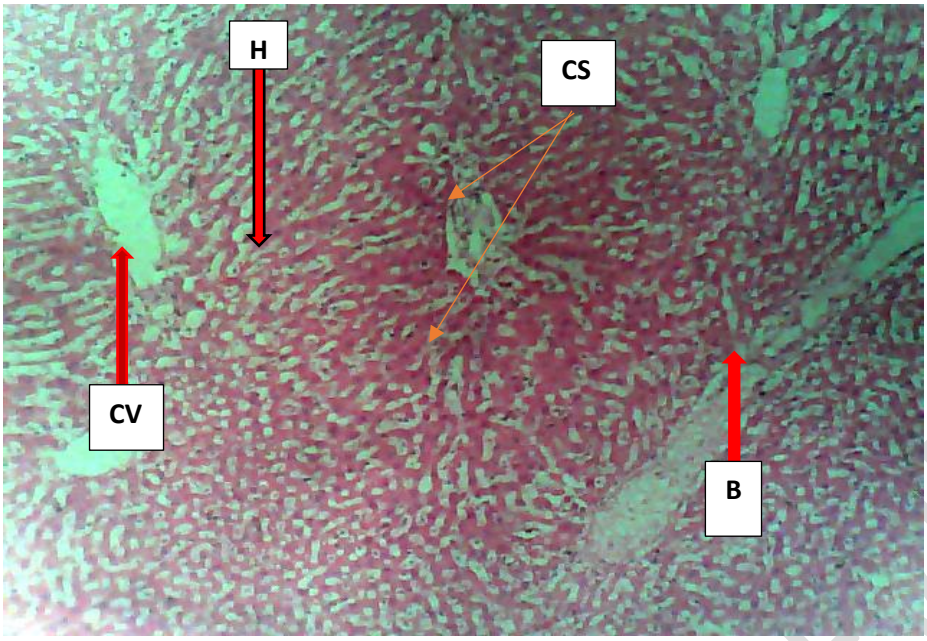

Figure 3.e: Photomicrograph of the liver of Wistar rat from female rats administered with 374.0 mg/kg herbal formulation Showing Distention of Central Vein (CV), Congested sinusoids (CS) constriction of blood vessel (B), and dilatation of vessels (V), H&E stain x100.

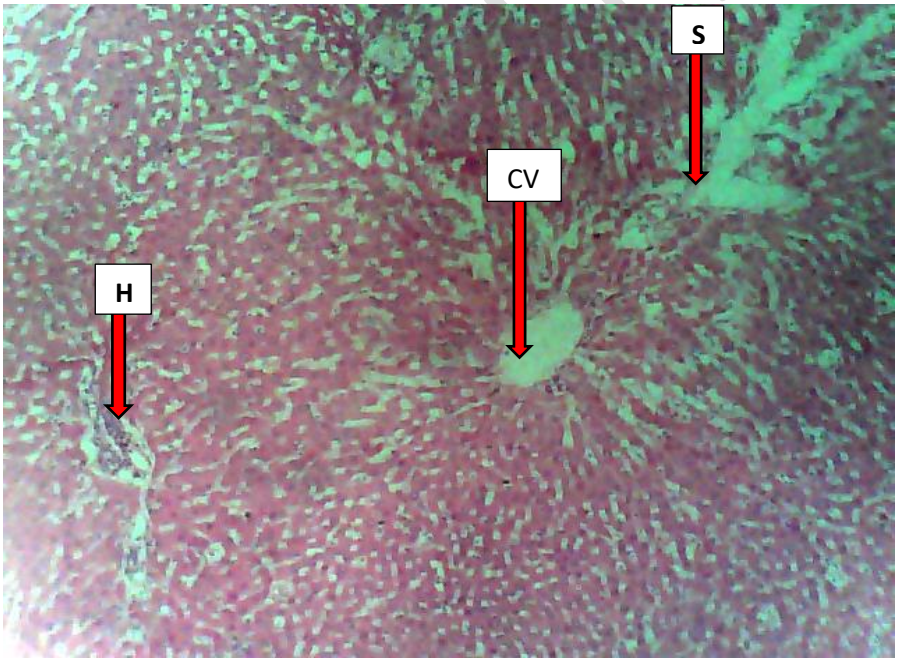

Figure 3.f: Photomicrograph of the liver of female rats administered with 187.0 mg/kg herbal formulation showing normal Central Vein (CV), Hepatocytes (H), and Sinusoids (S). H&E stain x100.