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 allotted 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 euthanized 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 374 mg/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 187 mg/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 374 mg/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

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

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

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

51

52 **Materials and Method**

53 Preparation of Stock solution

54 The herbal formulation (Batch number G-43221, NAFDAC registration number A7-9528 L) was
55 bought from a Pharmacy outlet in Mararaba, Nasarawa State, and the stock concentration was
56 obtained according to the method of [5]

57 10 mL of the herbal preparation was poured into four clean 50 ml beakers and placed in an electric
58 water bath (Sanyo, Japan). The temperature was set at 40 °C and the mixture was allowed to
59 evaporate. This was done to determine the marc of the herbal mixture. A laboratory weighing
60 balance was used to calculate the initial weights of the 50 mL beakers. Using the mathematical
61 formula below, the stock concentration of the herbal product was estimated by taking the average
62 of the variations in weight between the beakers and marc in 1 mL of solution:

63 Weight of beaker= X (g)

64 Weight of beaker + marc = Y (g)

65 Weight of Marc = Y – X (g)

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

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

68 Where N= the number of beakers used.

69 Experimental Animals

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

76 Acute Toxicity Determination

77 The polyherbal product's LD_{50} was determined using the Lorke method[6], with certain
78 modifications. Twenty (20) Swiss albino mice weighing 17-20 g were acclimatized and starved
79 for 12 hours. Animals were allotted into five groups with n=3. Various doses of the polyherbal
80 product were administered to the animals via the oral route as shown in Table 1. Within the first
81 24 hours after oral delivery, the animals were examined for visual symptoms of toxicity and
82 mortality. The calculated LD_{50} was used to determine the optimal doses to be given for the sub-
83 chronic toxicity experiments. The LD_{50} was determined using the following mathematical formula:

84 $LD_{50} = \sqrt{xy}$

85 Where x represents the maximal dose with 0% mortality and

86 y represents the minimum dose with first mortality.

87 Experimental Design

88 30 mature albino rats of both sexes were weighed and divided into six groups of five animals each
89 (n=5) at random and treated as described in Table 2. For the duration of the test period of 60 days,
90 the animals were given daily oral treatments[7,8]. Each group of animals was closely observed for
91 changes in body weight, behavioral changes, drinking and feeding habits, and overall health during
92 the entire period of study. At the end of the study, the animals were starved overnight and
93 slaughtered under anesthesia using diethyl ether. Blood was collected for biochemical analyses via
94 cardiac puncture into plain bottles. The liver was removed from each animal for histopathological
95 analyses. The Laboratory Animal Care and Use Manual,[9] was strictly adhered to for the handling
96 and care of all experimental animals used in this study.

97 **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

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

100

101 Biochemical Analysis

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

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

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

109 Where: ΔA 405 nm/ sec represents a change in absorbance per minute for the homogenate sample;
110 2742 represents the Extinction coefficient.

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

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

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

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

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

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

128 Histopathological Examination

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

138 Statistical Analysis

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

142 **Results and Discussion**

143 Acute Toxicity

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

145 Effect of Herbal Formulation on Body Weight

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

152 Biochemical results

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

158 GPT values reported in male groups given 187.0 mg/kg were substantially ($p<0.05$) higher than in
159 control male rats. GPT levels differed significantly between animals given 374.0 mg/kg and
160 animals given 187.0 mg/kg. The female rats given 374.0 mg/kg had significantly higher serum
161 GPT levels than the male rats given 374 mg/kg of the formulation and their respective controls.
162 However, no significant ($p>0.05$) difference in GPT values was seen between male mice given
163 187.0 mg/kg and female animals given the same dose of the formulation.

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

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

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

174 Histopathological Results

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

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

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

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

220 **Conclusion**

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

225 Ethical Approval:

226 As per international standards or university standards written ethical approval has been collected
227 and preserved by the author(s).

228 **Conflict of Interest:** There is no conflict of interest, according to the authors.

229 **Author's Declaration**

230 The authors hereby certify that the work contained in this article is original and that they will
231 accept responsibility for any claims arising from the content of this post.

Acknowledgment

Mr. Williams Ujah of the Department of Anatomy, Faculty of Basic Medical Sciences, Bingham University, Nigeria, is thanked by the authors for his significant contributions, particularly in the creation of photomicrographs and interpretation of slides for this study.

Authors' contributions

All authors worked together to complete this study. The experiment was designed by IUP, NPS, and DP, who also prepared the original draft of the book. The original draft was examined and edited by authors FTI, AI, and OJ. The author, BMI, was in charge of the literature searches. Author IUP performed the statistical analysis. Authors DP and NPS excised tissues from all the euthanized experimental animals. Author FTI revised the final manuscript. All authors contributed significantly and read and approved the final paper.

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312 **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

313 *Route of administration: orally, n=18*

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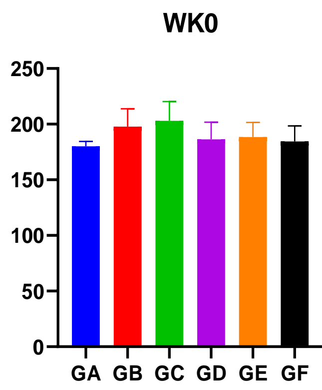

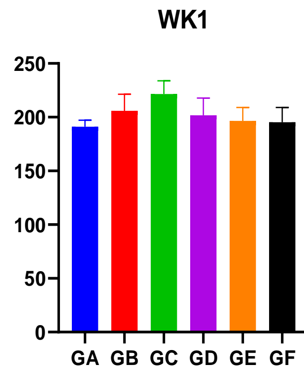

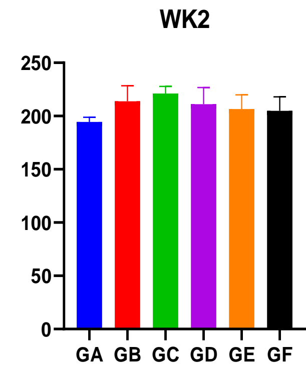

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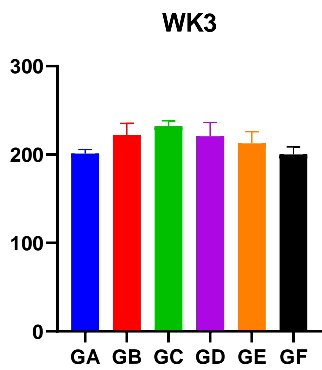

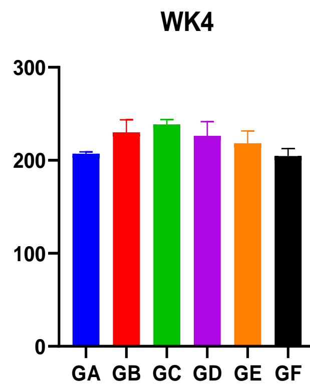

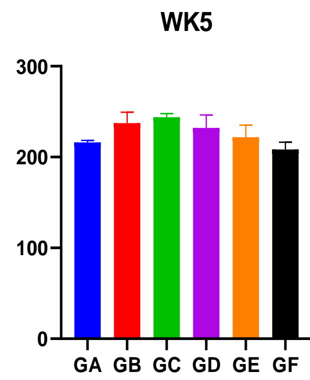

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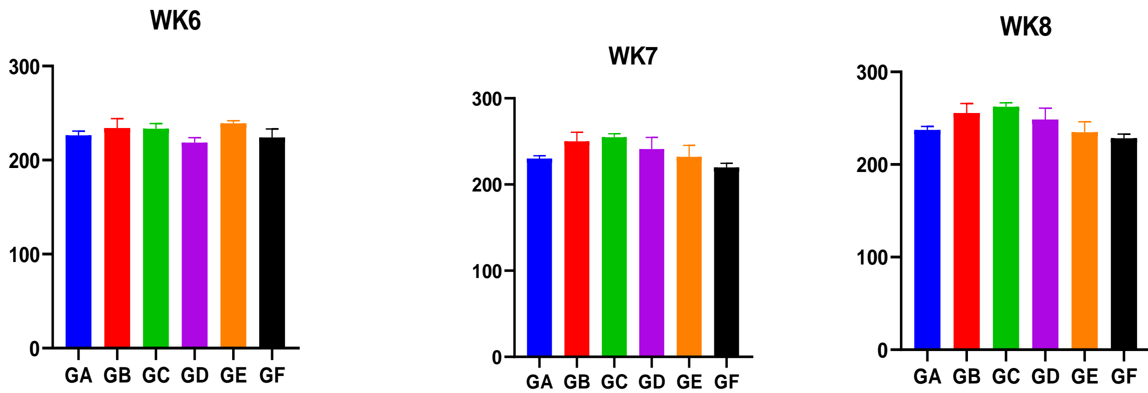

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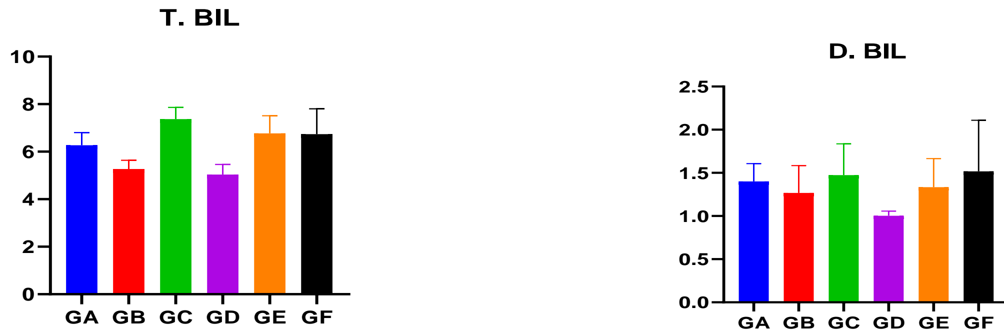
324 **Figure 1; KEY:** ***GA=Control males (CM), GB= Males rats given 374 mg/kg, GC= Female rats given 374***

325 ***mg/kg, GD= Control Females (CF), GE= Male rats given 187.0 mg/kg, GF= Female rats given 187.0***

326 ***mg/kg.***



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328 **A** **B**

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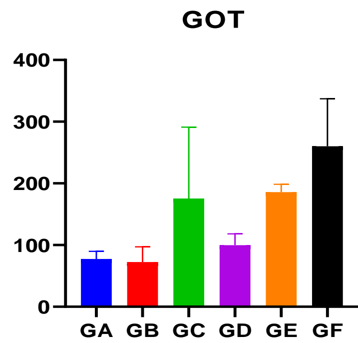
330 **C** **D**



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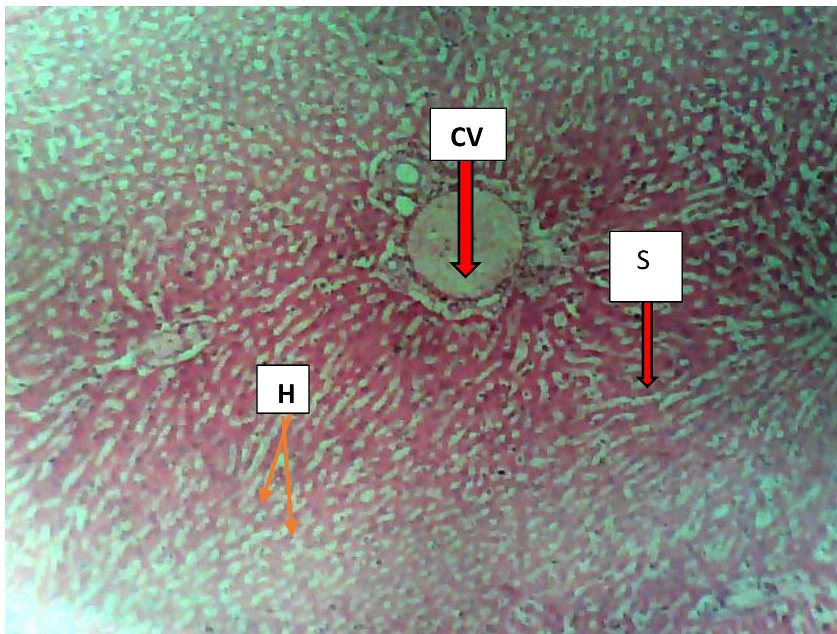
332 **E**

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



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

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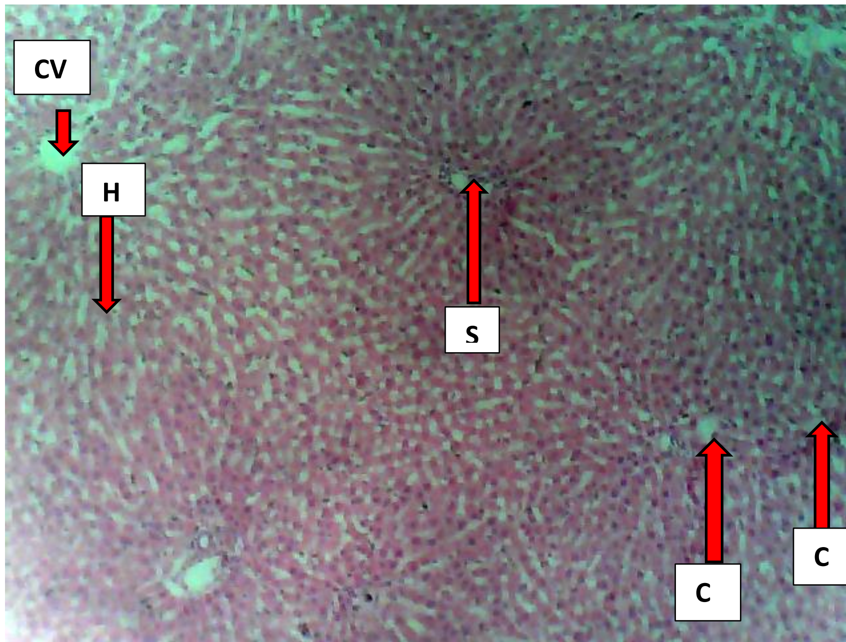

341 ***Figure 3.b: Photomicrograph of the liver of Wistar rat from female group (Control) showing normal***
342 ***Central Vein (CV), Hepatocytes (H), and Sinusoids (S) at different stages, H&E stain x100.***

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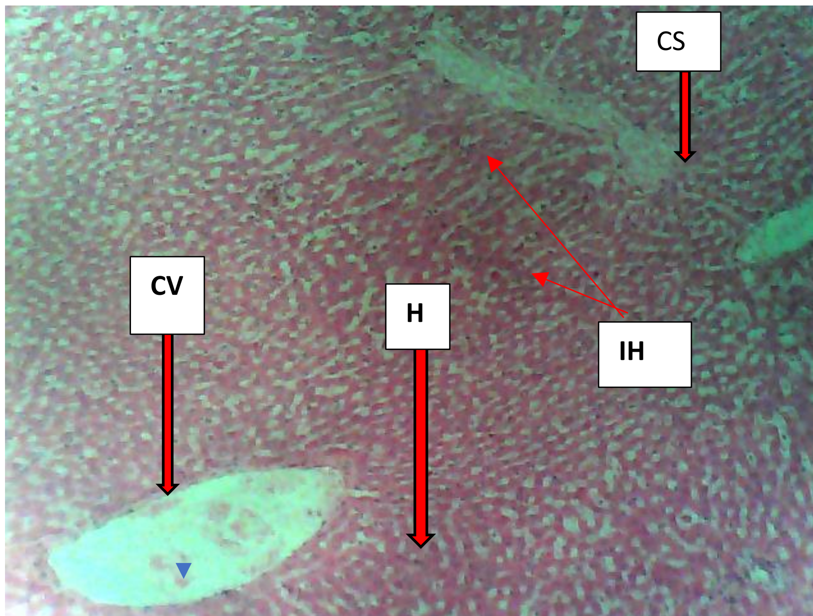

344 ***Figure 3.c: Photomicrograph of the liver of Wistar rat Administered with 374.0 mg/kg of herbal extract***
345 ***from Group of Male showing distended Central Vein (CV), Inflamed Hepatocytes (IH), and congested***
346 ***Sinusoids (CS), H&E stain x100.***

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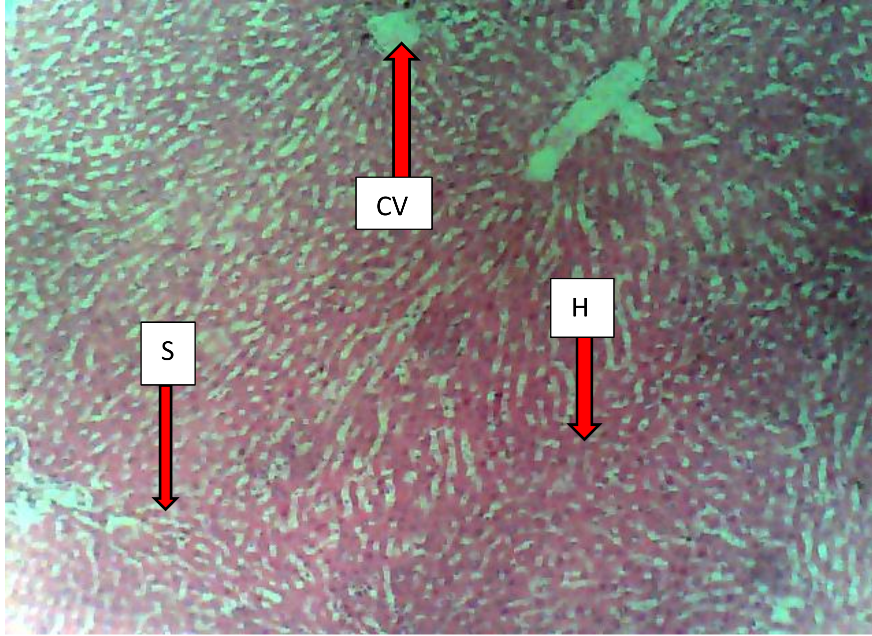

349 ***Figure 3.d: Photomicrograph of the liver of Wistar rat from male group given 187.0 mg/kg showing***
350 ***normal Central Vein (CV), Hepatocytes (H), and Sinusoids (S), H&E stain x100.***

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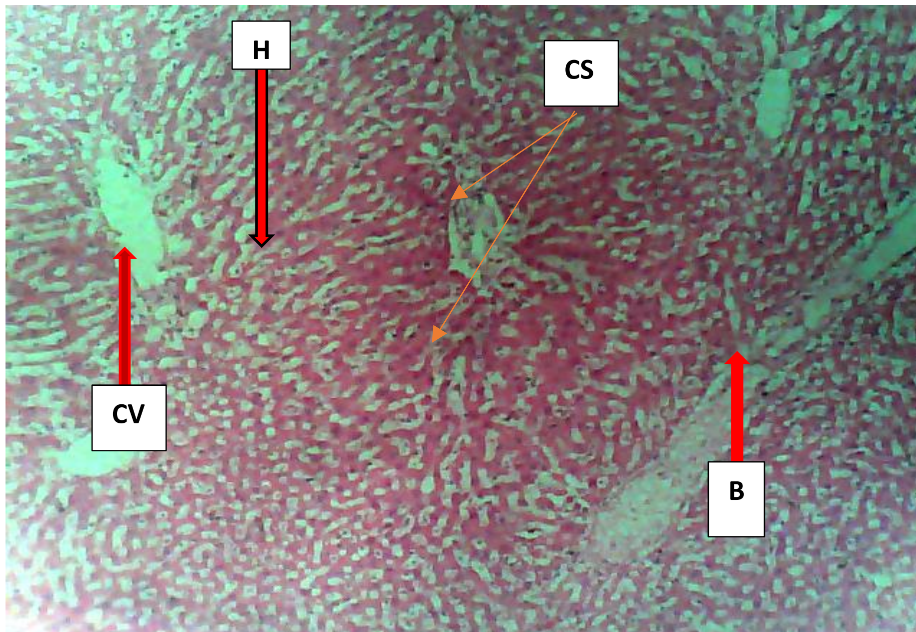

352 ***Figure 3.e: Photomicrograph of the liver of Wistar rat from female rats administered with***
353 ***374.0 mg/kg herbal formulation Showing Distention of Central Vein (CV), Congested***
354 ***sinusoids (CS) constriction of blood vessel (B), and dilatation of vessels (V), H&E stain x100.***
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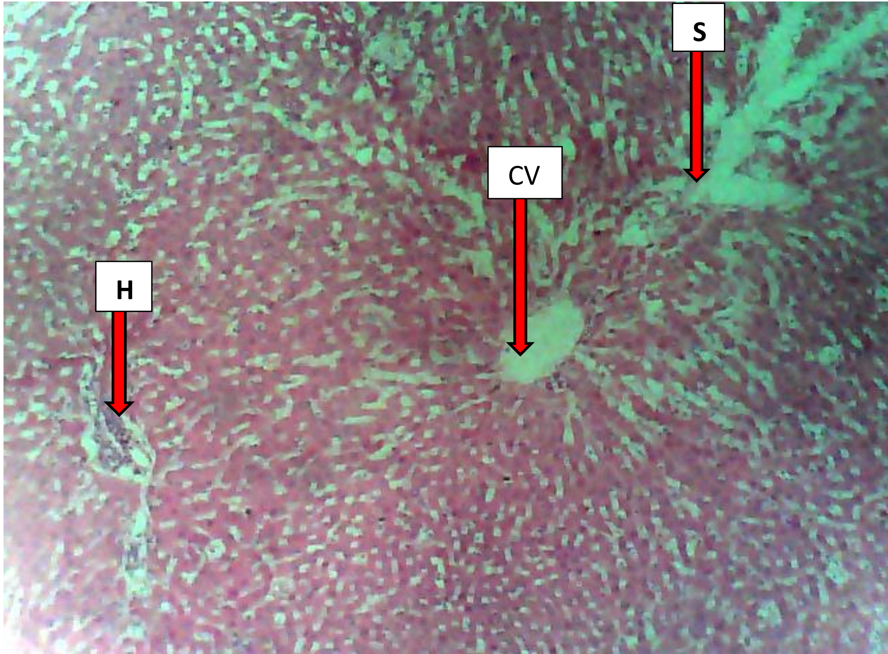

357 ***Figure 3.f: Photomicrograph of the liver of female rats administered with 187.0 mg/kg herbal***
358 ***formulation showing normal Central Vein (CV), Hepatocytes (H), and Sinusoids (S). H&E***
359 ***stain x100.***
360