

# Histological Study of Effect of Ethanol Stem Extracts of *Homalium Letestui* on Thioacetamide - Induced Injury in Albino Rat, Using Various Staining Techniques

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**Abstract:** - Introduction/Aim: Knowledge of the normal histology of the multitude of tissue types within the body is necessary for the recognition and understanding of disease. Proper staining and evaluation of tissue damage is essential in research. *Homalium letestui* is an evergreen tree. The plant has been of immense benefit to traditional users. A bark-decoction, combined with other medicinal plants, is taken by draught for orchitis, and bark-scrapings enter a prescription given to a newly-delivered woman. In this study the histological effect of the ethanol stem extract of *Homalium letestui* on rat thioacetamide induced liver injury was carried out using H&E and Gordon and Sweet silver impregnation Technique.

**Method:** Thirty six (36) rats were used for this work. Group one served as the positive control receiving normal saline, group two served as thioacetamide group, group 3 received silymarin 100 mg/mg, while group 4, 5 and 6 received 250, 500 and 750 mg/kg of the extract respectively. On the 8<sup>th</sup> day the animals in group 2-6 were administered 200mg/kg bw of thioacetamide dissolved in saline orally. Twenty hours later all animals were weighed again and sacrificed under light diethylether vapour. General staining procedure Hematoxylin and Eosin and the specific staining technique, Gordon and Sweet silver impregnation Technique were carried out on the liver. Haematological and chemopathological investigation were also done.

**Result:** In H&E stain, there was disorganization of the texture of hepatic cells with centrilobular necrosis, hyperplasia, vascular and cellular degeneration, polymorphonuclear aggregation, inflammation and fatty degeneration in the thioacetamide - treated rats. The liver sections of the rats treated with extract showed signs of protection as was evident by slight areas of vacuolation, cellular proliferation. Gordon and sweet impregnation technique showed portal tract degeneration while pretreated group revealed slight reticular fiber degeneration with much roughed fiber observed in the rats that received 750 mg/kg dose of the extract. This result with other parameters carried out.

**Conclusion:** result indicates that the plant may prevent or protect the liver architecture

**Keywords:** *Homalium letestui*, liver, thioacetamide

## I. INTRODUCTION

Each organ of a body consists of four basic types of tissues: epithelial, connective, muscle and nervous tissue. However, the cells in these tissues can be shaped differently when they are found in different organs. In fact, how the cells in a tissue are shaped can predict how that tissue functions. Histology is the microscopic study of animal and plant cell and tissues through staining and sectioning and examining them under a microscope (electron or light microscope). There are various methods used to study tissue characteristics and microscopic structures of the cells. Histological studies are used in forensic investigations, autopsy, diagnosis and in education. In addition, histology is used extensively in medicine especially in the study of diseased tissues to aid treatment<sup>1</sup>.

*Homalium letestui* occurs from Senegal east to the Central African Republic and south to western DR Congo and Cabinda. It prefers proximity to running water. The tree has magnificent clusters of rose-coloured flowers. The fruits are also showy and the young leaf-flush is red before turning green. The tree is thus attractive and worthy of cultivation<sup>2</sup>. Bark sap is applied as enema and bark pulp rubbed in to treat oedema. Bark decoctions are taken in mixtures to treat orchitis and as tonic for women after childbirth. Root extracts are administered to treat malaria. The tree is decorative with its showy flowers, fruits and reddish young leaves, and is sometimes planted as ornamental<sup>3</sup>. In Ivory Coast sap from the bark is used in enemas for the treatment of generalised oedemas while lees from the bark are rubbed over the area<sup>4</sup>. In Gabon a bark-decoction with other drug-plants is taken by

draught for orchitis, and bark-scrapings enter a prescription given to a newly-delivered woman. The Yoruba of Nigeria call on the plant in an incantation against small-pox, while the bark, finely ground to a powder, is blown by Liberian witchdoctors into a dragon's lair to stupefy it before slaying it<sup>5</sup>. Several work has been done to evaluate some of the folkloric claim of the plant. The present study was undertaken to evaluate the histological effect of ethanol stem extract of *Homalium letestui* on thioacetamide induced liver injury<sup>6,7,8,9,10,11,12</sup>.

## II. MATERIAL AND METHOD

### *Plants collection*

*Homalium letestui* (stem) was collected in a forest in Uruan area, AkwaIbom State, Nigeria. It was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU 382) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

### *Extraction*

The stem was washed and dried under shed for two weeks. The dried plant material was then cut into smaller pieces and grounded to powder. The powdered material was macerated in 70% ethanol. The liquid filtrate was evaporated to dryness *in vacuum* 40°C using rotary evaporator. The ethanol extract was stored at -4°C until used.

### *Animals*

Adult male albino rats were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

### *Animal treatment*

36 rats were weighed and divided into six groups with 6 animals per group. Treatment was as follows: Group 1 consisted of normal animals that were administered with normal saline (10 ml/kg) for eight days, Group 2, the thioacetamide group, received normal saline 10 ml/kg for eight days. Group 3 served as the standard group and rats in this group were administered 100 mg/kg body weight of silymarin orally for 8 days, while groups 4, 5 and 6 were administered p.o with 250, 500 and 750 mg/kg of *H. letestu* stem extract respectively daily for 8 days. On the 8<sup>th</sup> day the animals in group 2-6 were administered 200mg/kg bw of thioacetamide dissolved in saline orally. Twenty hours later all animals were weighed again and sacrificed under light diethylether vapour.

### *Hematological study*

Blood samples were collected from each rat by cardiac puncture immediately after the animals were sacrificed under diethyl ether anesthesia, using 21 gauge (21 G) needles

mounted on a 5 ml syringe into ethylene diamine tetra-acetic acid (EDTA) - coated sample bottles for analyzed. Hematological parameters such as full blood count (FBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and Total and differential white blood cell count (WBC). These parameters were analyzed using automatic hematological system.

### *Liver function test*

Serum was separated from the blood of each animal sacrificed and the sera were stored at -20°C in a freezer until used for biochemical determinations such as total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, total and direct bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols<sup>13</sup>.

### *Histopathological examinations*

The livers were processed and stained with haematoxylin and eosin (H&E) and by Gordon and Sweet<sup>14</sup> silver impregnation technique. Prepared slides of the organs were mounted on high-definition microscope. The result were interpreted by a Pathologist in the Department of Chemical Pathology, University of Uyo, Uyo.

Morphological changes in the excised organs of the sacrificed animals were observed and recorded. Histologic micrographs were taken.

### *Statistical Analysis and Data Evaluation*

Data obtained from these study were analyzed statistically using Students' t-test and ANOVA (One - way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5%, 1% and 0.1% level of significance i.e  $P \leq 0.05$ , 0.01 and 0.001.

## III. RESULT

### **Effect of treatment with ethanol stem extract of *Homalium letestui* on the blood hematological parameters of rats with thioacetamide-induced hepatotoxicity.**

The administration of thioacetamide (200 mg/kg bw) did not affect RBC, WBC, platelet count and haemoglobin concentration as well as PCV, basophils and lymphocytes percentages when compared to normal control (Table 1). However, there was significant ( $p < 0.01-0.001$ ) increase in the percentage of neutrophils in thioacetamide-treated rats and those of rats pretreated with middle and high doses of the extract (500 and 750 mg/kg). Eosinophils percentage was significantly ( $p < 0.05-0.001$ ) reduced in both thioacetamide and extract treated groups (Table 1).

### **Evaluation of effect of *Homalium letestui* stem on liver function test of Thioacetamide - induced liver injury in rats**

Administration of thioacetamide (200mg/kg bw) to rats caused a significant (p<0.001) elevation of enzymes levels such as AST, ALT , ALP, total cholesterol, total and direct bilirubin and decreases in total protein and albumin levels when compared to control. There was observable significant (p<0.01 - 0.001) non – dose dependent decreases of these enzymes levels and that of total cholesterol, total and direct bilirubin in the groups pre-administered with the stem extract of *H. letestui* (500 – 1000 mg/kg bw) when compared with the thioacetamide group. Total protein and albumin levels were significantly (p<0.05 - 0.001) elevated dose-dependently in the groups pre-treated with the stem extract when compared to the thioacetamide group (Table 2).

**Histopathological studies of rat liver in thioacetamide-induced hepatotoxicity**

Gordon and sweet impregnation technique showed portal triad degeneration and crowding of the reticular fiber in the thioacetamide group. Silymarin group and the rats pretreated with extract revealed slight reticular fiber degeneration with much roughed fiber observed in the rats that received 750

mg/kg bw dose of the extract. There were intact reticular fiber in the normal group (Figure 3-4)).

Using H&E technique, histopathological examination of liver sections in normal control group showed intact hepatic cells, sinusoidal spaces and central vein (group 1). There was disorganization of the texture of hepatic cells with centrilobular necrosis, hyperplasia, vascular and cellular degeneration, polymorphonuclear aggregation, inflammation and fatty degeneration in the thioacetamide -treated rats. The liver sections of the rats treated with stem extract of *H. letestui* (500 - 750 mg/kg bw) of groups 5 and 6 showed signs of protection as was evident by slight areas of vacuolation, cellular degeneration, hepatocytic hyperplasia, cellular proliferation, pyknotic nucleus and slight distortion in the area of reticular fibers, while the extract did not exert any effect at the low dose (group 4). Liver sections of the rats treated with silymarin (100 mg/kg bw) in group 3 showed significant reduction in fatty degeneration and absence of necrosis, inflammation and no reticular fiber distortion observed (Figure 1-2)).

Table 1: Effect of treatment with ethanol stem extract of *Homalium letestui* on the hematological parameters of rats with thioacetamide -induced hepatotoxicity.

Parameters Treatment Dose (mg/kg)	RBC (X 10 <sup>12</sup> /l)	PCV (%)	Hb (g/dl)	WBC (X 10 <sup>9</sup> /l)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Platelets (%)
Normal control	4.95±0.13	46.8±2.60	15.5±0.86	5.62±0.54	41.5±5.99	45.5±6.17	7.00±1.80	4.33±2.02	0.00±0.00	141.6±20.91
THA +Dist. Water	4.08±0.25	47.8±2.13	15.8±0.72	5.16±0.45	48.33±3.28 <sup>c</sup>	42.3±3.63	5.66±1.43 <sup>c</sup>	2.33±0.80 <sup>a</sup>	0.20±0.20	144.5±15.87
Silymarin 100 mg/kg + THA	5.15±0.85	57.8±3.33	17.25±1.10	4.80±0.43	42.1±6.96	48.6±7.00	5.16±2.35 <sup>d</sup>	2.00±0.85 <sup>a</sup>	0.00±0.00	154.0±22.37 <sup>a</sup>
HL. 250mg/kg + THA	5.11±0.28	54.8±1.77	18.8±0.59	4.66±0.48	52.6±8.14 <sup>c</sup>	41.3±3.63	5.00±1.36 <sup>c</sup>	1.00±0.81 <sup>ce</sup>	0.00±0.00	156.5±13.01 <sup>c</sup>
HL. 500mg/kg+ THA	4.80±0.16	49.0±2.12	16.3±0.70	5.85±0.69	44.3±5.30	45.8±4.84	5.0±1.73 <sup>cd</sup>	3.83±0.85 <sup>c</sup>	0.00±0.00	189.3±27.56 <sup>ce</sup>
HL. 750mg/kg+ THA	5.11±0.39	54.3±3.32	18.16±7.68	5.10±0.78	49.1±7.68 <sup>c</sup>	40.8±6.38	5.0±1.65 <sup>c</sup>	4.33±1.05 <sup>a</sup>	0.00±0.00	133.1±16.66 <sup>c</sup>

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control. dp< 0.05, ep< 0.01, fp< 0.001 when compared to paracetamol . n = 6.

Table 3: Effect of *H. letestui* on liver function of thioacetamide –induced liver injury in rats

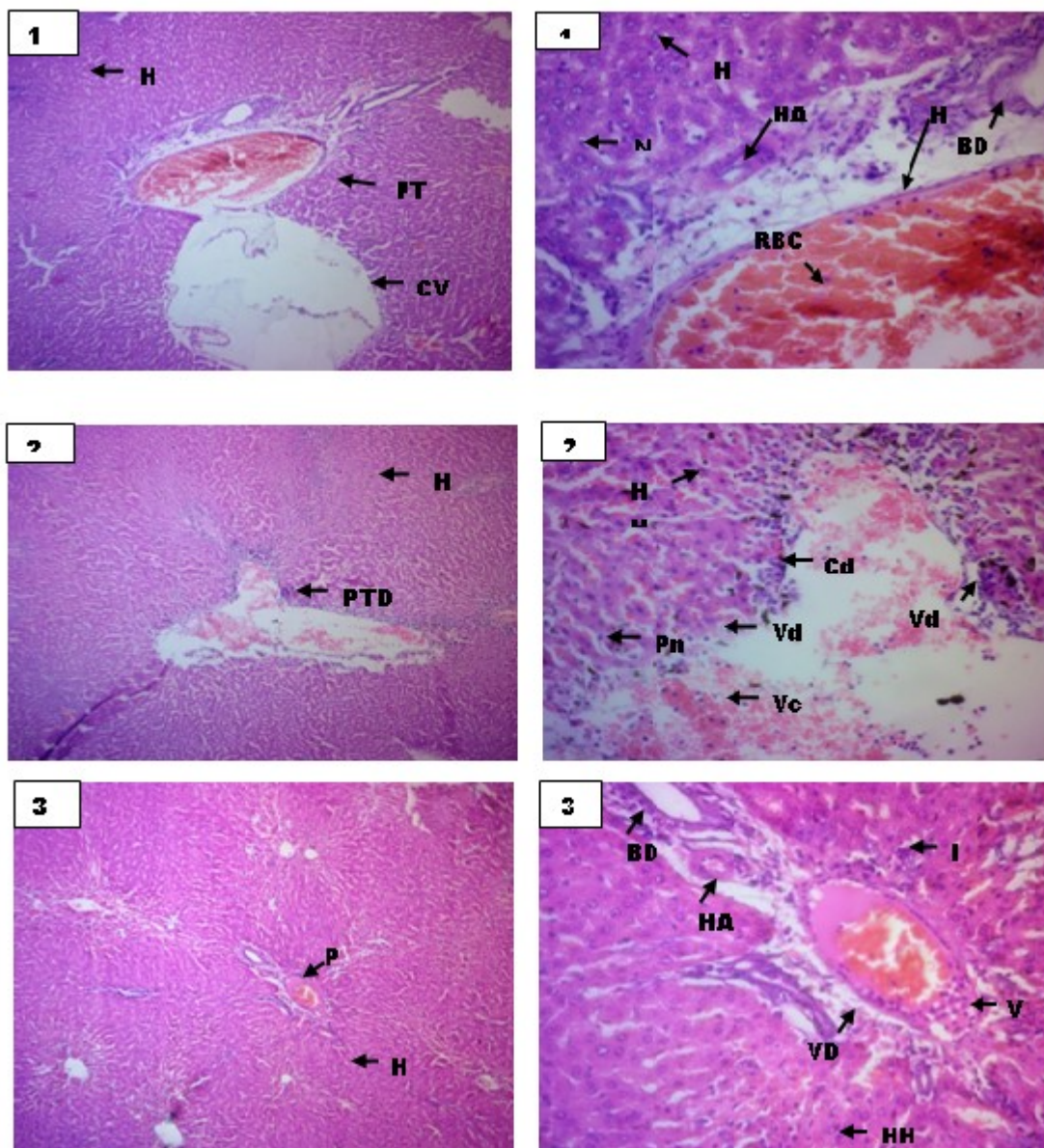
PARAMETERS/ TREATMENT	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	TOTAL BILIRUBIN (mg/dl)	CONJUGATE D BILIRUBIN (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL CHOLESTEROL Mmol/L
Normal control	5.96± 2.08	4.45±0.59	2.98±0.28	1.23±0.13	114.0±3.65	38.66±3.74	178.83±14.35	3.93± 0.29
THA +Dist. Water	2.95±0.58 <sup>c</sup>	2.58±0.22 <sup>c</sup>	7.41±0.25 <sup>c</sup>	2.53±0.13 <sup>c</sup>	170.1±3.25 <sup>c</sup>	86.62±5.20 <sup>c</sup>	281.32±10.37 <sup>c</sup>	7.53±0.45 <sup>c</sup>
Silymarin 100 mg/kg + THA	5.73±0.37 <sup>f</sup>	4.12±0.39 <sup>f</sup>	3.75±0.34 <sup>f</sup>	0.85±0.13 <sup>f</sup>	151.82±4.92 <sup>cd</sup>	61.50±6.11 <sup>bd</sup>	202.8±10.24 <sup>cf</sup>	4.05±0.39 <sup>f</sup>
Ext. 250 mg/kg + THA	6.13±0.84 <sup>f</sup>	4.67±0.20 <sup>f</sup>	5.16±0.39 <sup>af</sup>	1.60±0.05 <sup>d</sup>	163.1±6.22 <sup>c</sup>	80.30±10.32 <sup>c</sup>	224.0±6.19 <sup>cf</sup>	5.67±0.53 <sup>cf</sup>
Ext. 500 mg/kg+ THA	6.36±0.98 <sup>f</sup>	4.11±0.19 <sup>c</sup>	4.56±0.42 <sup>af</sup>	1.00±0.15 <sup>f</sup>	132.16±4.33 <sup>cf</sup>	80.66±6 .25 <sup>c</sup>	214.6±3.49 <sup>cf</sup>	6.63±0.14 <sup>bf</sup>
Ext. 750 mg/kg+ THA	6.25±1.05 <sup>f</sup>	4.15±0.33 <sup>f</sup>	4.35±0.25 <sup>f</sup>	0.86±0.06 <sup>b</sup>	150.5±4.35 <sup>ad</sup>	84.50±9.80 <sup>c</sup>	239.5±7.32 <sup>cc</sup>	6.46±0.11 <sup>f</sup>

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control.dp< 0.05, ep< 0.01, f< 0.001 when compared to paracetamol . n = 6

Table 3: Effect of *H. letestui* on weight of liver in thioacetamide –induced liver injury in rats

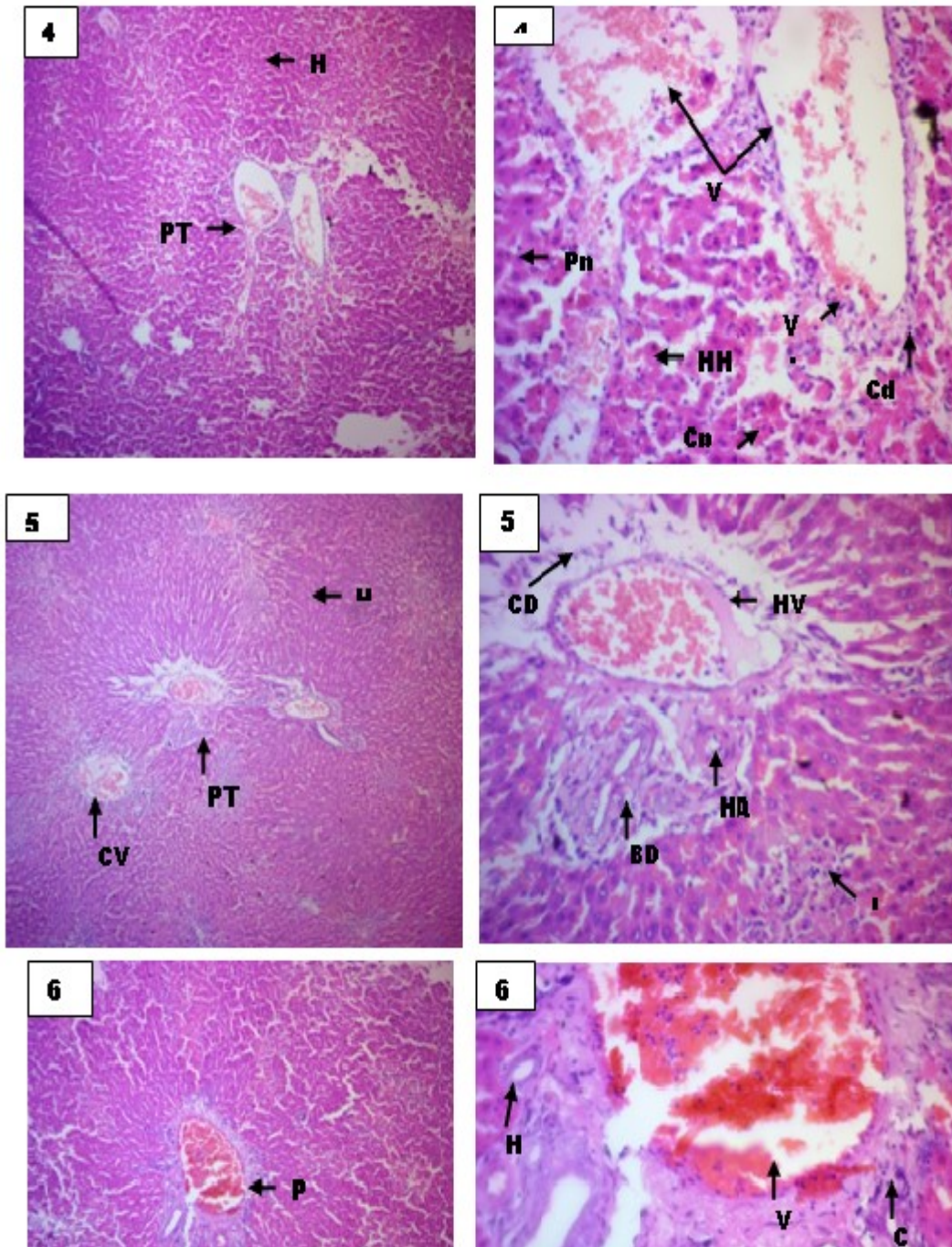
PARAMETERS/ TREATMENT	LIVER WEIGHT (g)
Normal control	6.53±0.23
THA +Dist. Water	8.46± 0.16 <sup>c</sup>
Silymarin 100 mg/kg + THA	6.56±0.12 <sup>f</sup>
Ext. 250 mg/kg + THA	7.63±0.20 <sup>bc</sup>
Ext. 500 mg/kg+ THA	6.76±0.14 <sup>f</sup>
Ext. 750 mg/kg+ THA	6.62±0.15 <sup>f</sup>

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control.dp< 0.05, ep< 0.01, f< 0.001 when compared to paracetamol . n =6



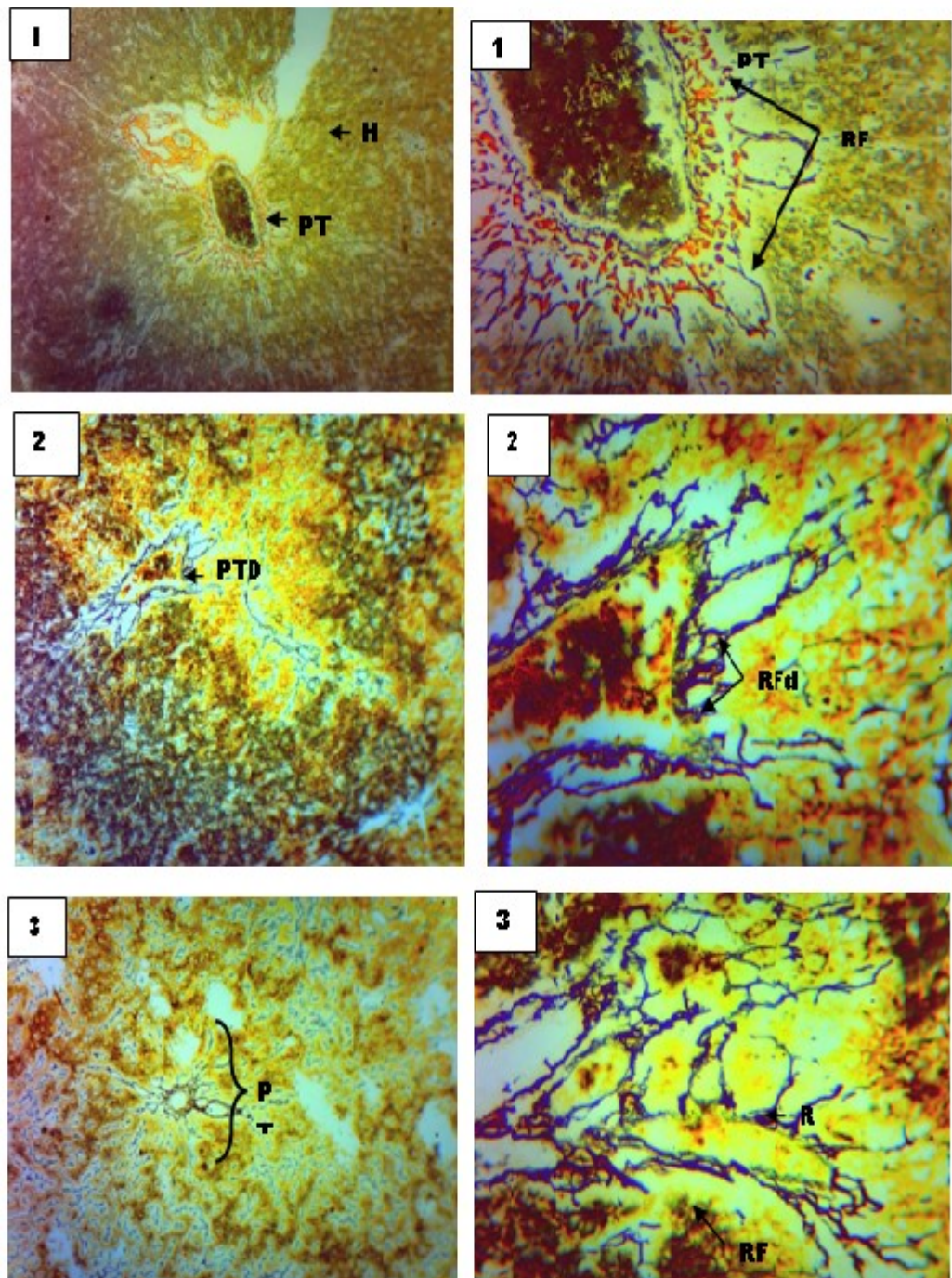
**Figure 1:** Histological sections of Livers of rats treated with Normal saline 10 ml/kg bw (1),Thioacetamide 200 mg/kg bw (2) and Silymarin100 mg/kg bw and Thioacetamide 200 mg/kg bw(3) at magnification A (x100) and B(x400) using H&E technique.

Keys: Bile duct (BD) Cellular degeneration (Cd), Portal triad (PT), Inflammation (I), Portal triad degeneration (PTD), Cellular Degeneration, Vascular congestion (Vc), Hepatic artery (HA), Vascular degeneration (Vd), Hepatocytic hyperplasia (HH), Hepatic artery (HA), Pyknotic nucleus (Pn)



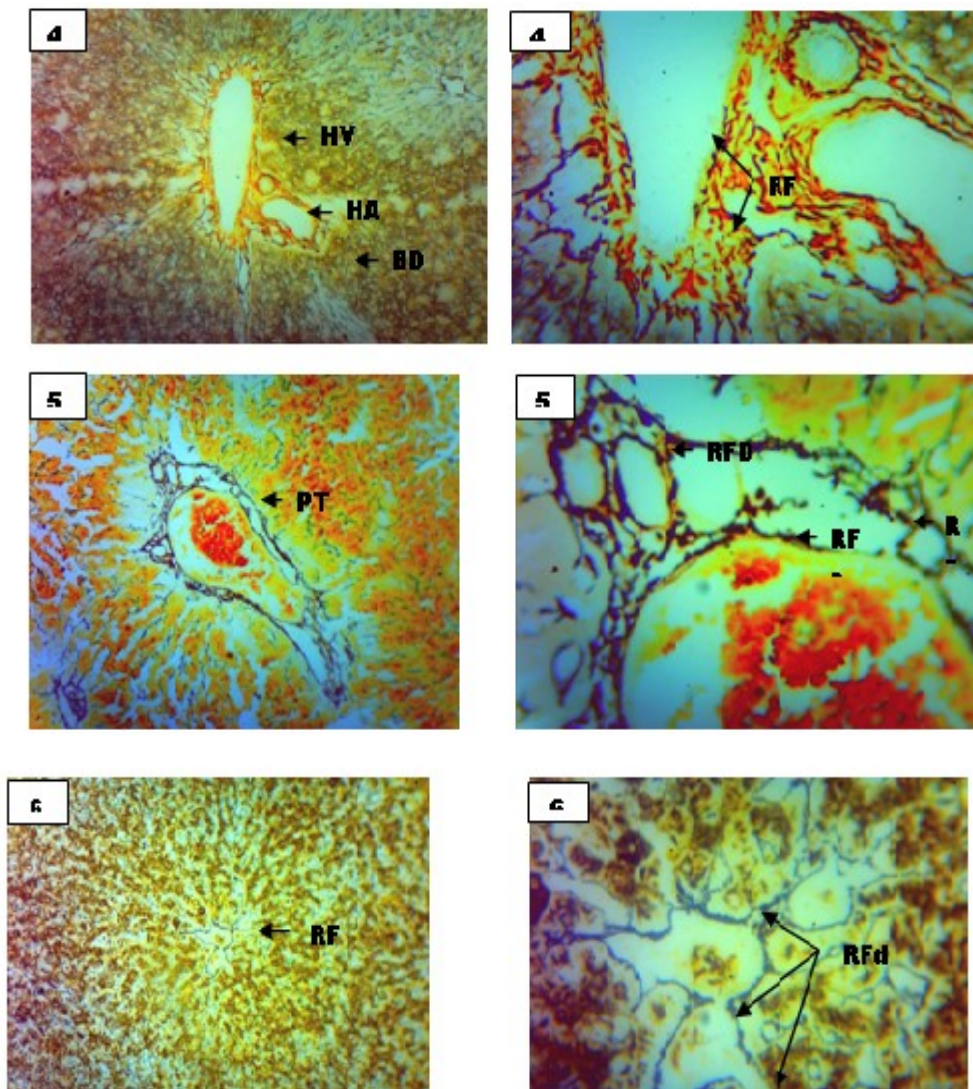
**Figure 2:** Histological sections of Livers of rats treated with *Homalium letestui*250 mg/kg bw (4) and Thioacetamide,*Homalium letestui*500 mg/kg bw and Thioacetamide 200 mg/kg bw (5) and *Homalium letestui*750 mg/kg bw and Thioacetamide 200 mg/kgbw (6) at magnification A (x100) and B(x400) stained with H&E technique.

Keys: Bile duct (BD) Cellular degeneration (Cd), Inflammation (I), vascular degeneration (Vd), Hepatocytic hyperplasia (H), Hepatic artery (HA), Pyknotic nucleus (Pn), Portal triad (PT) and Vascular congestion (Vc)



**Figure 3:** Histological sections of Livers of rats treated with Normal saline 10 ml/kg (1), Thioacetamide 200 mg/kg bw (2) and Silymarin 100 mg/kgbw and Thioacetamide 200 mg/kg bw (3) at magnification A (x100) and B(x400) stained with Gordon and Sweet silver impregnation Technique

**Keys:** Poetal triad (PT), Portal triad degeneration (PTD), Hepatocytes (H), Reticular fiber (RF) and Degenerated Reticular fiber (RFd)



**Plate XIII:** Histological sections of Livers of rats treated with *Homalium letestui* 250 mg/kg bw and Thioacetamide (4), *Homalium letestui* 500 mg/kg bw and Thioacetamide 200 mg/kg bw (5) and *Homalium letestui* 750 mg/kg bw and Thioacetamide 200 mg/kg bw (6) at magnification A (x100) and B(x400) stained with Gordon and Sweet silver impregnation Technique

**Keys:** Bile duct (BD), Poetal triad (PT), Hepatocytes (H), Hepatic artery, Reticular fiber (RF) and Degenerated Reticular fiber (RFd)

#### IV. DISCUSSION

The liver is an important organ responsible for the metabolism, bile secretion, elimination of many substances, blood detoxifications, synthesizes, and regulation of essential hormones. Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality<sup>15</sup>. The principal causative factors for the liver diseases in developed countries are excessive alcohol consumption, and viral-induced chronic liver diseases while in the developing countries the most frequent causes are environmental toxins, parasitic disease, hepatitis B and C viruses, and hepatotoxic

drugs (certain antibiotics, chemotherapeutic agents, high doses of paracetamol, carbon tetrachloride (CCL<sub>4</sub>), thioacetamide (TAA), etc. A number of herbals show promising activity, including Silymarin for liver cirrhosis, *Phyllanthus amarus* in chronic hepatitis B, glycyrrhizin to treat chronic viral hepatitis, and some herbal combinations from China and Japan that have been scientifically proven for treatment of liver diseases<sup>16</sup>. Silymarin, a flavonolignan from “milk thistle” *Silybum marianum*, is widely used for hepatoprotection. Silymarin offers good protection in different toxic models of induced liver cirrhosis experiments by using laboratory animals<sup>17</sup>.

Thioacetamide (TAA) is a hepatotoxicant agent that is converted by the liver enzymes to highly reactive toxic S-oxide derivatives which cause centrilobular necrosis. These metabolites interfere with the movement of RNA from the nucleus to the cytoplasm which leads to membrane injury, therefore they reduce the number of viable hepatocytes as well as rate of oxygen consumption<sup>18</sup>. Moreover, TAA decreases the volume of bile and its content (bile salts, cholic acid and deoxycholic acid). Rodents intoxicated with TAA have been demonstrated as good experimental model of liver cirrhosis and fibrosis, and have been used for evaluation of antihepatotoxic drugs<sup>19</sup>. It is well known that the mechanism of liver toxicity by TAA involved free radical chain reactions.

In this study, the haematological effect of the ethanol extract of the *Homalium letestui* stem extract on thioacetamide-induced toxicity in rat was investigated. The results demonstrated that thioacetamide exposure was associated with slight increase in Hb, PCV, neutrophils and slight decrease in lymphocytes, monocytes and RBC. The result agrees with Muddasir *et al.*<sup>20</sup> that acute TAA administration caused neutrophilia, thrombocytosis as well as increased hemoglobin concentration and decline of erythrocytic count. The depression in RBCs count and rise in Hb contents and PCV recorded in the present work is suggestive of a megaloblastic RBCs and could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation. According to Travlos *et al.*<sup>21</sup>, consistent erythrocyte damage is presumed to be related to direct oxidative injury to the red cells by the chemicals or to the pitting function of the spleen. Leucopenia is a clinical manifestation with decrease in circulating white blood cell (WBC). From the present study, the pretreated group had improved level of lymphocytes and monocytes, while there were improvements in some of the haematological parameters. Neutrophils and its derived cytokines play a crucial role in the development and manifestation of inflammation. The stimulation of neutrophils can lead to the production of oxygen-derived free radicals also called reactive oxygen species (ROS) that cause further cellular damage<sup>22</sup>. The formation of free radicals and cytotoxic oxygen metabolites probably impart a key role in various types of tissue degeneration and pathology such as aging, cancer and retinal degeneration<sup>23</sup>. In the present study, after intraperitoneal injection of TAA, there was significant elevation in the neutrophil count (neutrophilia) when compared to control which may be due to the free radicals resulting from TAA metabolism which caused liver injury and a proportion of these free radicals liberated into the blood may also affect the circulating cells and induce a significant change in their number. This significant neutrophilia might reflect its involvement in inflammation by forming various reactive oxygen species (ROS), inflammatory, metabolic and myeloproliferative disorders, tissue necrosis, acute hemorrhagia, malignant tumors or due to rapid release of young cells from the bone marrow<sup>24</sup>. Persistent increase in the

neutrophil level of all extract groups was observed in this work which may suggest that the extract could not effectively reverse the effect of elevation in neutrophil count induced by thioacetamide administration. It could also be that increase in neutrophils in pretreated groups may be a potentiation in immunological response to the toxicant<sup>25</sup>.

A marked reduction of serum total protein and albumin levels were observed in the thioacetamide-treated group when compared with the normal healthy animals. *Homalium letestui*-treatment caused significant improvements in the levels of these proteins in the pretreated group. Parallel findings of the effect thioacetamide on serum total protein and albumin levels in rat were also previously reported<sup>26</sup>. Also, the levels of plasma total protein in all the extract-treated rats were above that of Silymarin-treated rats, indicating that the plant can be of immense clinical importance in the management of liver diseases. Hepatic factors (ALP, ALT, and AST) were significantly increased in the thioacetamide administered rats, which agrees with previously described work<sup>27</sup>. Although, ethanol stem extract of *Homalium letestui* showed significant prospect in the liver function test it did not reduce the elevated liver enzymes levels to normal as in the normal control in the doses administered. Hematological and liver function parameter results were supported by the histopathological analysis of the organotoxic group. The extract showed slight cellular degeneration, inflammation, vascular congestion, pyknotic nucleus, slightly distorted basement membrane and hepatocyte regeneration when compared to the thioacetamide group that revealed complete degeneration of cellular features in the H&E staining technique. Gordon and Sweet Silver impregnation technique showed portal triad degeneration and crowding of the reticular fiber in the thioacetamide group. Silymarin and pretreated group revealed slight reticular fiber degeneration with much roughed reticular fiber observed in the rats that received 750 mg/kg dose of the extract. These biphasic results suggest that the plant may have a partial agonistic effect due to the phytochemical component of the plant extract. This shows that the plant extract is able to inhibit the free radical chain reaction of S-oxide derivatives of thioacetamide by preventing and reducing necrosis, interference in movement of RNA from the nucleus and decrease in volume of bile and its contents through the activities of its antioxidant constituents. This activity may have resulted from the presence of some polyphenolic compounds in the extract such as vanillin, 2-Coumaranone, 3, 4, 5-trimethoxy phenol and 4-phenyl isocoumarin, and 4-(3-hydroxy-1-propenyl)-2-methoxy phenol and  $\alpha$ -Terpineol which have been known to have antioxidant effect<sup>28</sup>. Other members of the family such as *Homalium brachybotrys*, *Flacourtia indica*, *Casearia grayi*, and *Scolopia braunii* have been reported to possess antioxidant activities due to presence of polyphenolic compounds that were also found in the extract<sup>29</sup>.



## V. CONCLUSION

The result of the investigation suggests that caution should be taken while consuming extract of the plant for certain antidote because at a particular dose it may be hepatoprotective and at another dose the effect may be reversed.

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