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Effect of Aqueous Whole Plant Extract of *Selaginella Myosurus* on Liver Markers and Haematological Indices of Albino Rats

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Abstract
So much has been done in screening herbal medicines for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far outnumber those of toxicity. This study was designed to investigate the effect of aqueous whole plant extract of *selaginella myosurus* on liver markers and haematological indices of albino rats. A total of thirty six (36) albino rats of both sexes weighing between 100.5g-149.5g were divided into nine groups of four rats per group. Group 1 received distilled water, while groups 2-5 and 6-9 received 400,600,800, and 1000mg/kgBW of extract for 7 and 14 days respectively. Rats were sacrificed 24hours after the last treatment and blood samples collected for biochemical and haematological investigation. Histology of the liver was conducted and photochemical screening of plant was done. Result revealed non-significant differences ($p>0.05$) in albumin, total protein and bilirubin levels as well as the activities of aspartate and alanine transaminases for all the extract treated groups when compared to control values. Significant reduction ($p>0.05$) was observed in the activities of alkaline phosphatase in all the extract treated groups except group 2 when compared to control. All the haematological parameters showed non-significant differences except platelet which significantly increased ($p<0.05$) in all the treated groups and neutrophils and lymphocytes in groups 2-4 only when compared to control values. Eosinophils, monocytes and basophils were not detected. Weight of liver significantly decreased ($p<0.05$) in groups 2-5 and non-significantly increased ($p>0.05$) in groups 6-9 in comparism with control. Quantitative phytochemical screening of plant revealed the presence of flavonoids, triterpenoids, saponins, tannin, steroid, cardiac glycoside, phenol in decreasing order (32.19 ± 0.23-13.10 ± 0.11) and absence of alkaloids, anthraquinone and cyanogenic glycosides. Histological observation for 1000 mg/kgbw treated groups for 7 and 14 days (groups 5 and 9 showed hepatocytic necrosis while groups 2-4 and 6-8 showed normal liver architecture. In conclusion, aqueous whole plant extract of *selaginella myosurus* may induce liver toxicity at doses $\geq$ 1000 mg/kgbw, boost platelet synthesis and immune response.

1. Introduction

Herbal medicines as preparations derived from naturally occurring plants with medicinal or preventive properties are a major component in all indigenous peoples' traditional medicine, including Ayurvedic, homeopathic, naturopathic, traditional oriental, Native American and African medicine [1]. Aside from the discovery of numerous orthodox drugs from the study of traditional cures and folk knowledge, the

efficacy of a number of botanicals has been proven scientifically. Herbal remedies are generally regarded as safe and are promoted to the public as being "natural" and completely "safe" [2], owing to long history of use [3]. The surge in popularity and patronage of herbal medicines necessitate concern based on adverse effects of potentially toxic constituents in plants (e.g. aristolochic acids, pyrrolizidine alkaloids, benzophenanthrine alkaloids, lectins, viscotoxins, saponins, diterpenes, cyanogenetic glycosides and furonocoumarins) which can be fatal [4] (Fennell *et al.,* 2004). Pharmacological and toxicological evaluations of medicinal plants are essential for drug development [5-7]. So much has been done in screening herbal medicines for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far outnumber those of toxicity. In view of the foregoing, this study was designed to investigate the toxicity of *selaginella myosurus* on the liver of albino rats in order to extrapolate the possible toxicological effect in human. The liver is the most complex and vital organ of vertebrates and some other animals. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemical necessary for digestion. It plays a major role in metabolism, including regulation of glycogen storage, decomposition of red blood cells and hormone production. It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction. Chemicals that cause liver injury are called hepatotoxins. Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories (e.g. ccl_4, paracetamol) and industries (e.g. lead, arsenic), natural chemicals (e.g. microcystins, aflatoxins) and herbal remedies (*Cascara sagrada*, ephedra) can also induce hepatotoxicity [8]. These agents are converted in chemically reactive metabolites in liver, which have the ability to interconnect with cellular macromolecules such as protein, lipids and nucleic acids, le ading to protein dysfunction, lipid per oxidation, DNA damage and oxidative stress. This damage of cellular function can dismiss in cell death and likely liver failure [8]. The toxicity of herbs may also result from the generation of reactive intermediates through metabolic activation of herbal constituents via phases I and II reactions within the human body. The resultant reactive intermediates can bind covalently to DNA and proteins, leading to organ toxicity, mutagenicity, and even carcinogenicity. These hepatotoxic agents activated some enzymes activity in the cytochrome p-450 system such as CYP2E1 also leads to oxidative stress. *In vitro* studies have also indicated the role of herbal reactive intermediates in irreversibly inhibiting various cytochrome enzymes (CYPs). However, the discrepancy of effects between *in vitro*, animal, and human studies reflects the significance of herbal dosing in the modulation of CYPs [9].

Selaginella myosurus is a medicinal plant that has not been widely used, either traditional or modern medicine [10]. Small amounts of the species are also used as ornamental plants and vegetables. All species of *Nusantara* have small leaves resembling scales, with two different sizes: the smaller median leaves in the inner row and the larger lateral leave in the outer rows [10, 11]. The family *Selaginellaceae Reichb has only one genus, Selaginella Pal. Beauv, consisting of 700- 750 species and* widespread in a cosmopolitan way [12, 10]. *Selaginella* can be found in the pharmacopoeia in Asia, Africa and Latin America, but not found in Europe and North America [13]. *Selaginella is* traditionally used to treat several diseases such as: injury, treatment of post-childbirth, cancer, skin diseases, headaches, fever, respiratory infections, urinary tract infections, menstrual disorders, liver disorders, fractures and arthritis. The parts used are all parts of the plants. From field studies in Indonesia, it is known that *Selaginella* is use to treat wounds, and for treatments before, during, and after giving birth, and to improve fitness and endurance of the body [14]. The result of the field study also shows that the herb is commonly used to treat wounds and bleeding, either external wounds or internal injuries such as menstrual disorders and postpartum haemorrhage, and also used as a tonic to improve fitness and stamina.

Selaginella contains a variety of secondary metabolites such as alkaloids, phenol (flavonoids, tannins, saponins), and terpenoids (triterpene, steroid) [15]. The main secondary metabolite of this plant is biflavonoid, whose type is various depending on the species. Biflavonoid that has been identified from *Selaginella*, among others amentoflavone, 2,8-biapigenin, delicaflavone, ginkgetin, heveaflavone, hinokiflavone, isocryptomerin, kayaflavone, ochnaflavone, podocarpusflavone A, robustaflavone, sumaflavone, and taiwaniaflavone. These compounds act as antioxidants, anti-inflammatory, anti-cancer, anti-allergic, antimicrobial, antifungal, antibacterial, antiviral, protective against ultra violet (UV) irradiation, vasorelaxant, heart boosters, antihypertensive, anti-clotting, and affect the metabolism enzymes [14]. Biflavonoid is a typical of secondary metabolites which is found only in *Selaginellales*, *Psilotales*, gymnosperms, and several species of Bryophytes and Angiosperms [16].

2. Materials and Methods

2.1. Apparatus/Equipment

Spectrophotometer (BSA 3000), SFRI France, Rotary evaporator, Centrifuge (Universal laboratory century), Hettich Zentrifugen, Metlar weighing balance, SIEMENS Advia 2120 Automated Analyzer

2.2. Reagents/Chemicals

All reagents and chemicals are of analytical grade.

2.3. Collection/Identification of Plant

Selaginella myosurus was collected in the surrounding bush of the University of Port Harcourt in Choba community of Obio/Akpor Local Government Area of Rivers state. A voucher specimen (UPH-NO. C-129) was authenticated by a botanist, Dr. N. L. Edwin-Wosu and deposited at the herbarium unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt.

2.4. Extract Preparation

The whole plant of *Selaginella myosurus* was washed with running tap water and air dried for 2 weeks before grinding into powdered form. The coarsely powdered plant material was macerated in a maceration jar for 24hours, with distilled water. Filtration was done using a glass funnel which was placed in a retort stand, using a Whatman filter paper. The filtrate was allowed for about 1-2 hours to observe any residue or sediment. After having a clear filtrate, it was then put in a rotary evaporator which separated the water from the extract, leaving the extract in a paste form. The extract was then poured into a crucible plate for drying on a steam bath at 40°C to 50°C. The crude extract was stored in a refrigerator pending usage.

2.5. Phytochemical Screening

Phytochemical screening of the whole plant of *Selaginella myosurus* was done using standard procedure as described by [17] Sofoware (1993) in the Department of Pharmacognosy, Faculty of Pharmacy, University of Port Harcourt.

2.6. Source of Animals

A total of thirty six (36) albino rats of both sexes weighing between 100.5g-149.5g were purchased from an animal breeding facility in Choba community, and were kept in the Department of Biochemistry, University of Port Harcourt Animal House, Choba park for one week acclimatization. The rats were fed with normal rat feed and water *ad libitum*.

2.7. Lethal Dose (LD_{50}) Determination

LD_{50} was done using an "up-and-down" procedure described by [18] Bruce, (1985). Three dose-groups of 1000mg/kg, 3000mg/kg, and 5000mg/kg were orally administered to 3 groups of rats (n=2 rats per group). The rats were observed for 24hours and for a period of 1 week. No death was recorded; therefore, safe doses of 400, 600, 800 and 1000mg/kgBW were selected.

2.8. Experimental Design

The rats were divided into ten (9) groups (n=4rats).

GROUP 1 (Control): 0.5 ml of distilled water was orally given to the animals in this group daily for 14 days.

GROUP 2 (400mg/kg b.w extract): A single daily dose of 400mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

GROUP 3 (600mg/kg b.w extract): A single daily dose of 600mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

GROUP 4 (800mg/kg b.w extract): A single daily dose of 800mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

GROUP 5 (1000mg/kg b.w extract): A single daily dose of 1000mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

GROUP 6 (400mg/kg b.w extract): A single daily dose of 400mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 14 days.

GROUP 7 (600mg/kg b.w extract): A single daily dose of 600mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

GROUP 8 (800mg/kg b.w extract): A single daily dose of 800mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

GROUP 9 (1000mg/kg b.w extract): A single daily dose of 1000mg/kg b.w of aqueous whole plant extract of *Selaginella myosurus* was orally administered to rats in this group for 7 days.

Sacrifice, collection and preparation of plasma

At the end of 7 and 14 days, all the animals were anaesthetized with chloroform before decapitated for collection of blood. The blood was stored in heparinised sample bottle, spun at 5000rpm using MSE centrifuge to obtain plasma for biochemical investigations, while that for haematology investigation was collected in EDTA sample bottles.

3. Biochemical Investigation

Plasma samples were tested for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by the method of [19], plasma alkaline phosphatase activity by the method of [20], and plasma total protein and albumin assessed by Biuret and BCG methods respectively. Bilirubin determination was done by Jendrassik and Crod method. Haemoglobin was determined by haemiglobinecyanide technique, Packed Cell Volume (PCV) by micro-haematocrit method and Leukocytes, Platelets White Blood Cells (WBC) and Red Blood Cells (RBC) Counts were done manually.

3.1. Histopathogical Studies

The rats were dissected using a set of dissection kit and livers of the control and treated groups were collected and fixed in 10% freshly prepared formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin

and eosin [21].

3.2. Statistical Analysis

All the values were reported as mean ± standard error of mean (M ± SEM). Statistical analysis was performed using SPSS version 20.0 (IBM, U.S.A). The data were analyzed using one-way analysis of variance (ANOVA) and significant difference were determined using post Hoc Turkey's test for multiple comparisons at $p < 0.05$.

4. Result

Table 1. *Effect of aqueous whole plant extract of selaginella myosurus on liver markers in albino rats.*

TREATMENT GROUPS	PARAMETERS					
	TP (g/l)	ALB (g/l)	TBIL (Umol/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 1 Water control	69.25± 1.11[a]	41.50±0.65	10.00±0.41	35.25±1.25	39.50±1.04	247.00±3.70[a]
Group2 400mg/kg BW EXT FOR 7 DAYS	63.50±0.65[a]	42.75±0.48	10.00±0.41	33.50±0.96	38.25±0.85	241.00±1.29
Group 3 600mg/kg BW EXT FOR 7 DAYS	68.00±0.91	43.25±0.48	9.25±0.48	33.50±0.87	40.00±0.91	223.25±2.69[a]
Group 4 800mg/kg BW EXT FOR 7 DAYS	69.00±0.61	43.75±0.48	9.25±0.63	34.25±0.63	40.25±1.38	219.25±1.89[a]
Group5 1000mg/kg BW EXT FOR 7 DAYS	71.25±0.41	44.00±0.41	9.25±0.49	34.50±0.65	40.00±0.91	216.25±2.29[a]
Group 6 400mg/kg BW EXT FOR 14 DAYS	66.75±1.31	42.50±1.04	9.50±0.65	34.50±0.65	37.50±0.65	233.23±1.38[a]
Group7 600mg/kg BW EXT FOR 14 DAYS	68.00±0.91	43.25±0.85	10.000.71	35.25±0.75	38.25±0.85	222.50±1.76[a]
Group 8 800mg/kg BW EXT FOR 14 DAYS	69.25±0.49	43.50±0.96	10.00±0.91	34.75±0.85	37.75±0.48	221.25±1.11[a]
Group9 1000mg/kg BW EXT FOR 14 DAYS	70.00±0.41	42.50±0.65	10.00±0.41	34.00±0.82	38.75±0.63	221.25±1.31[a]

Data are represented in Mean±Standard Error of Mean (M±SEM)
Similar superscripts represent significant different ($p<0.05$) in the same rows

Table 2. *Effect of aqueous whole plant extract of selaginella myosurus on haematological indices in albino rats.*

TREATMENT GROUPS ((M±SEM)	PARAMETERS									
	Hb (g/dl)	PCV (%)	RBC ($X10^{12}$/L)	WBC ($X10^{9}$/L)	PLATELET ($X10^{9}$/L)	NEUTRO PHIL	LYMPHO CYTE	E	M	B
Group 1 Water control	11.35±0.30	34.00±0.91	4.72±0.16	7.50±2.32	185.00±5.00[a]	25.50±0.50[a]	74.50±0.50[a]	-	-	-
Group 2 400mg/kg BW EXT FOR 7 DAYS	10.93±0.45	32.75±1.31	4.18±0.36	6.53±0.56	247.50±16.52[a]	37.50±1.44[a]	62.50±1.44[a]	-	-	-
Group 3 600mg/kg BW EXT FOR 7 DAYS	11.65±0.20	35.00±0.58	5.10±0.06	10.13±2.24	275.00±14.43[a]	38.00±1.78[a]	62.00±1.78[a]	-	-	-
Group 4 800mg/kg BW EXT FOR 7 DAYS	10.18±0.31	30.75±1.03	4.10±0.41	6.63±0.24	320.00±14.71[a]	38.00±2.71[a]	62.00±2.71[a]	-	-	-
Group 5 1000mg/kg BW EXT FOR 7 DAYS	10.50±0.39	31.50±1.19	4.03±0.30	9.83±0.10	275.00±2.89[a]	32.25±1.03	67.75±1.03	-	-	-
Group 6 400mg/kg BW EXT FOR 14 DAYS	10.93±0.54	32.75±1.60	4.28±0.42	6.25±0.48	270.00±17.32[a]	29.00±0.58	71.00±0.58	-	-	-
Group 7 600mg/kg BW EXT FOR 14 DAYS	12.90±0.36	38.75±1.03	5.60±0.23	8.50±0.29	265.00±8.66[a]	27.50±1.44	72.50±1.44	-	-	-
Group 8 800mg/kg BW EXT FOR 14 DAYS	11.50±0.12	34.50±0.29	4.90±0.06	5.25±0.43	260.00±5.78[a]	24.00±2.31	76.00±2.31	-	-	-
Group 9 1000mg/kg BW EXT FOR 14 DAYS	11.93±0.14	35.75±0.49	5.20±0.00	4.10±0.08	220.00±0.00	28.50±0.87	71.50±0.87	-	-	-

Data are represented in Mean±Standard Error of Mean (M±SEM)
Similar superscripts represent significant different ($p<0.05$) in the same row

Table 3. *The effect of aqueous whole plant extract of saleginella myosurus on weight of liver of albino rats.*

TREATMENT GROUPS	ORGAN WEIGTHS
	LIVER (g)
Group 1 Water control	5.60±0.37[a]
Group 2 400mg/kg BW EXT FOR 7 DAYS	4.93±0.12
Group 3 600mg/kg BW EXT FOR 7 DAYS	5.55±0.24
Group 4 800mg/kg BW EXT FOR 7 DAYS	3.86±0.13[a]
Group 5 1000mg/kg BW EXT FOR 7 DAYS	3.78±0.25[a]
Group 6 400mg/kg BW EXT FOR 14 DAYS	8.15±0.43[a]
Group 7 600mg/kg BW EXT FOR 14 DAYS	5.99±0.20
Group 8 800mg/kg BW EXT FOR 14 DAYS	5.81±0.06
Group 9 1000mg/kg BW EXT FOR 14 DAYS	6.32±0.00

Data are represented in Mean±Standard Error of Mean (M±SEM)
Similar superscripts represent significant different ($p<0.05$) in the same row

Table 4. *Qualitative phytochemical screening of whole plant extract of Selaginella myosurus.*

SECONDARY METABOLITES	TEST	RESULT
Alkaloids	Drangedorff	-ve
	Mayer	-ve
	Hager	-ve
Flavonoids	Shinoda	-ve
	Lead acetate	-ve
	Alkali	+ve
Tannins	$FeCl_3$	+ve
	Phlobatannins	+ve
	Gelatin	ND
	Albumin	ND
Anthraquinone	Free Anthraquinone	-ve
	Combined Anthraquinone	-ve
Triterpenoid/steroids	Liebermann-Buchard	+ve
	Salwoski	+ve
Fixed oil		+ve
Carbohydrates	Molisch	+ve
	Fehlings	+ve
Cardenolide	Keller Killani	+ve
	Kedde	+ve
Cyanogenic glycosides	Frothing	-ve
Saponins	Frothing	+ve
	Haemolysis	-ve
	Emulsion	+ve

Note: +ve = present
-ve = absent
ND = Not Detected

Table 5. *Quantitative phytochemical screening of whole plant extract of Selaginella myosorus.*

Secondary metabolite	Mean ± standard error of mean (m + SEM) (mg/100g)
Flavonoid	32.19± 0.23
Saponins	23.74± 0.20
Cardiac glycoside	15.28± 0.23
Steroid	16.53± 0.12
Terpenoid	26.24± 0.12
Tannin	18.74± 0.17
Phenol	13.10± 0.11

Histological examination of liver sections of rats treated with distilled water and varying doses of aqueous whole plant extract of *selagenella myosurus* for 7 and 14 days.

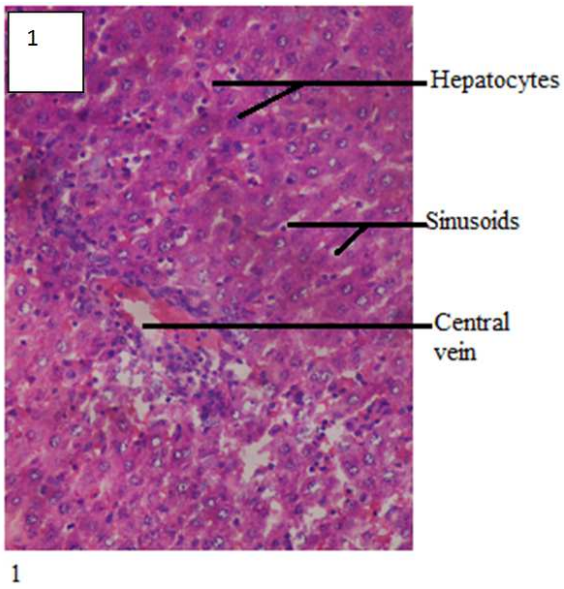


Fig. 1. *Liver section of control rat giving distilled water rat (Control) showing normal hepatocyte and sinusoid (H&E, x 400).*

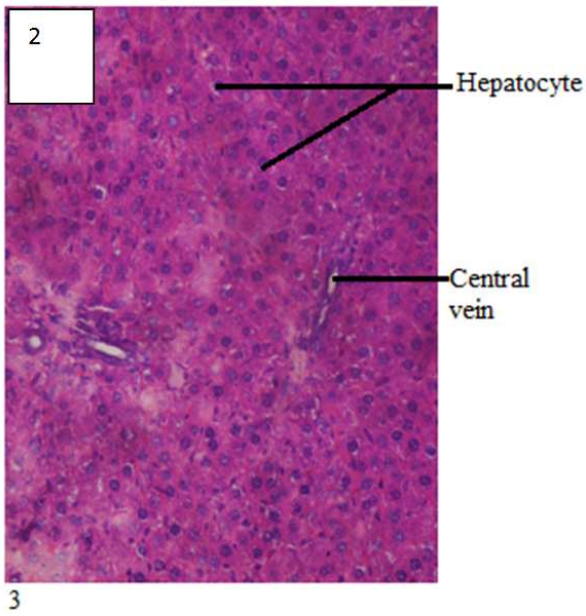


Fig. 2. *Liver section of rat treated with 600mg/kgbw aqueous whole plant extract of selagenella myosurus for 7days showing normal hepatocyte sinusoid and central vein (H&E, x 400).*

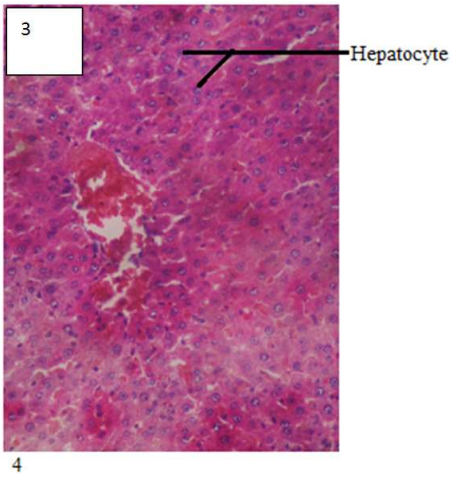
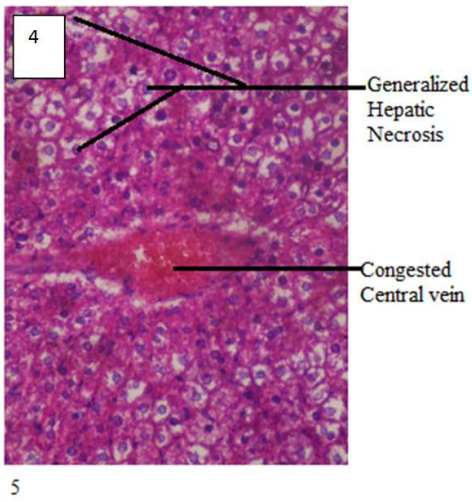
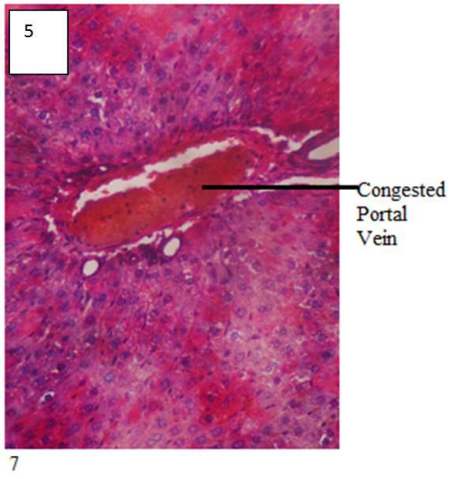


Fig. 3. *Liver section of rat treated with 800mg/kgbw aqueous whole plant extract of selagenella myosurus for 7days showing Normal hepatocyte (H&E, x 400).*



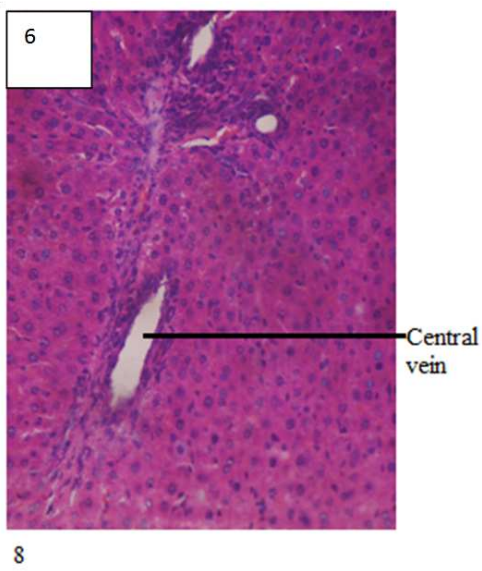
Generalized hepatocytic necrosis, congested central vein (H&E, x 400)

Fig. 4. *Liver section of rat treated with 1000mg/kgbw aqueous whole plant extract of selagenella myosurus for 7days showing.*



Showing normal hepatocyte congested portal vein (H&E, x 400)

Fig. 5. *Liver section of rat treated with 600mg/kgbw aqueous whole plant extract of selagenella myosurus for 14days.*



Showing normal hepatocyte and central vein (H&E, x 400)

Fig. 6. *Liver section of rat treated with 800mg/kgbw aqueous whole plant extract of selagenella myosurus for 14days.*

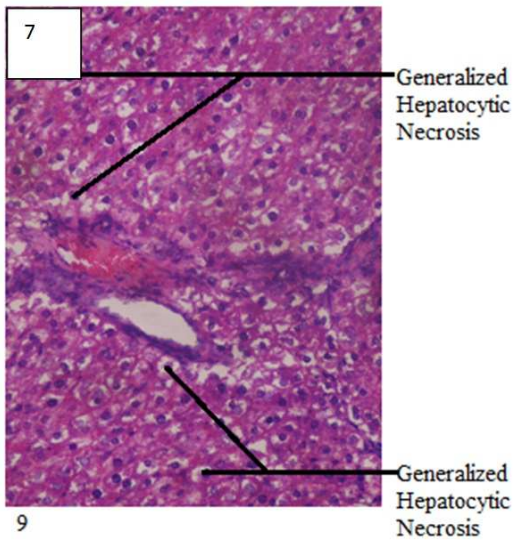


Fig. 7. *Liver section of rat treated with 1000mg/kgbw aqueous whole plant extract of selagenella myosurus for 14days showing generalized hepatocytic necrosis, (H&E, x 400).*

5. Discussion and Conclusion

Result in Table 1 revealed non-significant differences ($p>0.05$) in albumin, total protein and bilirubin levels as well as the activities of aspartate and alanine transaminases for all the extract treated groups when compared to control values. Significant reduction ($p>0.05$) was observed in the activities of alkaline phosphatase in all the extract treated groups except group 2 when compared to control.

Liver function tests are used to determine the presence or absence of liver disease, to make specific diagnoses, to determine severity, and to monitor the course of disease [22]. Some liver function tests (eg, bilirubin or albumin) measure identifiable physiologic functions. Most tests, however,

indicate injury to the liver (eg, aminotransferases and alkaline phosphatase [ALP]) or the reaction to that injury (eg, globulins or tissue antibodies) [23].

The liver is the major source of most the serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the a and b globulins [24]. Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function. Because the half life of albumin in serum is as long as 20 days, the serum albumin level is not a reliable indicator of hepatic protein synthesis in acute liver disease. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug related hepatotoxicity and obstructive jaundice. [25].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most commonly used indicators of cell necrosis. They are present in high concentration in liver cells where they catalyze the transfer of alanine and aspartate a-amino groups to the a-keto groups of ketoglutaric acid to produce pyruvic and oxaloacetic acids. Injury to liver cell membranes causes leakage of aminotransferases into the circulation [26-28]. Aminotransferases are sensitive but relatively nonspecific indicators of liver cell injury. ALT is the more specific of the two because, for the most part, it is confined to liver, whereas AST is present not only in liver but also in skeletal and cardiac muscle and kidney and red blood cells [29, 27-28]. Alkaline phosphatases are a family of zinc metaloenzymes, with a serine at the active center; they release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues. In liver, alkaline phosphatase is found histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes. Low levels of alkaline phosphatase occur in hypothyroidism, pernicious anemia, zinc deficiency and congenital hypophosphatasia [30]. Bilirubin is an endogenous anion derived from hemoglobin degradation from the RBC. Bilirubin is extracted from hepatic blood and transformed within the liver cell into more polar substances suitable for biliary excretion. Increases in total bilirubin (consisting of indirect and direct fractions) with an increase in the direct fraction occur with both cell necrosis and cholestasis, and are usua lly not useful in distinguishing between the two [22].

All the haematological parameters (Table 2) showed non significant differences except platelet which significantly increased in all the treated groups and neutrophils and lymphocytes counts in groups 2-4 only when compared to control values. Eosinophils, monocytes and basophils were not detected. According to [31, changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors. Packed Cell Volume, haemoglobin and red blood cell counts are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals [32, 33]. The fact that the blood indices (Hb, RBC and PCV), which are important for diagnosis of anaemia non significantly differed ($p>0.05$) in all the extract treated groups when compared to control value showed that the rats were not anaemic. Neutrophiles are essential for the immune system and lymphocytes essential for humoral and cell-mediated immunity responses.

Blood platelets are implicated in blood clotting. High platelet concentration as observed in this study suggests that the process of clot-formation (blood clotting) is enhanced thereby ensuring primary haemostatic role in circulation and preventing excessive loss of blood in the case of injury. Platelets are produced predominantly by the bone marrow megakaryocytes as a result of budding of the cytoplasmic membrane. Megakaryocytes are derived from the haemopoetic stem cell, which is stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin. Secondary metabolites found in this plant may have induced differentiation of haemopoetic stem cell by stimulation the secretion of various cytokines, including thrombopoietin.. This is supported by result of the field study conducted by [14] in Indonesia that the herb is commonly used to treat wounds and bleeding, either external wounds or internal injuries such as menstrual disorders and postpartum haemorrhage, and also used as a tonic to improve fitness and stamina. Tannins have been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses [34].

Weight of liver from Table 3, significantly decreased ($p<0.05$) in groups 2-5 and non significantly increased ($p>0.05$) in groups 6-9 in comparism with control.

Quantitative phytochemical screening of plant (Table 5) revealed the presence of flavonoids, triterpenoids, saponins, tannin, steroid, cardiac glycoside and phenol in decreasing order (32.19 ± 0.23-13.10 ± 0.11) and absence of alkaloids, anthraquinone and cyanogenic glycosides. It is well known that plants generally contain secondary metabolites, some of which exhibit physiological activity [35]. Saponins cause hypocholesterolemia by binding cholesterol, making it unavailable for absorption [36]. Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent anti-oxidant activity against the superoxide radical [37]. Its consumption has been documented not to be associated with mortality due to coronary heart disease. This may be as a result of its antioxidant activity and subsequent inhibitions of Low Density Lipoproteins (LDL) oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Tannins hasten the healing of wounds and inflamed mucous membrane [38]. The presence of these photochemical thus supports the medicinal uses of *selaginella myosurus.*

Histological pictures in Figures 2, 3, 5, 6 for rats treated with 600, 800 mg/kgbw for 7 days and 600,800 mg/kgbw for

14days revealed normal hepatocyte, sinusoid and central vein while 1000 mg/kgbw treated groups for 7 and 14 days showed hepatocytic necrosis (Figures 4 and 7) and normal hepatocyte architecture (Fig. 1) for control group. However there is no correlation between biochemical investigations and histological results for groups 5 and 9 treated 1000 mg/kgbw for 7 and 14 days only that alkaline phosphatase activities significantly reduced in extract treated groups. In conclusion, aqueous whole plant extract of *selaginella myosurus* could be termed safe, boost platelet synthesis and immune response.

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