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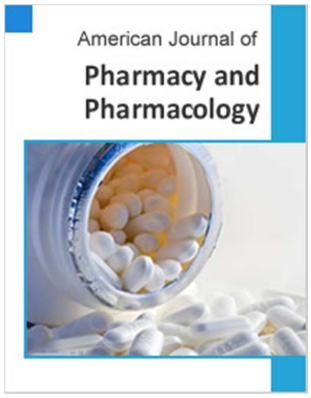

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Effect of Aqueous Whole Plant Extract of *Selaginella Myosurus* on Liver and Haematological Indices of Carbon Tetrachloride (CCl_4) Induced Acute Liver Injury in Wistar Rats

Peters D. E.*, Omeodu S. I., Egbordi C. E.

Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria

Email address
petersdikioye@ymail.com (Peters D. E.)

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Abstract
The study was designed to investigate the effect of aqueous whole plant extract of *selaginellamyosurus* on liver and haematological parameters of carbon tetrachloride (CCl_4) induced hepatotoxicity in wistar rats. Fifty (52) wistarrats of both sexes weighing 150-175g were divided into thirteen groups (±4g/group). Group 1 received distil water only, 2 and 3 received CCl_4 only, 4-7 received varying doses of 400 and 800mg/kgbw of extract, 8-11 received varying doses of 400 and 800mg/kgbw of extract after CCl_4 treatment and 12 and 13 received 100mg/kgbw of vitamin C after treating with CCl_4 all for 7 and 14 days. Rats were sacrificed and blood samples were collected for some biochemical and haematological analyses, liver was collected for histological investigation while phytochemical screening was conducted on the plant. Result showed significant elevation ($p<0.05$) of ALT, AST and ALP activities and TP level and non significant differences ($p>0.05$) in albumin and bilirubin levels when groups 2 and 3 were compared to group 1. Treatment with different doses of extract alone in all the groups did not show any significant change in all the parameters when compared to group 1. Groups 4, 5, 6, and 7 revealed non significant reduction ($p>0.05$) in the activities of ALT, AST and ALP and TP level when compared to groups 2 and 3. Vit C treatment for 14 days returned values of all the parameters to normal values. Groups 2-9 demonstrated non significant differences in Hb level, PCV, and RBC count when compared to control group. WBC, platelet and leukocyte counts significantly reduced ($p<0.05$) when some of the groups were compared to control group. Quantitative phytochemical screening of plant revealed the presence of flavonoids, triterpenoids, saponins, tannin, steroid, cardiac glycoside, phenol and absence of alkaloids, anthraquinone and cyanogenic glycosides. Histology result revealed regeneration of CCl_4 induced hepatocyte damage in some of the extract treated group. Therefore aqueous whole plant extract of *selagenellamyosurus* is safe at these doses and has ameliorating effect on CCl_4 induced hepatotoxicity.

1. Introduction

Many of the modern drugs mainly based on synthetic chemical compounds used in the management of hepatic injury and toxicity have been found to have harmful side effects

on human system (King & Perry, 2001). Herbal medicines are in great demand in various chemicals and drugs disordered hepatics, even in the developed world for primary health care because of their efficacy, safety, lesser side effects and narrow therapeutic window. Therefore, the use of herbal drugs is much safer than synthetic products available in the market (Singh *et al.,* 2012). This informed the undertaking of this study.

The Liver is the largest organ of the human body weighing approximately 1500g. The liver performs more than 500 vital metabolic functions (Gray & Lewis, 2005; Saukkonen *et al.,* 2006; Naruse *et al.,* 2007). It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream. It regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract. The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury (Saukkonen *et al.,* 2006). Also; 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. Chemicals that cause liver injury are called hepatotoxins. These agents are converted to chemically reactive metabolites in liver (by some enzymes in the cytochrome p-450 system such as CYP2E1) which have the ability to interconnect with cellular macromolecules such as protein, lipids and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress. This damage of cellular function can result to cell death and likely liver failure. Carbon tetrachloride (CCL_4) being one of these agents is used as a model drug for the study of hepatotoxicity in acute and chronic liver failure (Singh*et al.,* 2012). Alhassan *et al*., (2009) observed that high doses of the CCl_4 (90 – 120 mg/Kg) can induce massive liver damage and may persist for longer period compared to lower dosage, hence 120mg/kgbw was the choice for this work. CCl_4 is metabolized by CYP2E1, CYP2B and possibly CYP3A, to form the trichloromethyl radical, CCl_3. This CCl_3 radical can bind to cellular molecules damaging crucial cellular progression. This radical can also react with oxygen to form the tri-chlomethylperoxy radical CCl_3OO, a highly reactive species. The metabolites of CCl_4 cause the hepatic injury in the CCl_4 liver injury model. Single dose of CCl_4 to a rat produces centrilobular necrosis and fattychanges (Singh *et al.,* 2012). Many of the modern drugs mainly based on synthetic chemical compounds used in the management of hepatic injury and toxicity have been found to have harmful side effects on human system (King & Perry, 2001). Herbal medicines are in great demand in various chemicals and drugs disordered hepatics, even in the developed world for primary health care because of their efficacy, safety, lesser side effects and narrow therapeutic window. Therefore, the use of herbal drugs is much safer than synthetic products available in the market (Singh *et al.,* 2012). This has triggered off extensive research and development in the field of herbal medicine. In fact there is a growing demand for herbal medicine in most of the developed and developing countries of the world today (Handa *et al.,* 1999).

Selaginella myosurus is a shrub that can be found on a terrestrial habitat, on rocks, or rarely hemiepiphytic or epiphytic. Its stems can prostrate, creeping, decumbent, cespitose, climbing, or fully erect, articulate or not and greatly branched. *Selaginella* has been prescribed in traditional medicine of China and India, which has been thousands of years old. *Selaginella* can be found in the pharmacopoeia in Asia, Africa and Latin America, but not found in Europe and North America (Duke *et al.,* 2002). *Selaginella* 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, although sometimes they are called only a leaf. A whole plant maceration of *selaginellamyosurus* is used to treat headache in South-west region of Cameroon (Jiofack *et al.,* 2010). *Selaginella* is useful to treat wounds, menstrual disorders and for treatments before, during, and after giving birth, and to improve fitness and endurance of the body (tonic). In Gabon, a decoction of the stem and leaves of *Selaginellamyosurus* is used for rituals or for other cultural aspects (Sassen& Wan, 2006).

Selaginella species contain powerful inhibitor compounds which may act as primary antioxidants that react with free radicals. The scavenging effects of selected four *Selaginella* species are as follows: *Selaginellainvolvens>Selaginellaintermedia>Selaginellatenera>Selaginellainaequalifolia*. Sivaraman *et al.* reported that all the selected four species of *Selaginella* contain powerful inhibitor compounds which may act as primary antioxidants that react with free radicals (Sivaraman *et al.,* 2013). As in selected *Selaginella* species, the crude extracts of few other pteridophytes have already been reported to exhibit strong scavenging activities (Huang *et al.,* 2003; Haung *et al.,* 2005; Zhongxiang *et al.,* 2007; Lai & Lim, 2011). Hagerman *et al. (2007)* have reported that the high molecular Ferric reducing antioxidant power (FRAP) value. The reductive ability of the *Selaginella* species that was assessed by Sivaraman *et al.* suggests that all the extracts were able to donate electron. Hence, they should be able to donate electrons to free radicals in actual biological or food systems also, making the radicals stable. According to Oktay *et al.* (2003) a highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many plant species. Sivaraman *et al*. found out that ethanolic extracts of different *Selaginella* species possess significant antioxidant and free radical scavenging activities. Previously Gayathri *et al.* studied the antioxidant properties of *Selaginellainvolvens*, *Selaginelladelicatula* and

Selaginellawightii from the wild and tested the *in vitro* and *in vivo* lipid peroxidation, immunomodulatory property and hydroxyl radical scavenging activity (Gayathri *et al.,* 2005). They reported that the aqueous extract of *Selaginellainvolvens* has promising thymus growth stimulatory activity in adult mice and remarkable antilipid peroxidation property.

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

Selaginellamyosurus 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-Nwosu 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 *Selaginellamyosurus* 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 *Selaginellamyosurus* was done using standard procedure as described by Sofoware (1993) in the Department of Pharmacognosy, Faculty of Pharmacy, University of Port Harcourt.

2.6. Source of Animals

A total of fifty two (52) wistar rats of both sexes weighing between 150g-175g 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 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 thirteen (13) groups weight difference of (±4g/group) (n=4rats).

GROUP1 (Control): 0.5ml of distilled water was orally given to the animals in this group daily for 14 days.

GROUP2: A single dose of 120mg/kg b.w of CCl_4 administered intraperitoneally to rats in this group for 7 days.

GROUP3: A single dose of 120mg/kg b.w of CCl_4 administered intraperitoneally to rats in this group for 14 days.

GROUP4: A single daily dose of 400mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered to rats in this group for 7 days.

GROUP5: A single daily dose of 400mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered to rats in this group for 14 days.

GROUP6: A single daily dose of 800mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered to rats in this group for 7 days.

GROUP7: A single daily dose of 800mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered to rats in this group for 14 days.

GROUP8: A single daily dose of 400mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered for 7 days to rats intraperitoneally treated with CCl_4.

GROUP9: A single daily dose of 400mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered for 14 days to rats intraperitoneally treated with CCl_4.

GROUP10: A single daily dose of 800mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus*was orally administered for 7 days to rats intraperitoneally treated with CCl_4.

GROUP11: A single daily dose of 800mg/kg b.w of aqueous whole plant extract of *Selaginellamyosurus* was orally administered for 14 days to rats intraperitoneally treated with CCl_4.

GROUP12: A single daily dose of 100mg/kg b.w of Vit. Cwas orally administered for 7 days to rats administered CCl_4 intraperitoneally.

GROUP13: A single daily dose of 400mg/kg b.w of Vit. C

was orally administered for 14 days to rats administered CCl_4 intraperitoneally.

2.9. 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, spunat 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 alanineaminotransferase (ALT) and aspartate aminotransferase (AST) activities by the method of Reitman and Frankel (1957), plasma alkaline phosphatase activity by the method of Rec (1972), and plasma total protein and albumin assessed by Biuret and BCG methods respectively. Determination of Bilirubin was by Jendrassik and Crod method. Haemoglobin was determined by haemiglobinecyanide technique, Packed Cell Volume (PCV) determination was by micro-haematocrit method Leukocytes and Platelets count, White Blood Cells (WBC) Count and Red Blood Cells (RBC) Count were done manually.

3.1. Histopathogical Studies

The rats were dissected using a set of dissection kit and livers from 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 about5µm were mounted on glass slides and stained with haematoxylin and eosin (Lillie, 1965).

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 $p < 0.05$.

Table 1. *Effect of aqueous whole plant extract of Selaginellamyosurus on some biochemical parameters of CCl_4 induced hepatoxicity in wistar rats.*

TREATMENT GROUPS ((M±SEM)	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)	BIL. (umol/L)
Water control	45.75±3.33[a]	48.50±3.23[a]	238.00±4.60[a]	78.75±2.66[a]	35.50±1.55	7.75±0.49[a]
CCl_4 only 7 days	99.00±0.58[a]	66.00±1.73[a]	408.00±10.97[a]	116.50±1.44[a]	31.00±0.58	9.00±0.57
CCl_4 only for 14 days	95.50±2.22[a]	69.50±2.40[a]	426.50±16.69[a]	108.75±4.75[a]	32.25±2.02	9.75±0.85
400mg/kgbw Ext. 7days	33.50±0.96	38.25±0.85	241.00±1.29	63.50±0.65	42.75±0.48	10.00±0.41
400mg/kgbwExt. 14 days	40.50±1.02	44.00±2.04	236.50±1.84	75.50±1.43	39.00±1.22	8.50±0.21
800mg/kgbw Ext. 7days	34.25±0.63	40.25±1.38	219.25±1.89	69.00±0.61	43.75±0.48	9.25±0.63
800mg/kgbw Ext. 14 days	39.50±1.02	44.00±0.82	228.00±0.82	75.50±2.25	38.00±0.82	7.50±0.20
CCl_4+400mg/kgbw Ext for 7 days	87.25±3.79[a]	61.00±3.53[a]	353.25±12.83[a]	107.75±2.32[a]	33.25±1.11	9.75±0.75
CCl_4+400mg/kgbw Ext for 14 days	81.00±1.68[a]	62.00±1.78[a]	302.25±3.42[a]	81.75±2.9	39.00±0.91	9.75±0.48
CCl_4+800mg/kgbw Ext for 7 days	83.00±4.26[a]	53.75±1.80	324.00±14.44[a]	109.00±2.27[a]	34.50±1.32	9.75±0.85
CCl_4+800mg/kgbw Ext for 14 days	63.75±1.93[a]	55.50±1.85	269.00±3.76	84.75±3.09	36.50±1.55	8.75±0.48
CCl_4+100mg/kgbwVit. Cfor 7days	79.00±3.67[a]	54.50±0.61	286.50±2.65[a]	98.00±0.82[a]	34.50±0.20	11.50±0.20[a]
CCl_4+100mg/kgbwVit. C+for 14days	56.50±1.02	53.50±1.43	247.50±2.25	82.00±1.22	38.00±1.22	8.50±0.20

Data are represented in Mean±Standard error of mean (M±SEM)
Superscript a represents significant difference within the row

Table 2. *Effect of aqueous whole plant extract of Selaginellamyosurus on some haematological indices of CCl_4 induced hepatoxicity in wistar rats.*

TREATMENT GROUPS ((M±SEM)	PARAMETERS									
	Hb (g/dl)	PCV (%)	RBC ($X10^{12}$/L)	WBC ($X10^9$/L)	PLATELET ($X10^9$/L)	NEUTROPHIL	LEUCOCYTE	E	M	B
Water control	11.00±0.53	33.00±1.63	4.18±0.31	13.50±0.65	280.00±12.25	30.00±2.04	70.00±2.04[a]	-	-	-
CCl_4 onlyfor7days	11.35±0.78	34.00±2.31	4.25±0.43	8.85±0.20[a]	210.00±5.77	26.50±0.87	73.50±0.86	-	-	-
CCl_4 only for 14 days	12.08±0.72	36.25±2.17	4.70±0.43	8.05±0.49[a]	275.00±37.97	26.50±0.87	73.50±0.87	-	-	-
400mg/kgbw Ext. for 7 days only	10.93±0.45	32.75±1.31	4.18±0.36	6.53±0.56[a]	247.50±16.52[a]	35.50±1.44	62.50±1.44[a]	-	-	-
400mg/kgbw Ext. for 14 days only	10.85±0.06	32.50±0.20	4.10±0.04	4.25±0.10[a]	260.00±4.08	35.00±2.04	61.00±0.41[a]	-	-	-
800mg/kgbw Ext. for 7 days only	10.18±0.31	30.75±1.03	4.10±0.41	6.63±0.24[a]	220.00±14.71[a]	38.00±2.71	62.00±2.71[a]	-	-	-

TREATMENT GROUPS ((M±SEM)	PARAMETERS									
	Hb (g/dl)	PCV (%)	RBC ($X10^{12}$/L)	WBC ($X10^9$/L)	PLATELET ($X10^9$/L)	NEUTROPHIL	LEUCOCYTE	E	M	B
800mg/kgbw Ext. for 14 days only	10.50±0.08	31.50±0.20	3.90±0.40	4.50±0.20[a]	200.00±0.00[a]	37.50±1.02	62.50±1.02[a]	-	-	-
CCl_4+400mg/kgbw Ext for 7 days	10.83±0.28	32.50±0.87	4.18±0.20	8.73±1.33[a]	232.50±26.88	31.75±1.18	68.25±1.18	-	-	-
CCl_4+400mg/kgbw Ext for 14 days	11.78±0.08	35.25±0.25	4.83±0.06	4.18±0.19[a]	200.00±00[a]	30.25±2.72	69.75±2.72	-	-	-
CCl_4+800mg/kgbw Ext for 7 days	12.33±0.52	37.00±1.58	4.90±0.26	6.50±0.24[a]	200.00±8.16[a]	37.25±1.11	62.75±1.11[a]	-	-	-
CCl_4+800mg/kgbw Ext for 14 days	11.18±1.11	33.00±3.08	4.45±0.55	3.98±0.23[a]	190.00±5.77[a]	30.25±1.65	69.75±1.65	-	-	-
CCl_4+100mg/kgbw Vit. Cfor 7days	11.00±0.41	33.00±1.22	4.10±0.24	8.00±0.41[a]	200.00±0.00[a]	29.00±0.41	71.00±0.41	-	-	-
CCl_4+100mg/kgbw Vit. C+for 14days	12.00±0.29	36.00±0.51	4.60±0.16	7.75±0.71[a]	165.00±6.12[a]	31.00±0.41	69.00±0.41	-	-	-

Data are represented in Mean±Standard error of mean (M±SEM)
Superscript a represents significant difference within the row

Table 3. *Qualitative phytochemical screening of whole plant extract of Selaginellamyosurus.*

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 means present
-ve means absent
ND is Not Detected

Table 4. *Quantitative phytochemical analysis of whole plant extract of Selaginellamyosorus.*

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

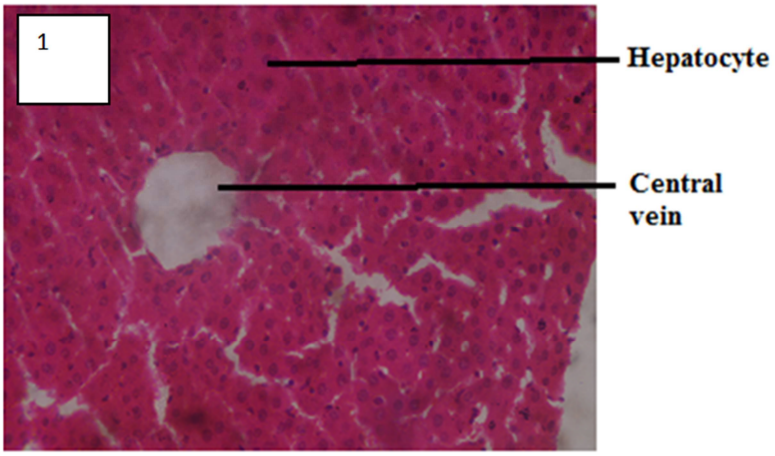

Fig. 1. *Photomicrograph of the liver tissue of rat treated with distilled water only as control. Arrows indicating normal hepatocyte and central vein (H&E) magnification X 400.*

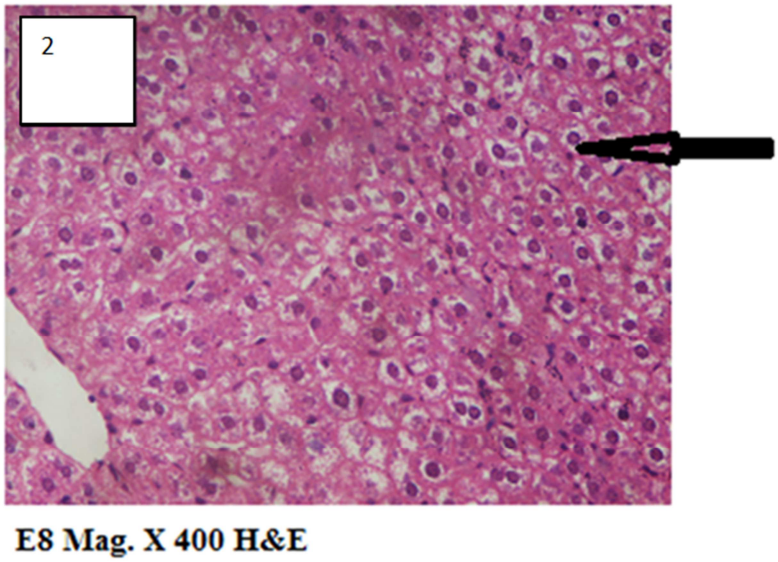

Fig. 2. *Photomicrograph of the liver tissue of rat treated with CCl_4 only for 14days (negative control). Arrow= ballooning necrosis of hepatocytes. (H&E) magnification X 400.*

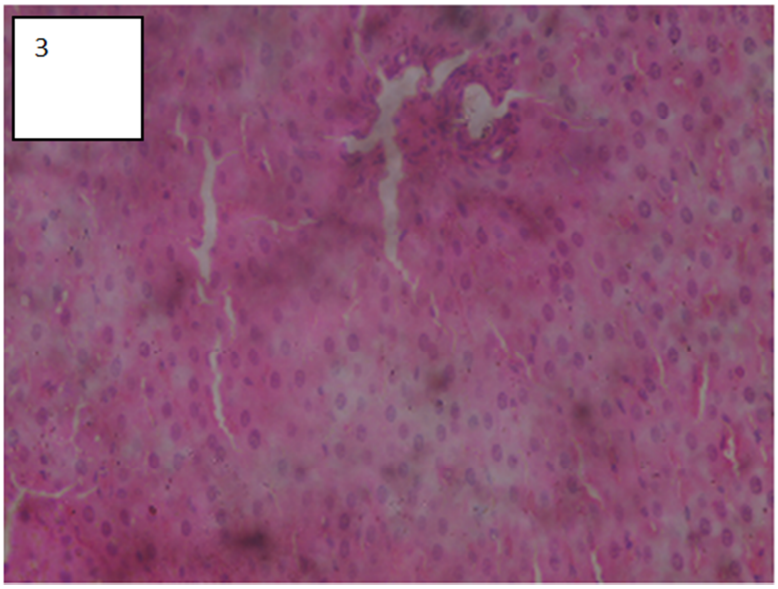

Fig. 3. *Photomicrograph of the liver tissue treated 400mg/kg bw extract only for 14days showing normal liver histology (H&E x400).*

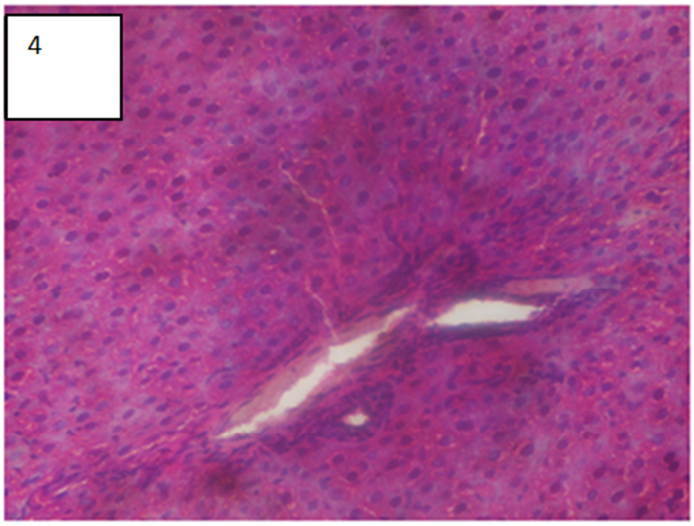

Fig. 4. *Photomicrograph of the liver of rat treated with 800mg/kgbw extract only for 14 days showing normal liver (H&E) X 400.*

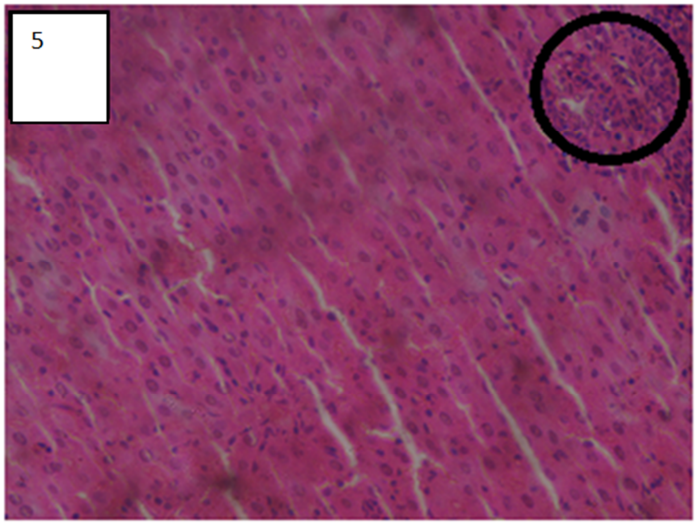

Fig. 5. *Photomicrograph of the liver tissue of rat treated with CCl_4 + 400mg/kgbw extract only for 7 days. Encircled area contains increased inflammatory cells. (H&E) X 40.*

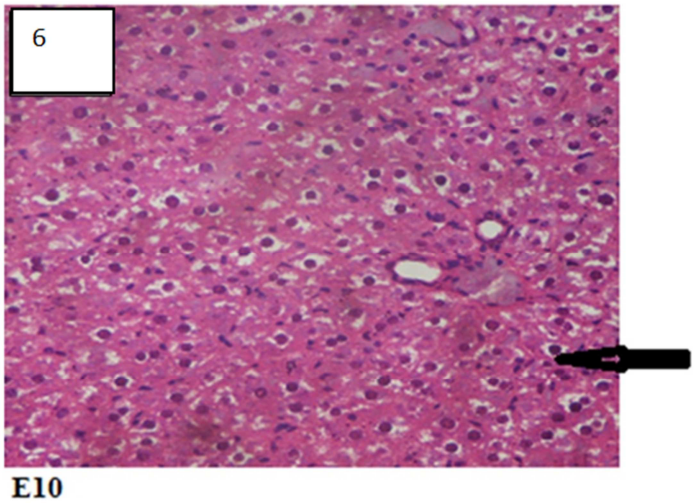

Fig. 6. *Photomicrograph of the liver tissue of rat treated with CCl_4 + 400mg/kgbw extract only for 14 days. Arrow indicating zone of necrotic tissue (H&E) X 40.*

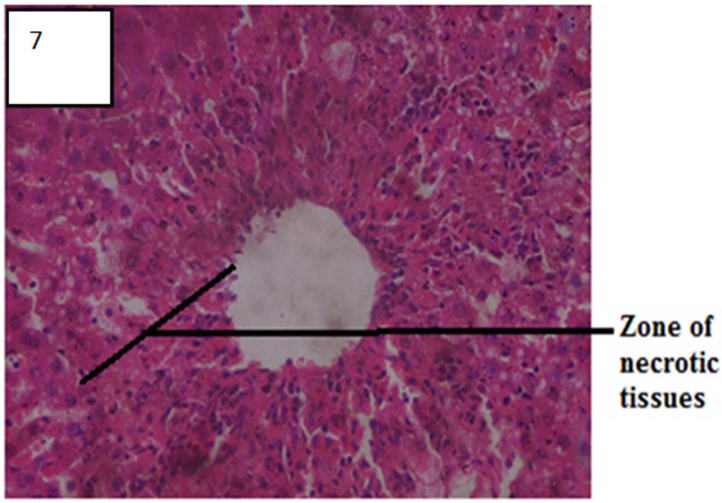

Fig. 7. *Photomicrograph of the liver tissue of rat treated with CCl_4 + 800mg extract only for 7 days. Arrow indicating zone of necrotic tissue (H&E) X 400.*

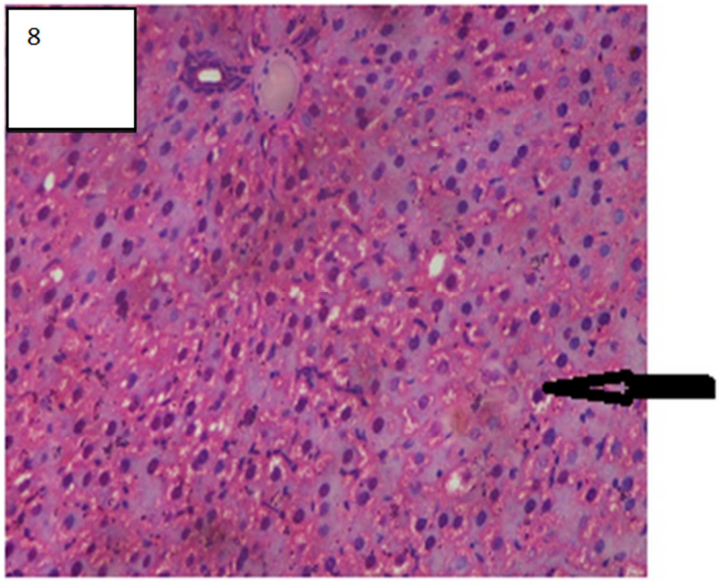

Fig. 8. *Photomicrograph of liver tissues treated with CCl_4 + 800mg/kg b.w extract for 14 days (H&E X400). Result shows normal histology.*

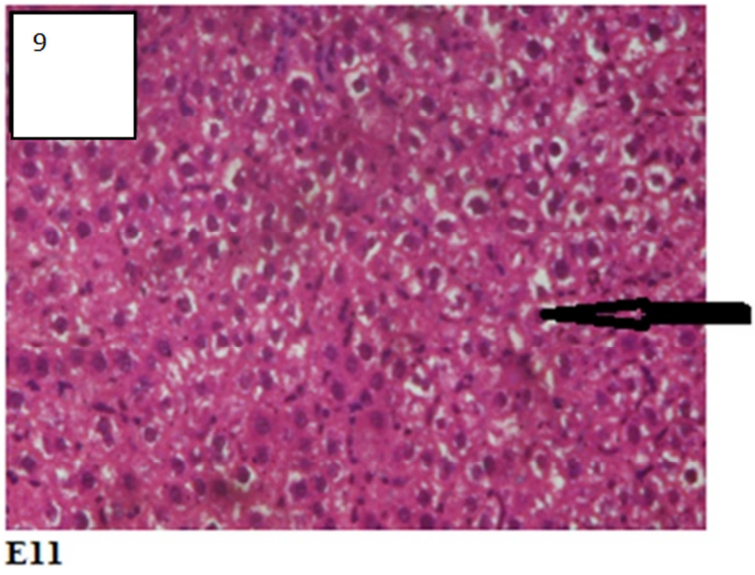

Fig. 9. *Photomicrograph of the liver tissue of rat treated with CCl_4 + Vitamin C for 7days as positive control. (H&E) X 400.*

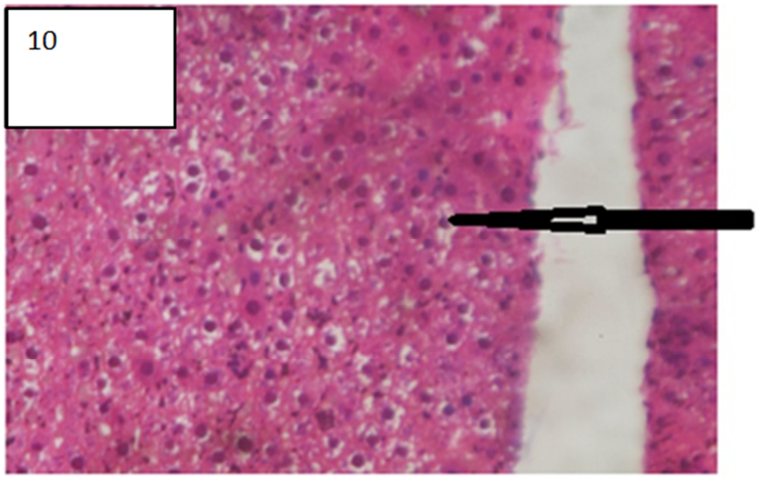

Fig. 10. *Photomicrograph of liver tissues treated with CCl_4 + 100mg/kg b.w Vitamin C for 14 days (H&E X400). Result shows normal liver histology.*

4. Discussion and Conclusion

Result from Table 1 showed significant elevation ($p<0.05$) of ALT, AST and ALP activities and TP level and non significant differences ($p>0.05$) in albumin and bilirubin levels when groups 2 and 3 were compared to group 1 indicating CCl_4 induced lipid peroxidation and liver damage as observed by Robbins and Cotran, (2006) and Alhassan*et al*., (2009). 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 α-amino groups to the α-keto groups of ketoglutaric acid toproduce pyruvic and oxaloacetic acids. Injury to liver cell membranes causesleakage of aminotransferases into the circulation (Lott and Wolf, 1986; Kew, 2000; Dufour *et al.,* 2000). Aminotransferasesare sensitive but relatively nonspecific indicators of liver cell injury. ALT isthe 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 cardiacmuscle and kidney and red blood cells (Kamath, 1996; Kew, 2000: Dufour *et al.,* 2000).

Treatment with different doses of extract alone in all the groups did not show any significant change in all the parameters when compared to group 1 suggesting the extract was safe at these doses.

Groups 4, 5, 6, and 7 revealed non significant reduction ($p>0.05$) in the activities of ALT, AST and ALP and TP level when compared to groups 2 and 3 indicating regeneration of liver cell from CCl_4 induced acute liver damage. Vit C treatment for 14 days returned values of all the parameters to normal range.

Groups 2-9 demonstrated non significant differences in Hb level, PCV, and RBC count when compared to control group. WBC, platelet and leukocyte counts significantly reduced ($p<L0.05$) when some of the groups were compared to control group. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are disposed to high risk of disease infection (Soetan *et al*., 2013).

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. The principal causes of carbon tetrachloride (CCl_4) induced hepatic damage are increased lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals (Poli, 1993; Ohta *et al*., 2000). When free radicals are formed, these highly reactive compounds will react non-enzymatically and potentially alter the structure and function of several important components such as cell membranes, signalling molecules, lipoproteins, proteins, carbohydrates, RNA, and DNA (Sies, 1997; Beckman and Ames, 1998; McCord, 2000; Blomhoff, 2005). Oxidative stress is important in the development of chronic degenerative diseases including liver and coronary heart diseases, cancer and aging (Ames *et al.,* 1993). Rajesh and Latha, (2004) showed that various herbal extracts couldprotect organs against CCl_4 induced oxidative stress by altering the levels of increased lipid peroxidation and enhancing the decrease activities of antioxidant enzymes. Phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C, E and carotenoids (Rice-Evans *et al.,* 1995; Rice-Evans *et al.,* 1996). It has been proposed that the antioxidant properties of phenolic compounds can be mediated by the following mechanisms: (1) scavenging radical species such as ROS/RNS; (2) suppressing ROS/RNS formationby inhibiting some enzymes or chelating trace metals involved in free radical production; (3) upregulatingor protecting antioxidant defense (Cotelle, 2001). Findings in the present study is supported by Sivaraman *et al.* (2013) who reported that four selected *Selaginella* species contain powerful inhibitor compounds which may act as primary antioxidants that react with free

radicals in this order *Selaginellainvolvens>Selaginellaintermedia>Selaginellatenera>Selaginellainaequalifolia*. As in selected *Selaginella* species, the crude extracts of few other pteridophytes have already been reported to exhibit strong scavenging activities (Huang *et al.,* 2003; Haung*et al.,* 2005; Zhongxiang *et al.,* 2007; Lai & Lim, 2011). Hagerman *et al. (2007)* have reported a high molecular Ferric reducing antioxidant power (FRAP) value. The reductive ability of the *Selaginella* species that was assessed by Sivaraman *et al.* suggests that all the extracts were able to donate electron. Hence, they should be able to donate electrons to free radicals in actual biological or food systems also, making the radicals stable. According to Oktay *et al*. (2003) a highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many plant species. Sivaraman *et al*. found out that ethanolic extracts of different *Selaginella* species possess significant antioxidant and free radical scavenging activities. Previously Gayathri *et al.* studied the antioxidant properties of *Selaginellainvolvens*, *Selaginelladelicatula* and *Selaginellawightii* from the wild and tested the *in vitro* and *in vivo* lipid peroxidation, immunomodulatory property and hydroxyl radical scavenging activity (Gayathri *et al.,* 2005). They reported that the aqueous extract of *Selaginellainvolvens* has promising thymus growth stimulatory activity in adult mice and remarkable antilipid peroxidation property.

Histology result revealed regeneration of CCl_4 induced hepatocyte damage. Therefore aqueous whole plant extract of *Selaginellamyosorus* is safe at these doses and has ameliorating effect on CCl_4 induced hepatotoxicity.

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