Original Research Article

Extract of *Icacina trichanta* improves lipid profile and CCl_4- induced histological changes in Wistar rats

ABSTRACT

Aim: This study was undertaken to investigate the effect of aqueous leaf extract of *Icacina trichanta* on lipid profile and CCl_4- induced histological changes in Wistar rats.

Study design: Liver damage was induced with CCl_4 and the rats were treated with aqueous extract of *Icacina trichanta.*

Place and duration of study: This study was undertaken at the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, between January to March 2014.

Methodology: Phytochemical screening, lipid profile analysis and histopathological studies were carried out. Thirty-five male rats were divided into seven groups of five rats each. Groups I (normal control) and III rats were not induced. Groups II (negative control), IV, V, VI and VII were induced with 1.0 ml CCl_4/kg body weight. Group II rats were not administered the extract, while those in group IV received 100 mg/kg bw of silymarin. Varied concentrations of the extract ranging from 200 to 400 mg/kg bw were administered to the test rats.

Results: Phytochemical analyses revealed the presence of alkaloids, saponins, tannins and polyphenols. The concentration of triacylglycerol (TG) in the CCl_4 control was significantly increased when compared with the normal control group (p<0.05). Concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) of the CCl_4 control were significantly reduced relative to the normal control and the test rats (p<0.05). There was no significant difference in the concentration of high-density lipoprotein cholesterol (HDL-C) among the groups (p > 0.05). Results of histopathological examinations showed an ameliorative effect of aqueous leaf extract of *Icacina trichanta* on hepatorenal toxicity.

Conclusion: Aqueous extract of *I. trichanta* leaf improves lipid profile and CCl_4-induced histological changes in Wistar rats.

Keywords: *Icacina trichanta*, Silymarin, Histology, Phytochemicals, Lipid profile, Cholesterol.

1.0 INTRODUCTION

The liver and kidney are vital organs of the human body involved in metabolism, detoxification, secretion and excretion of various endogenous and exogenous substances (Abdel-Misih and Bloomston, 2010). Liver cell injury induced by carbon tetrachloride (CCl_4) involves its initial metabolism to trichloromethyl free-radical by the mixed-function oxidase system of the endoplasmic reticulum (Junnila *et al*., 2000; Cui *et al.,* 2009). It is postulated that secondary mechanisms link CCl_4 metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl_4 metabolism or from peroxidative degeneration of membrane lipids (Kim *et al.,* 2010). The

possible involvement of radical species such as trichloromethyl (CCl_3), trichloromethylperoxy ($OOCCl_3$), and chlorine (Cl) free radicals, as well as phosgene and aldehydic products of lipid peroxidation, as toxic intermediates is discussed (Brattin *et al*., 1985). The pathogenesis of CCl_4 - induced renal dysfunction is not completely known. It may be due to the functional state of liver (Parola *et al*., 1993), or renal injury may develop independently to hepatic events, or can be attributed to CCl_4 induction of oxidative stress in many settings (Abraham *et al*., 1999); therefore, it might be expected to contribute to renal damage. *In vitro* and *in vivo* studies have shown that CCl_4 enhances lipid peroxidation and reduces the renal reduced/oxidized glutathione ratio in kidney cortex as well as renal microsomes and mitochondria (Tirkey *et al*., 2005).

Lipids are one of the necessary components which control cellular functions and homeostasis. The liver plays an essential role in lipid metabolism, several stages of lipid synthesis and transportation. Therefore, it is reasonable to expect an abnormal lipid profile in those with severe liver dysfunction. There is a prominent decline in plasma total cholesterol and triglycerol levels in patients with severe hepatitis and hepatic failure because of reduction in lipoprotein biosynthesis. For reduced liver biosynthetic capacity, low levels of triglycerol and total cholesterol is usually observed in chronic liver diseases (Halsted, 2004). Certain medicinal agents, chemicals and even herbal remedies may cause liver and kidney injuries. Today, a substantial number of drugs are developed from plants (Fabricant and Farnsworth, 2001) which are active against a number of diseases.

Icacina trichanta Oliv. is indigenous to West and Central Africa and can be found growing in the Savanna areas of Senegal, Gambia, Guinea Bissau, Northern Ghana, Benin and Nigeria. It is a perennial shrub with erect leafy shoot and broad elliptic simple, alternate leaves (Timothy and Idu, 2011). It is known as ibugo in Igbo and eso gbegbe in Yoruba. Different parts of the plant are used for ethnomedicinal purposes (Asuzu and Abubakar, 1995; Rufus, 2010). This study was undertaken to determine the effect of aqueous leaf extract of *Icacina trichanta* on lipid profile and CCl_4- induced histological changes in the liver and kidney of rats.

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

All reagents used were of analytical grade. Lipid profile assay kits were products of Randox Laboratories Limited (UK). All other chemicals were obtained from the British Drug House (BDH) (England), Merck (Germany) and Aldrich Chemical Company (USA).

2.2.1 Plant collection and preparation

Icacina trichanta leaves were obtained from a forest in Benin City and identified by Professor J.F. Bamidele of Plant Biology and Biotechnology Department, University of Benin, Benin City, Edo State, Nigeria. A sample was placed in the Herbarium (herbarium No: UBH_J 0186). The leaves were sun dried, pulverized and sieved.

2.2.2 Extraction and concentration

Extraction was by maceration over a 72 hrs period (Abu *et al*., 2015). A portion (100 g) of the powdered leaf was soaked in 1000 ml distilled water. The aqueous extract was filtered with a muslin cloth and freeze dried using a lyophilizer.

2.2.3 Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out on the aqueous, methanol, ethanol and acetone extracts to detect the presence of secondary metabolites using standard procedures. (Evans, 2002; Tiwari *et al*., 2011; Boakye *et al*., 2015).

2.3 Experimental Design

The CCl_4 model described by Obi *et al*., (2004) was employed for inducing liver damage (1.0 ml/kg bw CCl_4 diluted in vegetable oil (1:1) was orally administered). Thirty-five male albino rats weighing between 150 and 180 g were randomly assigned to seven groups of five rats per group as follows:

Group I (normal control): Rats in this group received 1.0 ml olive oil/kg bw only for 14 days.

Group II (negative control): Rats in this group were induced with CCl_4: olive oil (1:1), 1.0 ml/kg bw for 14 days.

Group III: Rats in this group received 400 mg extract/kg bw only for 14 days; they were not induced with CCl_4.

Group IV: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 100 mg silymarin/kg bw for 14 days.

Group V: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 200 mg extract/kg bw.

Group VI: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 300 mg extract /kg bw.

Group VII: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 400 mg extract /kg bw.

The rats were allowed free access to food and water.

2.4 Blood collection

At the end of the treatment period, blood samples were collected by direct cardiac puncture into sterile heparin containers. The liver and kidney of all experimental rats were harvested, washed in ice – cold saline, blotted dry and placed in plain containers. Weighted portions of the liver and kidney were placed in 10 % phosphosaline (pH 7.0) for histological examination.

2.5 Biochemical analysis

Lipid profile parameters were determined using Randox kits. Only LDL-C and very low-density lipoprotein cholesterol (VLDL-C) were determined by calculations using the Frieldwald equation as shown below:

VLDL = TG/5

LDL = TC – (TG/5) - HDL

2.6 Histological Examination of the Tissues

Portions of the liver and kidney were serially sectioned and fixed in 10 % formalin for 48 hrs. The specimen was then dehydrated through a graded series of alcohol and cleared in three changes of xylene before embeded in paraffin. Serial sections, each of 4 µm thickness, were made and stained with haematoxylin and eosin according to standard method. Histological assessment was performed under light microscopy. In every H and E section a minimum of 25 circular tubule were measured in two axes drawn perpendicular to each other using an image analyzer (Image Proplus, Version 3.0).

2.7 STATISTICAL ANALYSIS

Count data are presented as mean ± SEM. Statistical analysis was performed using GraphPad Prism Demo (6.07). Values of $p<0.05$ were considered statistically significant.

3.0 RESULTS

3.1 Phytochemical screening and yield of extract

The yield of the aqueous extract was 21.15%. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins and phenols (Table 1).

Table 1. Qualitative phytochemical screening

	Extracts			
Phytochemicals	**Aqueous**	**Methanol**	**Ethanol**	**Acetone**
Alkaloids	+	+	+	-
Flavonoids	-	-	-	-
Saponins	+	+	+	-
Glycoside	-	-	-	+
Tannins	+	+	+	-
Steroids	-	-	+	-
Phenols	+	+	+	-

(+) = detected; (-) = not detected

3.2 Weights of rats

Values of the same column with the superscript “a” were significantly decreased when compared with the normal control (Group 1), but increased when compared with the CCl_4 control (Group II) ($p < 0.05$). However, values on the same column with the superscript “b” were significantly increased when compared with the normal control group ($p < 0.05$). There were no significant differences in the weights of the kidneys among the groups ($p > 0.05$; Table 2).

Table 2. Effects of CCl_4, aqueous leaf extract of *I. trichanta* and silymarin on body and organ weights

Groups	Mean body weight gained (g)	Organ weight (g)	
		Liver	Kidney
I	37.53 ± 12.78	5.93 ± 0.48	0.50 ± 0.04
II	18.01 ± 2.81	8.67 ± 0.37	0.58 ± 0.01
III	48.74 ± 5.25	7.44 ± 1.29	0.66 ± 0.03
IV	23.28 ± 7.61[a]	8.39 ± 0.71[b]	0.61 ± 0.02
V	26.13 ± 9.06[a]	8.88 ± 0.66[b]	0.67 ± 0.06
VI	28.14 ± 5.49[a]	8.44 ± 0.63[b]	0.65 ± 0.06
VII	28.57 ± 6.35[a]	8.48 ± 0.86[b]	0.68 ± 0.01

Data are body weight gained and organ weight (n = 5).

3.3 Percentage weight gained and relative organ weight

Values of the same column with the superscript “a” were significantly decreased when compared with the normal control (Group 1), but increased when compared with the CCl_4 control (Group II) ($p<0.05$). Values on the same column with the superscript “b” and “c” were significantly increased when compared with the normal control group ($p < 0.05$). The results are shown in Table 3.

Table 3. Effects of CCl_4, aqueous leaf extract of *I. trichanta* and silymarin on percentage weight gained and relative organ weight

Groups	% Body weight Gained	Relative organ weight	
		Liver (x 10^{-1})	Kidney (x 10^{-2})
I	34.12 ± 3.84	1.58 ± 0.07	1.33 ± 0.08
II	10.94 ± 1.59	4.81 ± 0.95	3.22 ± 0.04
III	35.97 ± 3.07	1.53 ± 0.04	1.35 ± 0.04
IV	17.28 ± 2.11[a]	3.60 ± 0.73[b]	2.62 ± 0.11[c]
V	17.00 ± 2.92[a]	3.40 ± 0.29[b]	2.56 ± 0.19[c]

VI	19.95 ± 1.58[a]	3.00 ± 0.51[b]	2.31 ± 0.10[c]
VII	20.49 ± 2.19[a]	2.97 ± 0.39[b]	2.38 ± 0.09[c]

Data are percentage body weight gained and relative organ weight (n = 5).

4.4 Plasma concentrations of TC and HDL-C

Values with the same superscript were not significantly different (p>0.05), but were significantly different from those with a different superscript (p<0.05). The concentration of TC of the CCl_4 control was significantly reduced relative to those of the normal control and the other groups (p<0.05). There were no significant differences in the concentrations of HDL-C among the groups (p>0.05; Figure 1).

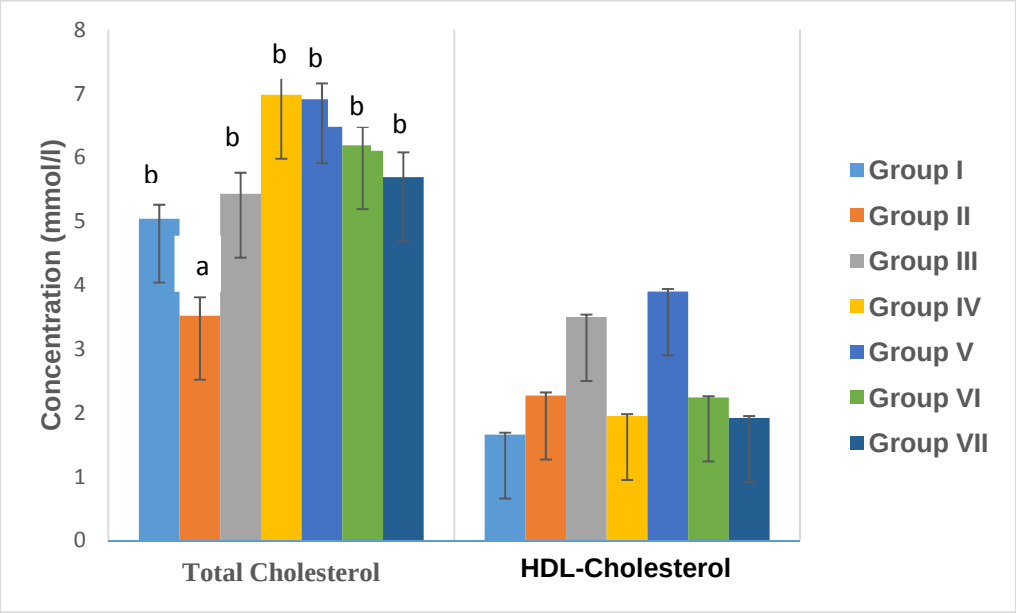

Figure 1. Effects of CCl_4, aqueous extract of *I. trichanta* and silymarin on plasma concentrations of TC and HDL-C

4.5 Plasma concentrations of TG and VLDL-C

The concentration of TG in the CCl_4 control was significantly increased when compared with the normal control group (p>0.05). The concentrations of VLDL-C of the CCl_4 control and treatment groups were significantly increased when compared with the normal control group (p<0.05).

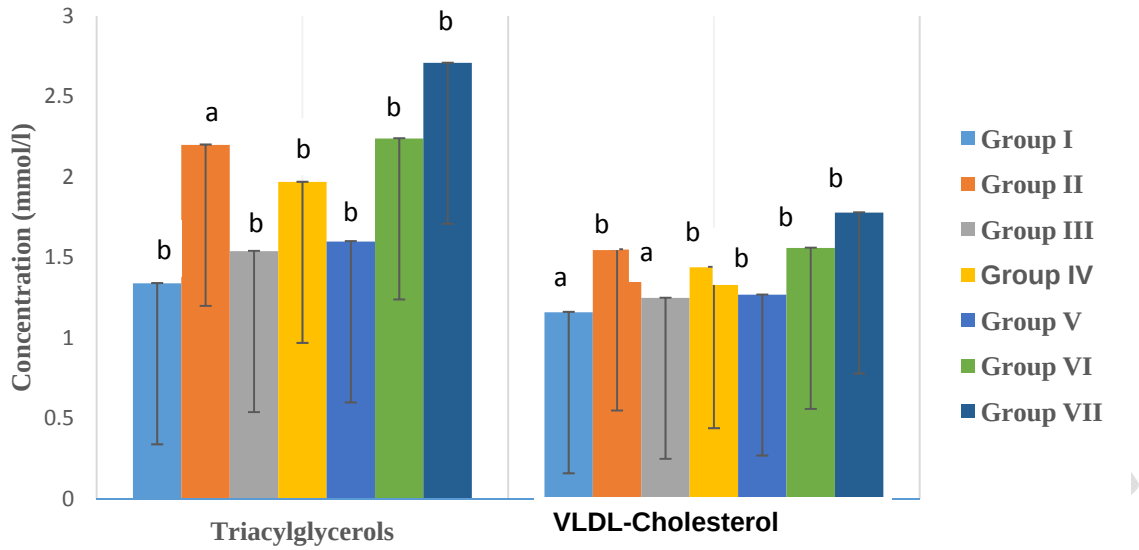

Figure 2. Effects of CCl_4, aqueous leaf extract of *I. trichanta* and silymarin on plasma concentrations of TG and VLDL-C

4.6 Plasma concentration of LDL-C

The concentration of LDL-C of the CCl_4 control was significantly decreased relative to normal control and the treatment groups (p<0.05).

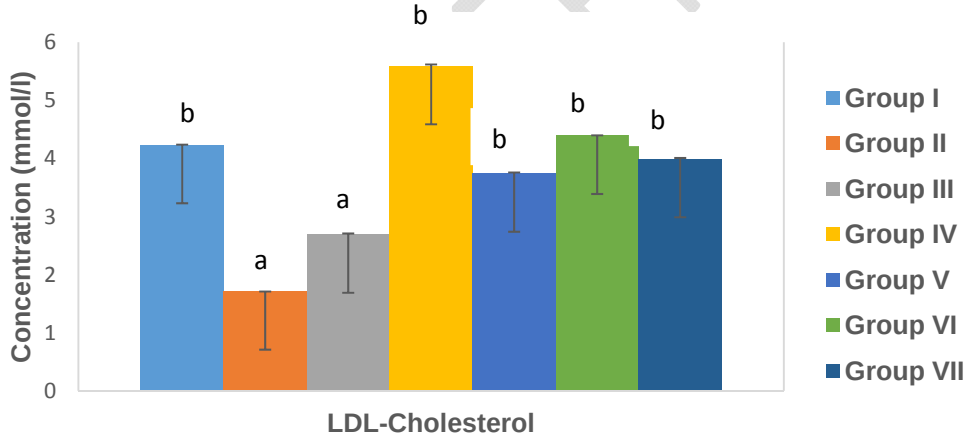

Figure 3. Effects of CCl_4, aqueous leaf extract of *I. trichanta* and silymarin on plasma concentration of LDL-C

4.7 Histological examinations of rats liver exposed to CCl_4, aqueous leaf extract of *I. trichanta* and silymarin

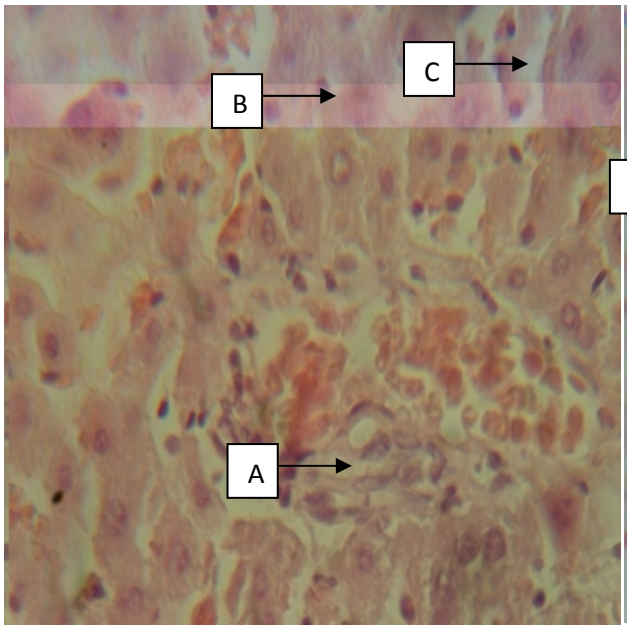

Plate 1. Control: Rat Liver composed of portal triad A, hepatocytes B and sinusoids C (H & E x 40)

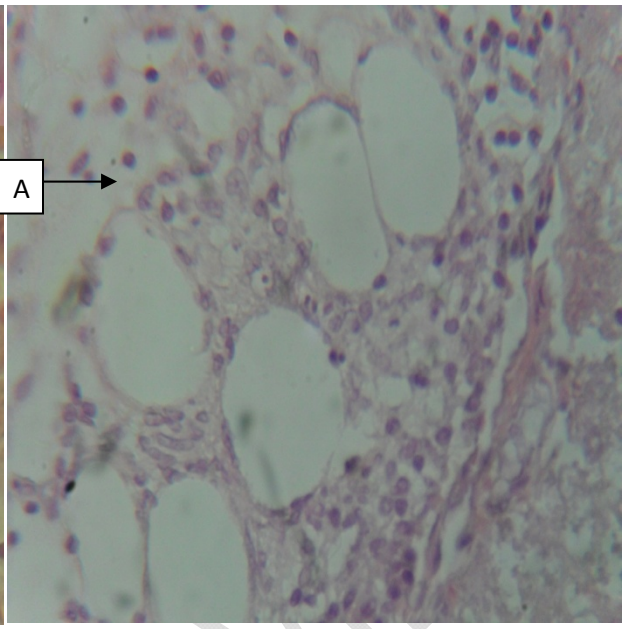

Plate 2. Rat Liver induced with CCl_4 (negative control) showing moderate macrovesicular steatosis A (H & E x 40)

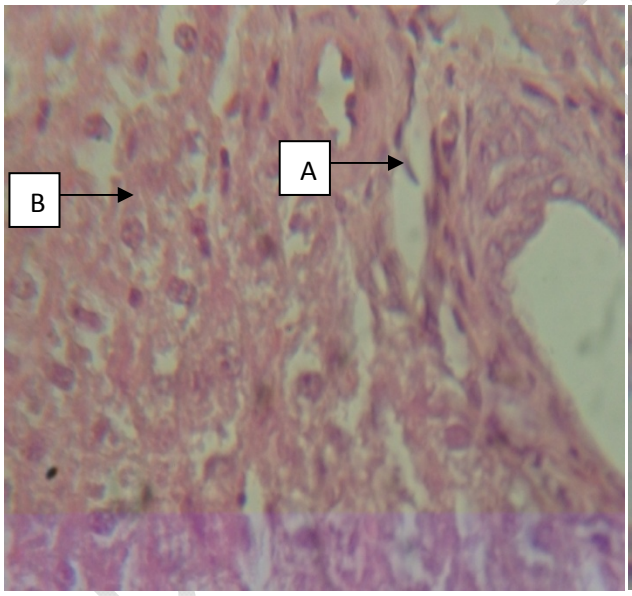

Plate 3. Rat Liver treated with crude plant leaf extract only (*Icacina trichanta*) showing unremarkable portal triad A and hepatocytes B (H & E x 40)

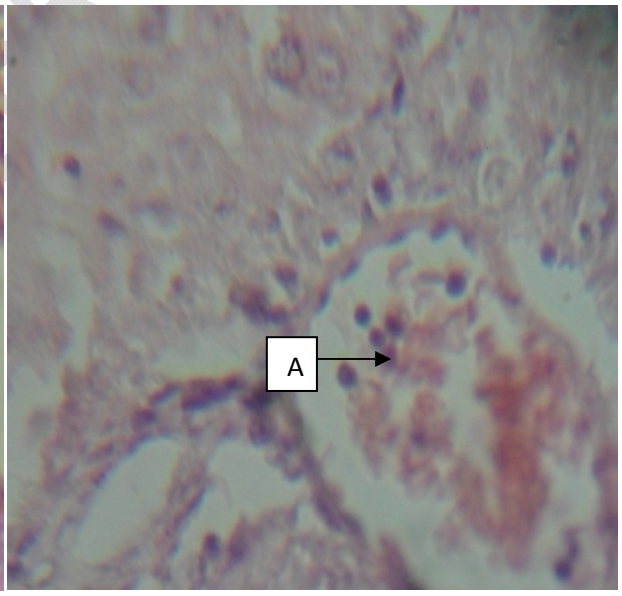

Plate 4. Rat Liver treated with CCl_4 and silymarin (100 mg/kg bw) showing mild portal congestion and dilatation A (H & E x 40)

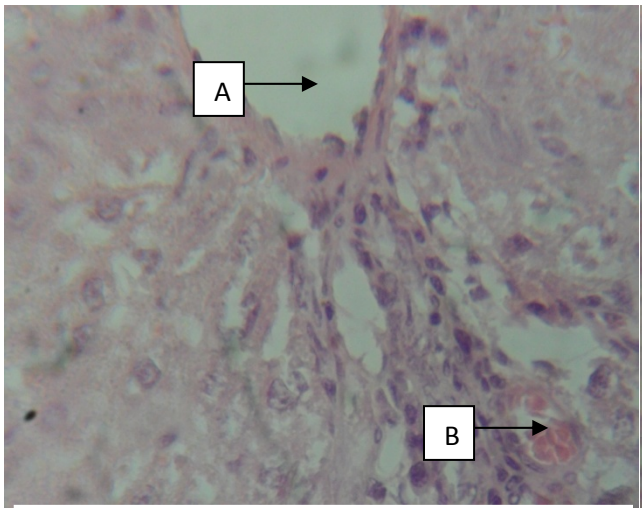

Plate 5. Rat Liver treated with CCl_4 and 200 mg/kg bwt aqueous leaf extract of *Icacina trichanta* showing mild portal dilatation A and vascular congestion B (H & E x 40)

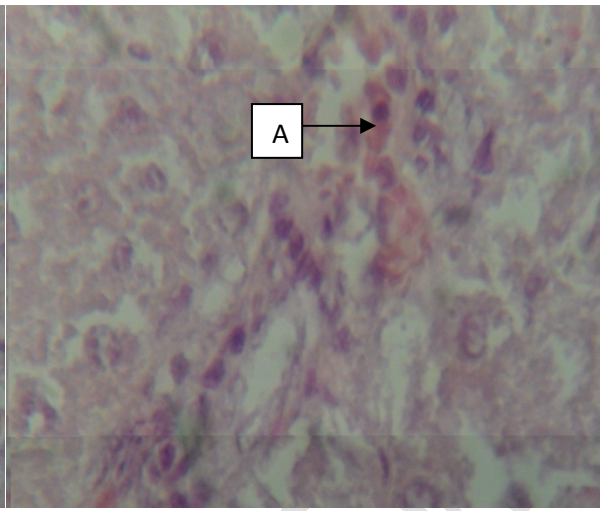

Plate 6. Rat Liver treated with CCl_4 and 300 mg/kg bw aqueous leaf extract of *Icacina trichanta* showing vascular congestion A (H & E x 40)

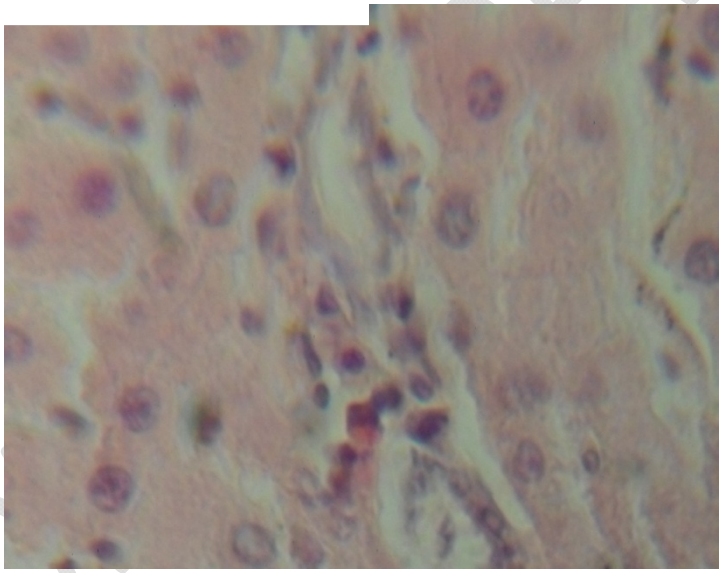

Plate 7. Rat Liver treated with CCl_4 and 400 mg/kg bw aqueous leaf extract of *Icacina trichanta* showing unremarkable liver architecture (H & E x 40)

4.8 Histological examinations of rats kidneys exposed to CCl_4, aqueous leaf extract of *I. trichanta* and silymarin

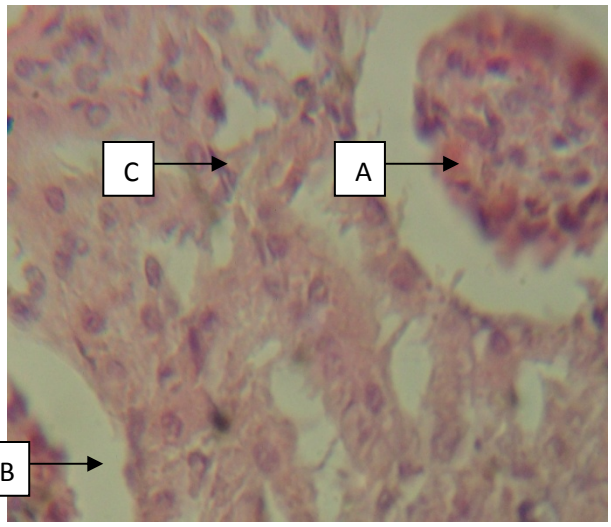

Plate 8. Control: Rat Kidney composed of glomeruli A, tubules B and interstitial space C (H & E x 40)

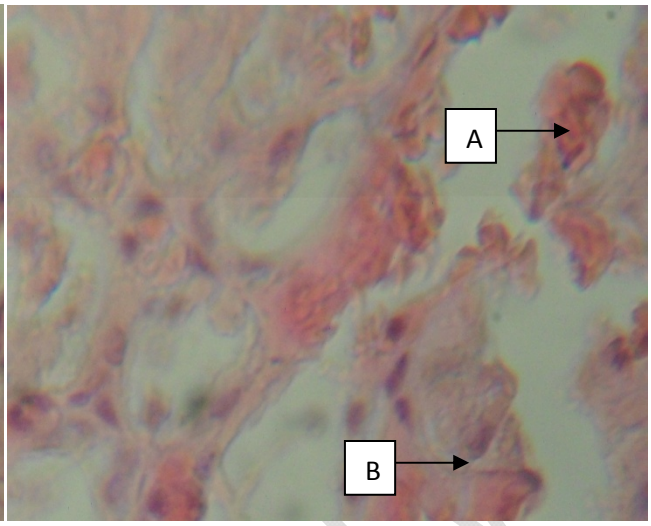

Plate 9. Rat Kidney induced with CCl_4 showing mild interstitial haemorrhage A and focal tubular necrosis B (H & E x 40)

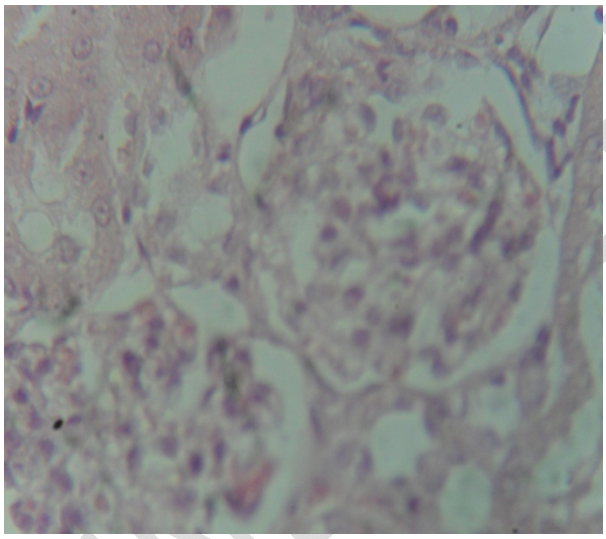

Plate 10. Rat Kidney treated with crude plant leaf extract only showing unremarkable nephron (H & E x 40)

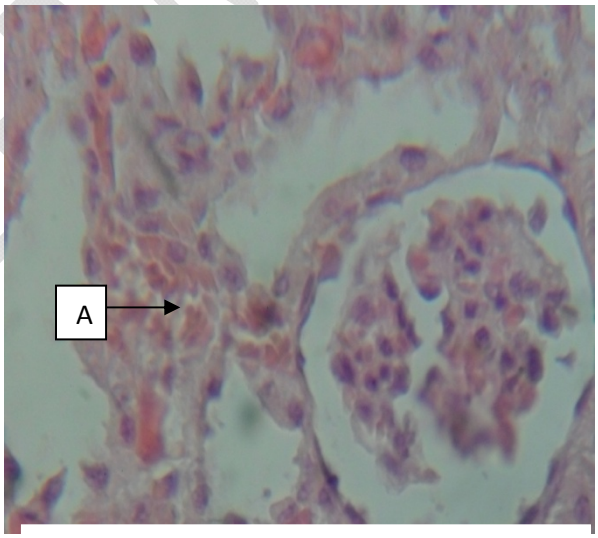

Plate 11. Rat Kidney treated with CCl_4 and silymarin (100 mg/kg bw) showing moderate interstitial congestion A (H & E x 40)

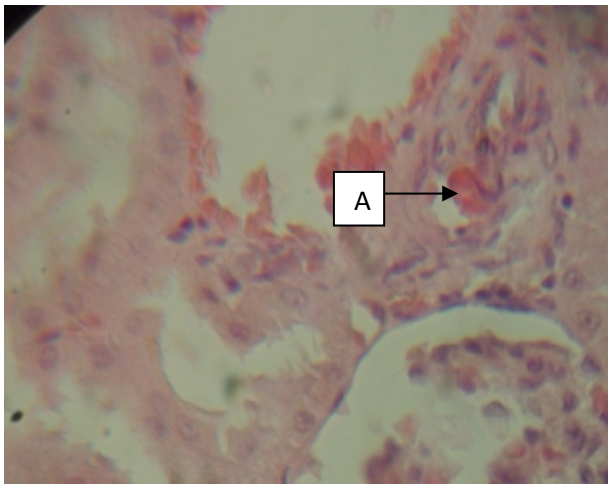

Plate 12. Rat Kidney treated with CCl_4 and 200 mg/kg bw aqueous leaf extract of *Icacina trichanta* showing mild interstitial congestion A (H & E x 40)

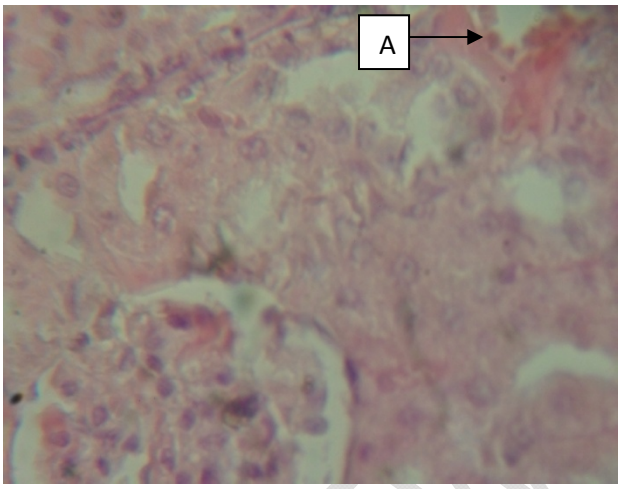

Plate 13. Rat Kidney treated with CCl_4 and 300 mg/kg bw aqueous leaf extract of *Icacina trichanta* showing mild interstitial congestion A (H & E x 40)

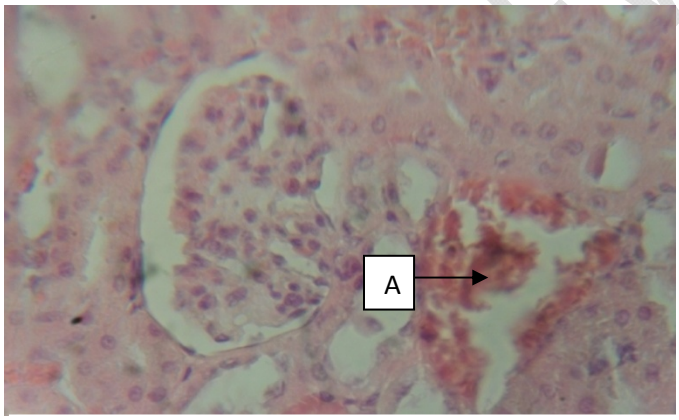

Plate 14. Rat Kidney treated with CCl_4 and 400 mg/kg bw aqueous leaf extract of *Icacina trichanta* showing mild interstitial congestion A (H & E x 40)

4.0 DISCUSSION

This study was undertaken to determine the effect of aqueous leaf extract of *I. trichanta* on lipid profile and CCl_4-induced histological changes in the liver and kidney of rats. In the present study, CCl_4 induction increases the synthesis of fatty acids and triacylglycerols from acetate. This could be due to the transport of acetate into the liver cell, resulting in increased substrate (acetate) availability. Venkatanarayana, *et al.* (2013) reported an elevated level of serum TC and TG in CCl_4 treated rats. In CCl_4 toxicity, the synthesis of cholesterol is increased (Boll *et al*., 2001). The liver plays an essential role in lipid metabolism, several stages of lipid synthesis and transportation. Therefore, it is reasonable to expect an abnormal lipid profile in those with severe liver dysfunction. There is a prominent decline in plasma TC and TG levels in patients with severe hepatitis and hepatic failure because of reduction in lipoprotein biosynthesis (Halsted, 2004).

In this study, there was significant reduction in the concentration of TG in the serum of CCl_4 + 200 mg/kg bw aqueous extract-treated rats. This could be due to the extract possessing hypocholesterolemic action at a lower dose (as opposed to higher doses) and this may be due to a decrease in the absorption of cholesterol or an increase in HDL-C (Kamalakkannan, *et al*., 2005; Shao, *et al*., 2012). The level of TC in the CCl_4 control group was significantly decreased when compared with the CCl_4 + 200 mg, 300 mg and 400 mg/kg bw groups, respectively. However, there were no significant differences in the levels of TC among the three groups containing graded doses of the aqueous extract. These values are comparable to that of silymarin group. These results appear to suggest that TC and LDL-C synthesis ability of the liver may be reduced with CCl_4 induction.

The protective property of the extract against CCl_4-induced oxidative stress in rats appears to be similar to those of silymarin and might be attributed to their antioxidant phytochemicals (Gazak, *et al*., 2007), which can prevent lipid peroxidation, changes in composition of membrane phospholipids, hepatic glutathione depletion and improve the functional markers of liver damage. Silymarin protects the liver against xenobiotic injury by controlling the liver secretion and uptake of plasma lipoprotein and increase the intracellular glutathione content with the scavenging of free radicals (Toklu, *et al*., 2008). Silymarin plays the role of an anti-inflammatory agent, through its ability to inhibit neutrophil infiltration and regulate the release of inflammatory mediators. Letteron *et al*., (1990) reported that silymarin prevents CCl_4-induced lipid peroxidation and hepatotoxicity in mice, firstly, by decreasing the metabolic activation of CCl_4 and secondly, by acting as a chain-breaking antioxidant. In addition, silymarin is able to stimulate protein synthesis resulting in production of new liver cells to replace older and damaged ones (Cecilia *et al*., 2009).

Histopathological studies provided supportive evidence for lipid profile analysis. Treatment with CCl_4 for 2 weeks (14 days) showed marked disruption of the structure of hepatocytes, induced steatosis (intrahepatocyte fat in-growth and inflammation) which was predominantly microvesicular. However, administration of graded doses of the aqueous leaf extract of *I. trichanta* showed marked regeneration of hepatocytes, which slightly affected the normal architecture of hepatocyte cords with few areas of discontinuity. Similarly, concurrent administration of CCl_4 and silymarin induced mild portal congestion and dilatation without any evidence of steatosis. Substituting silymarin with graded doses of aqueous leaf extract of *I. trichanta* (200 mg/kg bw, 300 mg/kg bw and 400 mg/kg bw) and concurrent treatment with CCl_4 produced mild portal dilatation and vascular congestion, as well as unremarkable hepatic lobular architecture. The 400 mg/kg bw dose, thus induced the best hepatoprotection.

In the kidneys, CCl_4 induced mild interstitial haemorrhage and focal tubular necrosis. Administration of silymarin, and graded doses of aqueous leaf extract of *I. trichanta* induced mild interstitial congestion and focal interstitial hemorrhage, with silymarin achieving the most remarkable nephroprotective effect.

5.0 CONCLUSION

The toxic hepatic injury induced by CCl_4 was blocked by treatment with graded doses of aqueous leaf extract of *I. trichanta*. However, the degree of hepatoprotection varied, with the 400 mg/kg bw producing the best protection.

6.0 REFERENCES

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