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Aqueous Leaf Extract of *Icacina trichanta* Oliv. Ameliorates CCl_4- Induced Liver Toxicity in Wistar Rats

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Abstract
This study was undertaken to determine the hepatoprotective effects of Icacina. trichanta over CCl_4-induced liver damage. Phytochemical analyses and determinations of ALT, AST, ALP, total protein, albumin, malondialdehyde, superoxide dismutase, catalase, vitamin E and A were carried out. Consequently, thirty-five male albino rats were divided into seven groups of five rats each. They were fed rats' chow and water ad libitum. Groups I (normal control) and III were not induced: while group I was not administered the extract, group III received 400 mg/kg b.wt I. trichanta extract. Groups II (negative control), IV, V, VI and VII were induced with 1.0 ml/kg b.wt of CCl_4. Group IV was administered 100 mg/kg b.wt of silymarin, while groups V, VI and VII were administered 200, 300 and 400 mg/kg b.wt of extract respectively. Results of phytochemical analyses revealed the presence of alkaloids, saponins, tannins and other polyphenolics in the aqueous extract. CCl_4 induction significantly raised the activities of liver function enzymes and lipid peroxidation status and this effect was ameliorated by both silymarin and the graded doses of extract. Levels of vitamin A and E were significantly raised by silymarin and the graded doses of extract when compared to the normal and negative controls. The graded doses of extract, particularly at 300 and 400 mg/kg b.wt dose levels compared favourably with silymarin in the treatment of liver damage.

Keywords: *Icacina trichanta*, Silymarin, Hepatoprotection, Phytochemicals, Lipid peroxidation

Introduction
The liver is a vital organ of the human body involved in metabolism, detoxication and excretion of various endogenous and exogenous substances. Certain medicinal agents, chemicals and even herbal remedies may cause liver injury. The use of conventional drugs for the management of liver disease suffers several adverse effects and this has led to increased dependence on complementary and alternative medicine ([1, 2]). Today, a substantial number of drugs are developed from plants ([3]) 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 ([4]). Different parts of *I. trichanta* are used for ethnomedicinal purposes: the tuber is used for the treatment of mumps ([5]), constipation, poisoning and malaria ([6]). Leaves of some species are known to have antiplasmodial activity. Silymarin is a single herbal drug formulation which is mostly used for the treatment of liver disease; the active principle has been isolated and chemically characterized ([7]). The active constituents of the plant consist of four flavolignans which are collectively known as the drug, silymarin. Silymarin is an antioxidant and free radical scavenger ([8]). This work compared the hepatoprotective properties of *I. trichanta* to those of this well researched hepatoprotective drug, silymarin.

Materials and Methods
Plant Collection and Preparation
Icacina trichanta leaves were obtained from a forest in Benin City and identified by Professor J. F. Bamidele of the 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 sundried, pulverized and sieved.

Extraction and Concentration
Extraction was by maceration over a 72 h period. Portion of the powdered leaf (100 g) was dissolved in 1000 ml of distilled water. The aqueous extract was filtered with a muslin cloth and freeze dried.

Qualitative Phytochemical Analysis
Qualitative Phytochemical analyses were carried out on the powdered sample to detect the presence of secondary plant metabolites using standard procedures ([9]).

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Methods

Experimental Design

A total of thirty-five adult male albino rats weighing between 180 and 200 g were randomly assigned to groups. The CCl_4 model described by Obi *et al* ([10]) was employed for induction of liver damage (1.0 ml/kg CCl_4 diluted in vegetable oil {1:1}). The rats were divided into seven groups of five rats each.

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

Group III: Rats in this group received 400 mg/kg b.wt aqueous extract of Icacina trichanta 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/kg b.wt silymarin for 14 days.

Group V: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 200 mg/kg b.wt aqueous extract of *I. trichanta*

Group VI: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 300 mg/kg b.wt aqueous extract of *I. trichanta*

Group VII: Rats in this group were induced with CCl_4: olive oil (1:1) and administered 400 mg/kg b.wt aqueous extract of *I. trichanta*

All the animals were allowed free access to food and water.

Blood and Tissue Sample Collection

At the end of the treatment, blood samples were collected by direct cardiac puncture into sterile containers with or without anticoagulant. The liver from both control and test animals were excised, washed in ice – cold saline, blotted dry and placed in plain containers. Liver homogenate was prepared in phosphate buffer 0.1 M, P^H 7.4 and used for biochemical analysis.

Biochemical Analysis

Catalase activity was determined by the method of Cohen *et al* ([11]), superoxide dismutase (SOD) activity by the method of Misra and Fridovich ([12]), and levels of malondialdehyde by the method of Guttridge and Wilkins ([13]). Plasma vitamin E concentration was determined by the method of Sauberlich *et al* ([14]). The method of Neeld and Pearson ([15]) was used to estimate plasma vitamin A concentration. Plasma activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured based on the colorimetric method of Reitman and Frankel ([16]) using Randox diagnostic kits. *Plasma Alkaline Phosphatase (ALP) activity* was estimated using the phenolphthalein monophosphate method ([17]). Plasma total protein was measured by the Biuret method ([18]), using Randox diagnostic kit. Plasma albumin concentration was estimated based on the Bromocresol Green dye – binding procedure ([19]).

Chemicals and Reagents

All reagents used were of analytical grade. ALT, AST, ALP, protein and albumin assay kits were products of Randox Laboratories Limited, UK. All other chemicals used were obtained from British Drug House (BDH), England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company, U.S.A.

Statistics

Data are presented as mean ± SEM and statistical analysis was performed using SPSS (19.0). Groups were compared using Duncan Multiple Test Range. Values of $p < 0.05$, were considered statistically significant.

Results

Table 1: Phytochemical constituents of the aqueous leaf extract of *I. trichanta*

Phytochemical	Inference
Alkaloids	Present
Flavonoids	Absent
Saponins	Present
Glycosides	Absent
Tannins	Present
Phenolics	Present

Phytochemical analysis of the aqueous extract of *I. trichanta* revealed the presence of alkaloids, saponins, tannins and phenolics

Table 2: Effects of aqueous leaf extract of *I. trichanta* and silymarin on organ weight in rats induced with CCl_4

Group	Liver weight(g)
I	5.94 ± 0.50^a
II	8.67 ± 0.70^b
III	7.44 ± 0.50^c
IV	8.39 ± 0.90^b
V	8.48 ± 1.10^b
VI	8.44 ± 0.80^b
VII	8.50 ± 0.90^b

Values with similar superscripts are insignificant and differ significantly ($p < 0.05$) from those with different superscript.

The negative control rats had significant increases in liver weight compared to the normal control. The reductions in liver weight observed in the silymarin control rats and those administered graded doses of the extract were not significant ($p > 0.05$) when compared to the negative control. Rats in group III that were administered extract only, no CCl4 induction also had significant increases ($p < 0.05$) in liver weight compared to control.

Table 3: Effects of aqueous leaf extract of *I. trichanta* and silymarin on activities of some liver enzymes in rats plasma induced with CCl_4

Group	ALT(U/L)	AST (U/L)	ALP(U/L)	Total Protein (g/dl)	Albumin (g/dl)
I	27.40 ± 0.90^a	47.17 ± 3.10^a	19.45 ± 0.26^a	75.37 ± 4.48^a	35.09 ± 2.10^a
II	40.50 ± 0.40^b	64.90 ± 5.40^b	74.30 ± 8.20^b	111.07 ± 12.30^b	27.08 ± 2.10^b
III	13.80 ± 0.30^c	37.90 ± 0.40^a	21.23 ± 0.30^a	57.43 ± 8.30^c	36.75 ± 0.60^a
IV	21.67 ± 0.30^a	56.67 ± 7.20^b	45.06 ± 3.10^c	80.20 ± 8.50^a	32.89 ± 3.30^a
V	28.40 ± 0.40^a	41.33 ± 3.33^a	57.80 ± 5.50^c	97.45 ± 7.40^b	32.05 ± 2.70^a
VI	20.50 ± 2.30^a	46.33 ± 4.09^a	55.75 ± 5.10^c	73.01 ± 5.60^a	33.29 ± 3.70^a
VII	23.50 ± 4.90^a	43.25 ± 2.00^a	58.80 ± 6.50^c	73.73 ± 9.90^a	35.21 ± 4.30^a

Similar superscripts denotes insignificance and different superscripts denotes significance at $p < 0.05$.

Group II (negative control) had significantly higher ($p < 0.05$) activities of ALT, AST, ALP and concentration of total protein; and lower levels of albumin compared to the normal control (group I). Group III rats (extract only, no CCl_4 induction) had significantly lower ($p < 0.05$) activity of ALT and total protein concentration compared to normal control, but differed insignificantly ($p > 0.05$) in AST, ALP and albumin relative to the normal control. Group IV rats (silymarin control) differed insignificantly ($p > 0.05$) in ALT and total protein, but had significantly higher ($p < 0.05$) AST and ALP activities compared to normal control. Compared to the negative control, group IV had significantly lower ($p < 0.05$) ALT, ALP activities and levels of total protein, and higher level of albumin, but differed insignificantly ($p > 0.05$) in AST activity. The rats administered graded doses of the extract (groups V, VI and VII) had significantly lower ($p < 0.05$) activities of ALT, AST, ALP and levels of total protein, and higher albumin levels compared to the negative control. Rats administered graded doses of the extract differed insignificantly ($p > 0.05$) in all parameters studied compared to the normal control, except in activities of ALP, which were significantly higher ($p < 0.05$) than in the normal control. Also, group V administered 200 mg/ kg I. trichanta had significantly higher ($p < 0.05$) total protein concentration compared to the normal control.

Table 4: Effects of aqueous leaf extract of *I. trichanta* and silymarin on the oxidative status of rats induced with CCl_4

Group	MDA (x 10^{-3})	SOD (x 10^{-5})	CAT (x 10^{-3})	Vit. A (mg/dl)	Vit. E (mg/dl)
I	1.67 ± 0.20^a	1.53 ± 0.20^a	1.24 ± 0.20^a	4.75 ± 0.60^a	39.72 ± 2.60^a
II	6.47 ± 0.70^b	2.07 ± 0.10^b	5.46 ± 0.40^b	2.57 ± 0.70^b	25.48 ± 2.10^b
III	1.70 ± 0.30^a	1.73 ± 0.10^a	1.77 ± 0.30^a	6.28 ± 0.20^c	31.33 ± 0.60^a
IV	3.26 ± 0.10^c	2.09 ± 0.10^b	2.56 ± 0.10^c	6.99 ± 0.10^c	38.08 ± 3.80^a
V	3.76 ± 0.20^c	1.74 ± 0.30^a	2.95 ± 0.50^c	6.38 ± 0.40^c	38.31 ± 3.70^a
VI	3.14 ± 0.10^c	1.96 ± 0.40^b	2.51 ± 0.70^c	6.87 ± 1.10^c	31.97 ± 3.90^a
VII	3.85 ± 0.10^c	1.99 ± 0.60^b	2.72 ± 0.50^c	6.98 ± 1.90^c	39.61 ± 3.30^a

Similar superscripts denotes insignificance and different superscripts denotes significance at $p < 0.05$. (SOD in units/mg protein, MDA in moles/mg wet tissue, CAT in units/mg protein).
Group II rats (negative control) had significantly higher ($p < 0.05$) MDA levels, SOD and catalase activities and significantly lower ($p < 0.05$) levels of vitamin A and vitamin E compared to the normal control. Group III rats (extract only, no induction) differ insignificantly ($p < 0.05$) from the normal control in activities of SOD, catalase and levels of MDA and vitamin E, but had significantly higher ($p < 0.05$) vitamin A levels. Group IV rats (silymarin control) had significantly lower ($p < 0.05$) MDA levels, lower catalase activity and significantly higher ($p < 0.05$) levels of vitamin A and E than in the negative control. Rats administered graded doses of the extract had significantly lower ($p < 0.05$) MDA level, lower catalase activity, but higher levels of vitamins A and E when compared to the negative control. Rats administered graded doses of extract differed insignificantly ($p < 0.05$) from the silymarin control in activities of SOD (with the exception of rats in group 1), catalase, and levels of MDA, vitamins A and E.

Discussion

Induction with CCl_4 increased the liver weights of rats; this effect was not ameliorated on administration of silymarin and graded doses of the extract. Rats administered only the aqueous extract of *I. trichanta*, no induction (group III) also had increase in liver weight. This set of rats (group III) was included in the experimental set – up in order to evaluate the safety of the plant extract at the highest dose of administration. The results of this study show that the plant extract may be safe at the highest dose of administration. Carbon tetrachloride is one of the most potent hepatotoxins which generates free radicals that trigger a cascade of events resulting in hepatic fibrosis ([10]).
The enzymatic activities of ALT, AST and ALP were studied to evaluate liver malfunctions; activities of liver enzymes are usually raised in acute hepatoxicity ([10]). Increase in ALT, AST and ALP activities on induction with CCl_4 is indicative of a hepatoxic effect ([20]). Rapid and extensive lipid peroxidation of the membrane lipids has been proposed as the basis of CCl_4 hepatocellular toxicity ([21]). Significant increases in the activities of ALT, AST and ALP observed in the negative control rats (group II) was indicative of the hepatotoxic effect of CCl_4.
A rather shocking observation was the unusually high activity of catalase observed in the negative control rats. Activity of SOD was also not significantly affected although non-enzyme antioxidants like levels of vitamin A and E were reduced compared to the normal control. The test rats were not significantly altered in activities of SOD and had significantly lower activities of catalase, but higher levels of vitamins A and E compared to the negative control. In spite of the high activity of catalase observed in the negative control rats, the lipid peroxidation status was high. The reason for the high activity of catalase is not known, however the high lipid peroxidation status of these rats (negative control rats) is in agreement with the results of other studies ([22, 23]). The increase in lipid peroxidation status shows that the abnormally high increase in activity of catalase did not produce any beneficial effect; it is not clear however, if excessively high activity of catalase can produce a pro-oxidant effect. Clinical trials with a limited number of antioxidants have shown that excess supplementation with certain antioxidants may be harmful ([24, 25]).
The effects of CCl_4 induction on the liver were ameliorated on administration of both silymarin and the graded doses of the extract. They significantly reduced activities of ALT, ALP and levels of MDA; and increased levels of vitamins A and E. Activities of AST were not significantly reduced in rats administered silymarin but were significantly reduced in rats administered graded doses of the extract; this could be an advantage in the use of *I.trichanta* over silymarin. These results are in agreement with those reported by Udeh and Nwaehujor ([26]).
The exact mechanism by which *I. trichanta* ameliorates CCl_4-induced hepatotoxicity is not known. However, the presence of some phytochemicals in the aqueous extract especially the polyphenols may be responsible for this. This is in line with an earlier report that there is a strong correlation between phenolic content and antioxidant activity ([27]).

Conclusion

I. trichanta is hepatoprotective; aqueous extract of this plant reversed CCl_4-induced liver damage in rats, and it compares favourably with silymarin in the treatment of liver damage.

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