Global Scientific JOURNALS

GSJ: Volume 8, Issue 1, January 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

THE EFFECT OF CHLOROFORM EXTRACT OF *CHASMENTHERA DEPENDENS* ON CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICITY

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ABSTRACT

This **Background:** *Chasmenthera dependens* have *been* reported to have many biological activities such as anti-fungal and anti-inflammatory activity. This study was aimed at evaluating the effect of the plant extract against CCl_4 induced hepatotoxicity.
Materials and Methods: Phytochemical and acute toxicity studies of the chloroform extract were carried-out. Twenty-four female albino rats divided into six groups of four rats each were used. Group I served as normal control. Groups II, III, IV, V and VI were injected (i.p) with CCl_4 (1ml/kg, 1:1 v/v CCl_4 and olive oil) every seventy-two hour for 10 days. Groups III, IV, V and VI were administered orally with 200, 400 and 600mg/kg of the extract and 200 mg/kg of Stalvan (standard drug) respectively. Group II served as the experimental control. After ten days, the animals were sacrificed. The serum and liver homogenate activities of alanine transaminase, aspartate transaminase, alkaline phosphatase and total bilirubin levels were measured. Data obtained were analyzed using SPSS version 20.0.
Results: The extract shows no lethality at 5000mg/kg of the extract. The phytochemical result showed the presence of phenolics, flavonoids, terpenoids, steroids, alkaloids, tannnins, carbohydrate and reducing sugar. The chloroform extract of *C.dependens* exhibited a strong hepato-protective as well as hepato-curative effect as it significantly ($p<0.05$) and dose-dependently reduced CCl_4 induced elevation of liver enzyme activities (AST, ALP and ALT) and total bilirubin levels.
Conclusion: The results of this study suggest that *C. dependens* extract exhibited strong inhibition against CCl_4 induced liver damage.

KeyWords
Carbon tetrachloride, hepatoprotective, hepatocurative, liver enzymes, *chasmenthera dependens*, hepatotoxicity, liver damage

Introduction

Liver is a vital organ that plays a major role in metabolism and excretion of xenobiotics from the body (Kumar, 2012). It has a wide range of functions, including detoxification, protein synthesis, and production of bio chemicals necessary for digestion. Liver diseases remain a public health challenge, for which the development of new pharmaceutical treatments is required (Nwidu *et al*., 2017). Generally, liver injury is considered a result of exposure to high levels of environmental toxins, which are associated with metabolic dysfunction, ranging from the transient elevation of liver enzymes to life-threatening hepatic fibrosis, liver cirrhosis and even hepatocellular carcinoma (Srivastava and Srivastava, 2018). Lots of liver damage, ranging from subclinical icteric hepatitis to necro inflammatory hepatitis, cirrhosis and carcinoma, has been proven to be associated with redox imbalance and oxidative stress (McGill *et al*., 2012; Roskams *et al*., 2003). Hence, antioxidants are frequently used to treat oxidative liver injury, and the consumption of antioxidants is known to be an important means of preventing or delaying the appearance of liver diseases (Huang *et al*., 2018). However, despite the frequent and numerous occurrence of liver damage, its high morbidity and high mortality rates, its medical management is currently inadequate. This is because inorganic drugs often have side effects; hence the need for research on suitable herbal drugs that could replace the chemical ones.

Chasmenthera dependens is a local climbing shrub that grows majorly in Savannah and forest regions of Anagola, Ethopia, Nigeria, Somalia, South Africa and Sierra Leone. It is a member of the family-Menispermaceae and locally known by different native names. *C dependens* is a medicinal plant used for the treatment of several diseases (Ogunlesi, *et al.,* 2008). In West Africa, the leaves and stem sap are locally applied to treat sprain and bruises. They are used as dressing for fractures or mixed with shear butter as an embalmment to treat pain and stiffness (Odugbemi, 2008). The leaves are used for the treatment of arthritis and rheumatism. The bark is chewed as a remedy for venereal discharges or as a general tonic for physical or nervous weakness causing inflammatory and exhausting illness (Okiei *et al*., 2009). The methanol extract of the dried leaves has also been reported to have analgesic and anti-inflammatory effect on laboratory animals (Morebise, *et al.,* 2001) while the aqueous and ethanol crude extract of the leaves have been reported to have anti fungal activity (Adekunle and Okoli, 2002). The constituents of the plant include: alkaloids, palmatine, colombamine and jateorhizine (Okiei *et al.,* 2009). A photochemical investigation of the stem led to the isolation of quaternary alkaloids including pseudocolumbamine, magnoflorine and non-phenolic alkaloids (Ogunlesi *et al.,* 2010). Berberine sulphate in the plant has been reported to inhibit leishmania. However, of all the works done on *C. dependens*, its effect on hepatotoxicity has not been researched.

Aims

The aim of this research is to evaluate the hepato-protective as well as the hepato-curative effect of the chloroform extract of *Chasmenthera dependens* root on CCl_4 induced hepatotoxicity.

Material and Methods

Animals

Wistar rats of both sexes weighing 103-200g were used for the experiment. They were obtained from the animal house of the department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka. The rats were feed *ad libitum* on water and grower mash purchase at the Nsukka market.

Plant Material

Chasmenthera dependens root were obtained from the environs of Nsukka, Nigeria and identified by Mr. Alfred Ozioko of the department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Voucher specimen was deposited in the herbaceous unit of the department for reference purposes.

Chemicals and Reagents

All chemicals used in this study were of analytical grade. The reagents used for all the assays were commercial kits.

Extraction

The roots of *Chasmenthera dependens* were dried under room temperature (25°C to 45°C) for two weeks, after which the roots were sliced into smaller bit and pulverized into coarse form with a milling machine. The coarse form was soaked in 50% Chloroform in a 3,000ml conical flask and the top was sealed with foil and masking tape to prevent evaporation. The system was swirled vigorously and allowed to stand for 48hours before filtering with the help of cotton wool and a white filter cloth. The resulting chloroform extract was concentrated and evaporated to dryness using a rotary evaporator at an optimum temperature of between 37°C and 40°C. The resulting extract was kept under fan and allowed to dry completely. The weight of the dry extract was determined.

Phytochemical Analysis

Preliminary phytochemical analysis was carried out on the chloroform extract of *Chasmenthera dependens* using standard methods.

Acute Toxicity Test

Locke's (1983) method was used to determine the acute toxicity of the extract. The extract was found to be relatively safe and doses of 200mg/kg, 400mg/kg and 600mg/kg were chosen as concentration of the extract to be administered to the rats.

Experimental Design

A modification of Karthikeyan and Deepa (2010) design was used. Twenty-four (24) Wistar rats of both sexes were randomly divided into six groups of four (4) rats each and housed in separate cages. The rats were acclimated for seven days and fasted for 12 hours

prior to the experiment. Group I served as normal control. Groups II, III, IV, V and VI were injected i.p with CCl_4 (1ml/kg, 1:1 v/v CCl_4 and olive oil) every seventy-two hour for 10 days. Groups III, IV, V and VI were administered orally with 200, 400 and 600mg/kg of the extract and 200 mg/kg of Stalvan (standard drug) respectively. Group II served as the experimental control. After ten days, the animals were sacrificed.

Preparation of serum

Bloods were obtained from the rats by eye puncture technique and collected into centrifuge tubes. Part of the liver was also collected from each rat and homogenized. The homogenate were put into a centrifuge tubes. Both the blood and the homogenized liver were centrifugated for 10minutes at 3000rev/hr in a bench centrifuge. The clear supernant was used for the biochemical analysis.

Biochemical Analysis

The serum and homogenate alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) were evaluated using Randox reagent enzyme kit based on Reitman and Frankel (1957) method and King and Kind (1954) method. Total bilirubin (T.Bil) was also estimated using reagent kit based on Jendrassic and Grof (1938) reaction.

Statistical Analysis

Data obtained were analyzed using SPSS version 20.0. All values are expressed as mean ± SD. Results were analyzed by one-way ANOVA and difference between means was assessed by Duncan's New multiple range $P>0.05$ were considered statistically significant.

Result

Acute Toxicity

Table 1: Result of acute toxicity test for the chloroform extract of *Chasmenthera dependens* root

Phase one

Groups	Number of Mice	Dose mg/kg	Dead (%)
1	3	10	0
2	3	100	0
3	3	1000	0

Phase two

Groups	Number of Mice	Dose mg/kg	Dead (%)
1	3	1600	0
2	3	2900	0
3	3	5000	0

The result of the toxicity test showed that the chloroform extract of *Chasmenthera dependens* has no toxic effect on the mice recorded for up to 500mg/kg body weight of the extract.

Phytochemical analysis of the chloroform extract of *Chasmenthera dependens* root.

Table 2: Result of phytochemical test of the chloroform extract of *Chasmenthera dependens* root

Phytochemicals	Qualitative Analysis	Quantitative Analysis
	Bioavaliability	Amount (mg/100g)
Alkaliods	+++	1975 ± 68.84
Carbohydrate	++	449.64 ± 59.30
Flavonoids	+++	319.79 ± 98.93
Glycoside	ND	-
Phenolics	++	4907.25 ± 75.33
Reducing Sugar	+	80.80 ± 36.19
Saponins	ND	-
Steriods	+++	4.55 ± 0.32
Tannins	++	22.47 ± 11.44
Terpenoids	++	674.51 ± 98.93

Values are mean ± SD (n=3)

+++ - Present in high concentration

++ - Present in moderate concentration

\+ - Present in small concentration

ND - Not Detected

The phytochemical study of the chloroform extract of *C. dependens* shows it contains a high concentration of flavonoids, steroids and alkaloids, a moderate concentration of phenolics, tannins, terpenoids and carbohydrates, and a trace quantity of reducing sugars.

Result of Biochemical Analysis

Table 3: Result of the effect of chloroform extract of *Chasmenthera dependens* root on biochemical parameters

	Group I	Group II	Group III	Group IV	Group V	Group VI
Parameters	Normal control	Experimental control	CCl_4 + 200mg/kg of extract	CCl_4 + 400mg/kg of extract	CCl_4 + 600mg/kg of extract	CCl_4 + 200mg/kg of stalvan (standard drug)
Serum Parameter						
AST	234.05 ± 8.505	371.50 ± 21.794 ^{abc}	300.75 ± 11.899 ^{ab}	302.75 ± 2.217 ^{ab}	279.00 ± 4.243 ^{abc}	304.75 ± 3.202 ^{ab}
ALT	64.25 ± 1.708	183.75 ± 6.344 ^{abc}	157.75 ± 2.363 ^{abc}	130.00 ± 3.916	129.75 ± 0.957 ^{ab}	126.00 ± 5.228
ALP	21.28 ± 1.636	42.08 ± 1.826 ^{abc}	33.20 ± 2.992 ^{abc}	27.78 ± 2.858 ^{abc}	19.33 ± 1.164 ^{b}	19.85 ± 1.399 ^{b}
T.Bil	0.51 ± 0.105	2.74 ± 0.097 ^{abc}	1.31 ± 0.117 ^{abc}	1.21 ± 0.041 ^{ab}	0.82 ± 0.109 ^{abc}	1.04 ± 1.399
Homogenate Parameter						
AST	334.50 ± 14.708	304.25 ± 18.118 ^{c}	303.25 ± 20.451 ^{c}	298.75 ± 5.679 ^{ac}	301.00 ± 4.546 ^{ac}	265.25 ± 15.756 ^{ab}
ALT	260.75 ± 5.679	257.25 ± 6.898	257.75 ± 14.728	266.25 ± 5.679	253.50 ± 12.50	268.75 ± 3.775
ALP	16.95 ± 2.149	13.48 ± 0.768 ^{c}	12.90 ± 0.648 ^{c}	18.18 ± 2.339	15.18 ± 3.796 ^{c}	20.40 ± 1.470 ^{b}
T.Bil	0.89 ± 0.267	2.91 ± 0.304 ^{abc}	1.62 ± 0.073	1.62 ± 0.078 ^{ab}	1.54 ± 0.067 ^{ab}	1.60 ± 0.210 ^{ab}

Values are mean ± SD; Results with different superscript (a, b, c) on the same row are statistically significant ($P < 0.05$)

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Serum AST Level.

The serum concentration of group II (Experimental control) was significantly higher than that of group I (normal control). The serum concentration of AST in groups III, IV and V (treated with 200mg/kg, 400mg/kg and 600mg/kg of extract after CCl_4 administration) reduced significantly when compared to group II. However, among the treated groups, only group V (treated with 600mg/kg of extract) reduced significantly when compared to group VI which was treated with standard drug. The serum concentration of AST in groups III, IV and V were significantly higher than that of group I.

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Serum ALT Level.

The serum concentration of ALT for group II (the experimental control) was significantly higher than those of group I (normal control). The serum concentration of ALT in groups III, IV and V reduced significantly when compared to group II (Experimental control). However, the ALT concentration of group VI which was treated with standard drug was significantly lower than those of group II and group III (treated with 200mg/kg of extract) but was not significantly different from those of groups IV and V (treated with 400mg/kg and 600mg/kg respectively). The serum concentration of ALT in both extract and standard drug treated groups (II, III, IV, V and VI) were found to be significantly higher than those of the normal control (group I).

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Serum ALP Level.

The serum concentration of ALP for group II (Experimental control) was significantly higher than that of group I (normal control). Treatment with the extract gave a significant reduction in serum ALP concentration when compared to group II. The ALP concentration of group VI which was treated with standard drug reduced significantly when compared to group II, III and IV but was not significantly different from groups V (treated with 600mg/kg). The serum concentration of ALP in group I was nevertheless significantly lower when compared to group III and IV, but was not significantly different from those of groups V and VI.

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Serum Total Bilirubin (T.Bil) Level.

The serum concentration of T.Bil for group II was significantly higher than that of group I. The serum concentration of both the extract and standard drug treated groups were significantly lower when compare to that of the group II. However, the serum concentration of T.Bil for group VI which was treated with standard drug were found to be significantly lower than that of group III (treated with 200mg/kg of extract) but not significantly different from that of group VI (treated with 400mg/kg of extract). The serum T.Bil concentration of group V (treated with 600mg/kg of extract) was also found to be significantly lower when compared to group VI

(treated with 200mg/kg of standard drug). Nevertheless, the serum concentration of T.Bil in groups III, IV, V and VI were significantly high when compared to group I.

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Homogenate ALP Level.
The homogenate concentration of AST for group II was not significantly different from that of group I. The homogenate concentration of groups II, III, IV and V were significantly high when compared to group VI. However, the homogenate concentration of groups IV, V and VI (treated with 400mg/kg, 600mg/kg and standard drug respectively) reduced significantly when compared to group I (Normal control).

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Homogenate ALT Level.
The homogenate concentration of ALT for all groups showed no significant difference from group I. Neither were the extract treated groups significantly different from group II (Experimental control) nor group VI (treated with standard drug).

The Effect of the Chloroform Extract of *Chasmenthera dependens* root on Homogenate ALP Level.
The homogenate concentration of ALP for group II was neither significantly different from group I nor the extract treated groups (groups III, IV and V). However the homogenate concentration of ALP for group VI treated with standard drug was significantly high when compared to groups II, III, and V.

The Effect of Chloroform Extract of *Chasmenthera dependens* root on Homogenate T.Bil
The homogenate concentration of T.Bil for group II was significantly high when compared to group I. The homogenate concentration of T.Bil for groups III, IV, V and VI reduced significantly when compared to group II. However, the homogenate concentration of T.Bil for group VI treated with standard drug was not significantly different from those of the extract treated groups (groups III, IV and V).

Discussion

CCl_4 is a well known hepatotoxic agent in laboratory animals (Domenicali *et al.,* 2009). Exposure to CCl_4 has been reported to induce free radical generation in the body tissues especially in the liver, kidney and blood (Pohl *et al*., 1984). The first metabolite of CCl_4; trichloromethyl radical, is believed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological conditions such as atherosclerosis, lipid peroxidation, cancer and cell apoptosis. Liver damage caused by acute exposure to CCl_4 shows clinical symptoms such as elevation of liver enzymes in the blood, liver inflammation and high serum concentration of bilirubin as seen in jaundice (Huang *et al.,* 2018). The liver enzymes found within organs are released into the blood stream following cellular necrosis and cell membrane permeability, and are used as diagnostics measures of liver damage (Anosike *et al.,* 2008). Results from this study showing a reduction in CCl_4 induced elevation of liver enzymes at the administration of *Chasmenthera dependens* extract suggest a protective effect of the extract against CCl_4 induced toxicity and therefore ameliorated liver damage.

In the acute toxicity study the LD50 of *Chasmenthera dependens* extract, no death was witnessed even with 5 times of the effective dose. This indicates the high margin of safety of the extract. Hence, it will be very efficient as a hepatoprotective and hepato-curative drug alternative since it will have no toxic side effect even at high dosage.

The antioxidant properties of the extract were evident in both the qualitative and quantitative phytochemistry of the extract (Table The phytochemical analysis of this work reveals that the chloroform extract of *Chasmenthera dependens* has high concentration of phenolics and flavonoids. Phenolics and flavonoids are natural antioxidants that help to scavenge reactive species in the body which can lead to oxidative stress (Sarian *et al*., 2017). Hence, these natural antioxidants help to protect the liver from damage as well as enhance the recovery of the liver by scavenging the offending metabolites.

Total bilirubin levels (Tab. 3) were significantly increased in the CCl_4 treated rats as compared to the control. The administration of the extract at all doses lead to a significant reduction ($P<0.05$) in both serum and liver homogenate concentrations of total bilirubin. Report from Anosike *et al.,* (2008) also showed a marked rise in bilirubin level after CCl_4 administration as seen in group two which is the experimental control. Bilirubin which is a major breakdown product of haemoglobin rises when there is liver injury or damage; leading to the discoloration of the skin known as jaundice (Yang *et al*., 2014). Elevation of total bilirubin which result from decrease uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct and conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts (Anosike *et al*., 2008). The reduction of CCl_4 induced increase in total bilirubin level by *C. dependens* extract further shows its protective as well as curative effect against CCl_4 induced liver toxicity. The extract perhaps protects the liver cell from damage and enhances liver cell regeneration. Hence, the extract up-regulates bilirubin uptake and conjugation by the liver and subsequent its secretion into the bile ducts.

The hepatoprotective and hepato-curative effect of the chloroform extract of *C. dependens* is further evident in the significant ($P < 0.05$) reduction of CCl_4 induced elevation in the levels of ALT, AST and ALP when compare with group II (the untreated group). Treatment with CCl_4 also induced elevation in serum enzyme concentration of ALT, AST and ALP in the study conducted by Anosike *et al*., (2008). In this study, although administration of extract reduced the serum concentration of AST, ALT and ALP significantly in the

treated groups when compare with the experimental control group, there was no significant difference in the liver homogenate enzyme level between the treated and experimental control groups.

The effectiveness of the extract increases as the dosage increases (Tab.3). The extract shows better hepatoprotective and hepatocurative effect at 600mg/kg body weight. The serum concentration of AST and total bilirubin when treated with 600mg/kg body weight (group V) were significantly more effective than the standard drug group used. Hence, from the knowledge of the phytochemistry of the extract it is obvious that flavonoids and phenolics are very effectively antioxidants in the treatment of hepatotoxicity.

Conclusion

The phytochemical analysis of the chloroform extract of Chasmenthera dependens show that it contains high concentration of flavonoids and phenols which are popularly known for their potency in scavenging free radicals. The extract show an execellent hepatoprotective and hepatocurative effect on CCl_4 induced hepatotoxicity from the results of this study. Since the extract recorded no death at high dosage of LD_{50} and the hepatoprotective effect of the extract increased as the dosage increases, *Chasmenthera dependens* extract would be an efficient alternative to chemical drugs in the treatment of hepatotoxicity.

Acknowledgment

The authors wish to Mr. Innocent Okagu, Mr. Alfred Oziko and Mr. Augustine Mmadueke for their invaluable support to the progress of this work.

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