

Hepatoprotective Effect of the Aqueous Leaf Extract of *Andrographis paniculata* Nees Against Carbon Tetrachloride – Induced Hepatotoxicity in Rats

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ABSTRACT: Hepatoprotective effect of the aqueous leaf extract of *Andrographis paniculata* was investigated against CCl₄ – induced hepatic injury in rats. Significant (P<0.05) increase of serum levels of alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin (TBL), direct bilirubin (DBL), total cholesterol (CHL), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and malondialdehyde (MDA) in CCl₄ intoxicated rats were restored to normal levels when treated with the extract and CCl₄. Significant (P<0.05) decrease of serum levels of total protein (TP), albumin (ALB), high density lipoprotein (HDL) and reduced glutathione (GSH) in CCl₄ intoxicated rats were restored to normal levels when treated with the extract and CCl₄. The LD₅₀ of the leaf extract was greater than 3000 mg/kg. The study demonstrated that *A. paniculata* possesses significant hepatoprotective effects and may be the source of lead compound in the management of liver diseases.

Key words: Hepatoprotective, *Andrographis paniculata*, Carbon Tetrachloride, Liver Disease.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion (Ahsan *et al.*, 2009). It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). So it has a surprising role in the maintenance, performance and homeostasis of the body (Ahsan *et al.*, 2009). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion of reduced glutathione levels. In addition, serum levels of many biochemical markers like transaminases, alkaline phosphatase, bilirubin, triglycerides and cholesterol are elevated in liver disease (Mascolo *et al.*, 1988). Liver diseases pose a serious challenge to international public health (Ahsan *et al.*, 2009). Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Rao *et al.*, 2005). Moreover, there are still no specific treatments in modern medicine that give protection to the liver against damage or help to regenerate hepatic cells (Chatterjee, 2000; Chattopadhyay, 2003).

Recently, the use of medicinal plants to cure various forms of liver diseases and dysfunctions is becoming increasingly popular and has received wide acceptance (Oyagbemi and Odetola, 2010). Moreover, a large number of medicinal plants have been found to offer some hepatoprotection (Handa and Sharma, 1990; Trivedi and Rawal, 2000; Ghosh *et al.*, 2007; Prakash *et al.*, 2008; Khan *et al.*, 2009; Ahsan *et al.*, 2009; Adeneye *et al.*, 2009; Oyagbemi and Odetola, 2010).

Andrographis paniculata Nees is a herbaceous plant, commonly known as "King of Bitters", in the family Acanthaceae (Jarukamjorn and Nemoto, 2008). *A. paniculata* grows erect to a height of 30 – 110 cm with glabrous leaves and white flowers with rose-purple spots on the petals (Sabu, 2006). It grows abundantly in southeastern Asia – India, Pakistan and Indonesia but is cultivated extensively in China, Thailand (Sandberg, 1994), the East and West Indies, and Mauritius (Gupta *et al.*, 1990). Because of its well – known medicinal properties, it is cultivated quite easily. *A. paniculata* has been used in Asia to treat variety of chronic and infectious diseases (Alpha Omega Labs, 2008). It has been traditionally used as a remedy against diabetes (Norhaida, 1994), hypertension (Ahmad and Asmawi, 1993), inflammation (Patarapanich *et al.*, 2007) and cobra bite (Selvanayagam *et al.*, 1994). Extensive

research revealed that *A. paniculata* has a surprisingly broad range of pharmacological effects and some of them are extremely beneficial and include anticancer (Kumar et al., 2004), anti HIV (Chang and Yeung, 1988), anti AIDS (Stephen and Comac, 2000), hepatoprotective (Handa and Sharma, 1990) and immunostimulatory (Puri et al., 1993). Other pharmacological effects include; antimalarial (Misra et al., 1992), antityphoid (Sabu, 2006), antiviral (Wiar et al., 2005), antifungal (Sabu, 2006), antibacterial (Mishra et al., 2009), antidiarrhoeal (Gupta et al., 1993), antipyretic (Vedavathy and Rao, 1991), anti-inflammatory (Chang and But, 1986), antidiabetic (Zhang and Tan, 2000), antithrombotic (Yeung et al., 1987), cardiovascular (Tan and Zhang, 2004), antivenom (Chang and But, 1986), antifertility (Sakila et al., 2009) and psychopharmacological activity (Mandal et al., 2001). *A. paniculata* may be a promising treatment for the alleviation of subjective symptoms of respiratory tract infections (Coon and Ernst, 2004). It may also be beneficial for those with chronic fatigue syndrome and fibromyalgia (Khan, 2007).

The aim of the present study was to determine the LD₅₀ and the hepatoprotective effect of the aqueous leaf extract of *A. paniculata* against carbon tetrachloride intoxicated rats.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *A. paniculata* plant were obtained from University Research Farm (Kwalkwalawa), Usmanu Danfodiyo University Sokoto. The plant was identified at Botany Unit, of the same Institution. A voucher specimen was also deposited in the Herbarium of the same Institution for reference.

Preparation of plant material

The leaves of *A. paniculata* were thoroughly rinsed in tap water twice. The leaves were open- air –dried under shade, cut into small pieces and pulverised into coarse powder (using pestle and mortar). The powder was stored in an airtight bottle until required.

Preparation of aqueous extract

Two hundred (200g) of the powdered sample was mixed with 2000ml of distilled water, in a conical flask. The mixture was stirred severally covered overnight at room temperature, filtered using Whatman filter paper (15 cm). The filtrate was evaporated to complete

dryness at 40°C, producing a fine and chocolate colour solid residue. The dried residue was scrapped, weighed and the percentage yield was calculated. The dried residue was stored in a capped bottle.

From the dried residue, a fresh solution was prepared on each day of the experiment.

Experimental animals

Wistar rats weighing between 180 to 200 g were purchased from Animal House, Department of Biological Sciences of Usmanu Danfodiyo University, Sokoto. The animals were kept in metal cages in a well ventilated room and allowed to acclimatize for 14 days. They were fed standard diet (Grand Cereals and Oil Mills Limited, Jos, Nigeria) and were provided clean tap water *ad libitum*. The experiment was performed according to ethical guidelines of OECD (2001).

Determination of LD₅₀

Aqueous leaf extract of *A. paniculata* (3000mg/kg body weight) was administered orally to five rats (one after the other at an interval of 48 hours) in a single dose using oro-gastric tube. The control group received distilled water. Observations of toxic symptoms were made and recorded systematically, at one, two, four and six hours after administration. The number of survivors was noted after 48 hours for each group of animals. The toxicological effects were assessed on the basis of mortality and expressed as LD₅₀ and calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD, 2001).

Hepatoprotective activity test

Carbon tetrachloride induction of hepatotoxicity was done according to procedures of Rao et al. (2005) with some modifications.

Experimental design

A total of 30 rats were used. The rats were randomly divided, into six groups of five rats each, as follows: Group A (normal control untreated rats) received 1ml daily dose of liquid paraffin (1ml/kg body weight, *per os*) for five days.

Group B, C, D, E, and F were administered standard diet and tap water on day one.

Group B (induction control) were administered 30% carbon tetrachloride in liquid paraffin (1ml/kg body weight, *i.p.*) for four days (from day two to five).

Group C received 30% CCl₄ in liquid paraffin (1ml/kg body weight, *i.p.*) and Silymarin, a known antihepatotoxic drug (Sigma Chemicals Company USA), at a dose of 100mg/kg, *per os*, for four days (from day two to five).

Groups D, E, and F (test groups) were treated with 30% CCl₄ in liquid paraffin (1ml/kg body weight, *i.p.*) and a daily dose of 100, 200 and 300 mg/kg body weight (orally) of aqueous leaf extract of *A. paniculata* respectively, for four days (from day two to five).

Clinical chemistry

On the sixth day, all the animals were sacrificed under chloroform anaesthesia and blood and liver samples were collected. The blood collected was allowed to clot for 30 minutes. Serum was separated by centrifuging at 3000 rpm for 5 minutes. The supernatant was collected using Pasteur pipette into the sample bottles. The serum was used for biochemical estimations. Section of the liver was perfused with cold 0.86% KCl, homogenised, and centrifuged to obtain post mitochondrial supernatant for estimation of liver reduced glutathione and malondialdehyde content. The activities of serum transaminases (ALT and AST), alkaline phosphatase, albumin, total protein, bilirubin (total and direct), total cholesterol, triglycerides and high density lipoprotein were assayed by Randox (assay kit) methods of Reitman and Frankel (1957), Sood (1999), Doumas *et al.* (1971), Gornall *et al.* (1949), Jendrassik and Grof (1938), Trinder (1969), Tietz (1990), and Lopez-Virella *et al.* (1977) respectively. Serum levels of low density lipoprotein and very low density lipoprotein were calculated by using Friedewald formula (Friedewald *et al.*, 1972). Post mitochondrial supernatant was used for the assay of reduced glutathione and malondialdehyde content by the methods of Patterson and Lazarow (1955) and Hartman (1983) respectively.

RESULTS AND DISCUSSION

Groups of rats treated with CCl₄ exhibited significant ($p < 0.05$) increase in the activity of alanine aminotransferase (ALT), aspartate amino transferase (AST), total bilirubin (TBL), direct bilirubin (DBL) and alkaline phosphatase (ALP) when compared to normal control rats. However, serum total protein (TP) and albumin (ALB) were significantly ($p < 0.05$) reduced in CCl₄- treated rats (Table 1).

Table 2 shows that groups of rats treated with CCl₄ demonstrated significant ($p < 0.05$) increase in the serum levels of total cholesterol (CHL), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and malondialdehyde (MDA) when compared to normal control rats. However, serum levels of high density lipoprotein (HDL) and reduced glutathione (GSH) were significantly reduced in CCl₄- treated rats.

Acute toxicity test at 3000 mg/kg body weight of aqueous leaf extract of *A. paniculata* produced no mortality after 48 hours of observation. The median lethal dosage (LD₅₀) of the aqueous leaf extract was therefore estimated to be greater than 3000 mg/kg body weight. The extract did not produce any negative behavioural changes such as restlessness, excitement, respiratory distress, convulsions or coma. However, a reduction in weight of the rats was observed. The reduction in weight might be due to reduced food and water intake, which might be secondary to feeling of fullness and loss of appetite after administration (Joseph *et al.*, 1989). Despite the listed side effects, the high value of the LD₅₀ showed that the aqueous leaf extract of *A. paniculata* was practically non-toxic.

Carbon tetrachloride is a simple molecule which, when administered to a variety of species, causes centrilobular hepatic necrosis and fatty liver. Low doses of carbon tetrachloride cause only fatty liver and destruction of hepatic cytochrome P-450. However, chronic administration or exposure leads to liver cirrhosis and in some instances liver cancer and kidney damage (Timbrell, 1987). Simultaneous administration of the plant extract and CCl₄ produced an effect that was almost similar to that produced by silymarin. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxin is the index of its protective value (Yadav and Dixit, 2003). The hepatotoxic effects induced by CCl₄ arise from its metabolite •CCl₃, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage (Bishayee *et al.*, 1995; Nan *et al.*, 2002). As shown in Table 1, induction of hepatic damage with CCl₄ was marked by a significant ($P < 0.05$) increase in serum levels of marker enzymes (ALT, AST and ALP) and bilirubin (TBL and DBL) in the induction control rats (Group B) when compared to normal control untreated rats (Group A).

Treatment with 100, 200 and 300 mg/kg (orally) of *A. paniculata* aqueous leaf extract was marked by significant ($P < 0.05$) decrease in serum levels of marker enzymes (ALT, AST and ALP) and bilirubin (TBL and DBL), in a dose – dependent manner, when compared to Group B values. Induction with CCl_4 was marked by a significant ($P < 0.05$) decrease in serum levels of total protein (TP) and albumin (ALB). Treatment with 100, 200 and 300 mg/kg (orally) of *A. paniculata* aqueous leaf extract was marked by significant ($P < 0.05$) increase in serum levels of TP and ALB, in dose – dependent manner, when compared to Group B. As shown by the results, CCl_4 doses induced acute hepatic damage as evidenced by a marked elevation in the serum levels of the liver enzymes, ALT, AST and ALP, and a significant decrease in the circulatory levels of TP and ALB, which are in conformity with earlier reports of the deleterious biochemical effects of CCl_4 on hepatic injury (Fadhel and Amran, 2002; Rajesh and Latha, 2004; Nagano *et al.*, 2007). It is well documented in literature that CCl_4 is metabolised by mixed – function oxidase system in the endoplasmic reticulum of the liver to the highly reactive trichloromethyl radical, and this reactive metabolite leads to auto – oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes both functional and morphological distortion of the cell membrane (Recknagel and Glende, 1973). The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum marker enzymes of acute hepatocellular damage namely ALT and AST, and ALP as a marker for hepatobiliary damage (Bhattacharyya *et al.*, 2003). However, of these marker enzymes, ALT is the most reliable. AST is known to be present in abundance in the cardiac muscle, skeletal muscle, kidneys and testes, and ALP is abundant in the growing bone. Thus, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of these enzymes (Friedman *et al.*, 1996). Extract treatment significantly attenuated the acute elevation of these Table 2 shows the dose – dependent preventive effect of oral doses of *A. paniculata* aqueous leaf extract against the deleterious effect of CCl_4 intoxication on the serum lipid profile. The liver is known to be involved in the syntheses of triglyceride and cholesterol which are synthesised from a substrate, acetyl CoA (produced through fatty acid oxidation) (West *et al.*, 1966). The hepatoprotective effects of the oral doses of *A. paniculata* aqueous leaf extract were determined using

serum triglyceride, total cholesterol, HDL, LDL and VLDL as measuring parameters of liver function since they are synthesised *de novo* in the liver. Significant ($P < 0.05$) increase in CHL, TG, LDL and VLDL was observed in the CCl_4 -intoxicated group. Significant ($P < 0.05$) decrease in HDL was observed in CCl_4 -intoxicated group. The inhibition of protein synthesis and disturbance of phospholipids metabolism might be responsible for the abnormal levels of lipoproteins in the serum. Treatment with aqueous leaf extract of *A. paniculata* significantly reversed these changes. This is an indication that the extract preserved hepatic protein synthesis and phospholipids metabolism.

Enzymes in dose – dependent manner; demonstrating *A. paniculata* aqueous leaf extract has hepatoprotective effect. CCl_4 induction was also associated with significant decrease in the serum levels of albumin and total protein. However, treatment with *A. paniculata* aqueous leaf extract protected the liver from the deleterious effect of the toxin by ameliorating the decrease in the circulatory levels of albumin and total protein in dose – dependent manner. CCl_4 induction causes degeneration of hepatocytes and blockade of the bile ducts which result into significant increase in the serum levels of total bilirubin and direct bilirubin (Saraswat *et al.*, 1993). Treatment with *A. paniculata* aqueous leaf extract normalised the elevated serum levels of total bilirubin and direct bilirubin. Thus, reduction in the levels of ALT and AST towards the normal value is an indication of regeneration process. Reduction in the levels of ALP, total bilirubin and direct bilirubin suggests the stabilisation of the biliary function. An increase in the serum levels of total protein and albumin suggests the stabilisation of endoplasmic reticulum, leading to protein synthesis. Reduced glutathione (GSH) is a naturally occurring substance that is abundant in many living creatures. GSH is an intracellular reductant and plays major role in catalysis, metabolism and transport (Ghosh *et al.*, 2007). GSH functions as free radical scavenger and in the repair of radical caused biological damage (Moron *et al.*, 1979). GSH, through its significant reducing power, contributes to the recycling of other antioxidants such as vitamins C and E that have become oxidised. Reduced GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4 (Kidd, 1997).

Table 1: Effect of aqueous leaf extract of *Andrographis paniculata* on serum liver function indices in CCl₄ – induced hepatic injury in rats

GROUP	Treatment	Dose (mg/kg)	ALT (U/l)	AST (U/l)	TP (g/dl)	ALB (g/dl)	TBL (µmol/l)	DBL (µmol/l)	ALP (U/l)
A	Paraffin	-	9.34 ± 0.26	9.56 ± 0.44	7.74 ± 0.10	4.18 ± 0.17	14.29 ± 0.24	2.46 ± 0.28	108.19 ± 3.20
B	-	-	14.66 ± 0.29 α	14.06 ± 0.38 α	5.65 ± 0.09 α	3.14 ± 0.17 α	21.87 ± 0.20 α	6.05 ± 0.23 α	304.15 ± 3.74 α
C	Silymarin	100	9.38 ± 0.22 β	9.94 ± 0.42 β	7.52 ± 0.08 β	4.15 ± 0.11 β	14.43 ± 0.13 β	2.51 ± 0.28 β	114.26 ± 3.96 β
D	APALE	100	10.98 ± 0.24 αβγ	11.02 ± 0.45 β	7.23 ± 0.08 αβ	3.84 ± 0.14 β	15.21 ± 0.19 β	3.89 ± 0.14 αβγ	142.97 ± 4.04 αβγ
E	APALE	200	10.53 ± 0.16 αβγ	10.70 ± 0.48 β	7.29 ± 0.13 αβ	3.94 ± 0.13 β	14.97 ± 0.31 β	3.54 ± 0.25 αβ	127.51 ± 3.54 αβ
F	APALE	300	10.05 ± 0.33 β	10.26 ± 0.56 β	7.38 ± 0.10 β	3.98 ± 0.16 β	14.58 ± 0.21 β	2.56 ± 0.23 β	120.34 ± 3.66 β

Values are means ± standard error of mean, n = 6, comparisons were made between:

"1"- Group A vs B, C, D, E and F; "2"- Group B vs C, D, E and F; "3"- Group C vs D, E and F, using Bonferroni multiple comparisons test.

APALE= *Andrographis paniculata* aqueous leaf extract.

ALT= Alanine transaminase, AST= Aspartate transaminase, TP= Total protein, ALB= Albumin, TBL= Total bilirubin, DBL= Direct bilirubin, ALP= Alkaline phosphatase.

α = represents statistical significance vs A: p<0.05

β = represents statistical significance vs B: p<0.05

γ = represents statistical significance vs C: p<0.05

Table 2: Effect of aqueous leaf extract of *Andrographis paniculata* on lipid profile, GSH and MDA in CCl₄-induced hepatic injury in rats

GROUP	Treatment	Dose (mg/kg)	CHL (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	GSH (mg/dl)	MDA (nmol/ml)
A	Paraffin	-	120.89 ±	116.40 ±	46.09 ±	51.53 ±	23.28 ±	218.57 ±	3.22 ± 0.02
B	-	-	212.44 ±	207.21 ±	31.44 ±	138.97 ±	41.44 ±	122.38 ±	5.53 ± 0.02 α
			2.29 α	3.27 α	1.20 α	2.50 α	0.65 α	3.58 α	
C	Silymarin	100	121.33 ±	119.28 ±	42.70 ±	54.78 ±	23.85 ±	215.71 ±	3.26 ± 0.02 β
			3.49 β	2.98 β	0.65 β	3.66 β	0.60 β	2.57 β	
D	APALE	100	147.56 ±	140.54 ±	41.35 ±	78.09 ±	28.11 ±	255.24 ±	3.47 ± 0.02
			2.69 αβγ	2.85 αβγ	0.81 αβ	2.48 αβγ	0.57 αβγ	2.95 αβγ	αβγ
E	APALE	200	144.44 ±	138.38 ±	41.81 ±	74.96 ±	27.68 ±	264.29 ±	3.42 ± 0.02
			6.00 αβγ	3.34 αβγ	0.72 αβ	6.48 αβγ	0.67 αβγ	2.26 αβγ	αβγ
F	APALE	300	143.56 ±	137.30 ±	42.48 ±	73.62 ±	27.46 ±	304.28 ±	3.31 ± 0.02 αβ
			3.95 αβγ	2.57 αβγ	0.85 β	3.06 αβγ	0.51 αβγ	4.66 αβα	

Values are means ± standard error of mean, n = 6, comparisons were made between:

"1"- Group A vs B, C, D, E and F; "2"- Group B vs C, D, E and F; "3"- Group C vs D, E and F, using Bonferroni multiple comparisons test.

APALE= *Andrographis paniculata* aqueous leaf extract.

CHL= Total cholesterol, TG= Triglycerides, HDL= High density lipoprotein, LDL= Low density lipoprotein, VLDL= Very low density lipoprotein, GSH= Reduced glutathione, MDA= Malondialdehyde.

α = represents statistical significance vs A: p<0.05

β = represents statistical significance vs B: p<0.05

γ = represents statistical significance vs C: p<0.05

A significant ($p < 0.05$) decrease in hepatic tissue GSH level was observed in the CCl_4 treated group while there was significant ($p < 0.05$) increase in GSH level in the groups treated with the plant extract. Hence, exogenous aqueous leaf extract of *A. paniculata* supplementation might provide the means of recovering reduced GSH levels to prevent hepatic injuries. The increase in hepatic GSH level in rats treated with the extract and CCl_4 might be due to *de novo* synthesis or GSH regeneration.

The level of lipid peroxides is a measure of membrane damage and alterations in structure and function of cellular membranes. A significant ($p < 0.05$) elevation of lipid peroxides in the liver of rats treated with CCl_4 was observed. The increase in MDA levels in the liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals (Achiliya *et al.*, 2004). Treatment with aqueous leaf extract of *A. paniculata* significantly reversed these changes.

It has been earlier reported that decreasing the metabolic activation of carbon tetrachloride, the antioxidant activity, prevention of generation of reactive oxygen species and scavenging of generated free radicals or by combination of these are important mechanisms in the protection against CCl_4 -induced hepatic lesion (Yutin *et al.*, 1990; Bhattacharyya *et al.*, 2003). The biochemical and antioxidant findings obtained suggest that the extract might be mediating its protective effects either by decreasing the metabolic activation of carbon tetrachloride, or by acting as a chain – breaking antioxidant for scavenging free radicals or by combination of these effects. Moreover, previous studies have reported that protective effects of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities (Lanhers *et al.*, 1991; Adeneye *et al.*, 2009). The presence of secondary metabolites might thus be accounting for the hepatoprotective effect of *A. paniculata* aqueous leaf extract and could be via antioxidant or free radicals scavenging activities.

The study demonstrated that *A. paniculata* possesses significant hepatoprotective effects and may be source of lead compound in the management of liver diseases. Further studies regarding the isolation and

characterisation of the active principles responsible for hepatoprotective properties are recommended.

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