African Scientist Vol. 24, No. 4 December 31, 2023
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2023060/24407

Hepato-Protective and Curative Activities of Leaf Extracts of *Andrographis paniculata* in Carbon Tetrachloride-Induced Damage in Experimental Male Rats

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(Received December 14, 2023; Accepted in revised form December 20, 2023)

ABSTRACT: The liver is a vital organ in the body that performs many functions. Many of these functions generate free radicals, which degenerate to loss of liver function. The antioxidants in the body neutralize the effect of the free radicals generated. The body is healthy when the antioxidants adequately neutralize free radicals. Carbon tetrachloride is usually used to model experimental liver damage in animals. This research investigated the possible protective and curative effect of the aqueous and ethanol extracts of *Andrographis paniculata* leaf on CCl_4-induced liver damage in male rats. Liver damage was induced by intraperitoneal injection of 1.25 ml CCl_4 in olive oil (1:1 v/v) per kilogram body weight of animals. The animals were treated with 300 mg of the aqueous and ethanol extracts of *A. paniculata* per kg body weight of animals, pre-and post-CCl_4 administration. The levels of total protein, albumin and reduced glutathione were reduced. Also, the activities of SOD, CAT and GPx were reduced, while the levels of MDA and total bilirubin were elevated. These alterations were reversed in the animals administered with the extracts of *A. paniculata*. This study suggested the protective and curative potential of *A. paniculata* extracts in CCl_4-induced liver damage.

Keywords: Liver damage, CCl_4, SOD, Total protein, MDA

Introduction

The liver is a crucial organ that functions in many life processes, including digestion, excretion, immunity, synthesis of plasma proteins, metabolism of xenobiotics, and vitamin storage (Allameh *et al*., 2023). Many processes in the liver lead to the generation of free radicals that damage the anatomy and physiology of the liver (Upadhyay *et al*., 2022). Due to its multiple functions, the liver is an object of toxicity as a result of the unchecked production of free radicals (Upadhyay *et al*., 2022). Liver diseases are common causes of death worldwide, and medicinal plants with protective and curative potential on the liver have been sourced (Ouassou *et al*., 2021).

Carbon tetrachloride (CCl4) is a synthetic, colourless, nonflammable liquid with a pleasant smell. It has been used as a solvent, pesticide, cleaning agent, degreasing agent, and in refrigerant production, propellant for aerosol cans, fire extinguishers and spot removals. Carbon tetrachloride was phased out due to its toxic effects (NCBI, 2024). It is highly toxic to the liver, kidney and other body organs. Hence, it is employed in the induction of experimental liver and kidney damage (El-Haskoury *et al*., 2021). Dehalogenation of CCl_4 by Cytochrome P450 to reactive trichloromethyl and trichloromethyl peroxyl radicals triggers the peroxidation of membrane lipids, which initiates organ damage (El-Haskoury *et al*., 2021).

Andrographis paniculata is an invasive plant in the Acanthaceae family found throughout Asia, some parts of America and Africa (Phuong *et al*., 2023). The plant is found in various locations: beaches, plains, hills,

cultivated and disturbed regions, growing up to a height of over one metre, leaf length between two and twelve centimetres, and flowering between December and April (Hossain *et al*., 2021). *A. paniculata* has been used in the various systems of folk medicine as a treatment for malaria, cold, cough, vitiligo, scabies, liver problems, hypertension, diabetes, diarrhoea, anorexia, indigestion, splenomegaly, helminthiasis, pelvic inflammation, cervical erosion, chicken pox, hepatitis, leprosy, gonorrhoea, herpes zoster, peptic and neonatal subcutaneous annular ulcer (Boopathi, 2000; Sheeja *et* al., 2007; Jarukamjorn *et* al., 2008; Akbar, 2011; Kabir *et al*., 2014).
The extracts of *A. paniculata* have been reported to possess antidiabetic, antioxidant, *in vitro* α-amylase inhibitory, and *in vitro* and *in vivo* anti-plasmodial activities (Owoade *et al*., 2021; Nonso *et al*., 2023; Isunu *et al*., 2023). Some compounds isolated from *A. paniculata* have been reported to possess pharmacological activities: neoandrographolide (hepatoprotective, antioxidant, antiparasitic, anti-herpes simplex virus) (Batkhuu *et al*., 2002); andrographolide (analgesic, antipyretic, antiulcerogenic, antiretroviral, anticancer, antidiabetic, antiinflammatory, immunomodulatory, cardioprotective and hepatoprotective) (Cheung *et al*., 2005; Lin *et al*., 2008); and 14-deoxyandrographolide (vasorelaxant, uterine smooth muscle relaxant, hypotensive, hepatoprotective, platelet activating factor antagonist) (Burgos *et al*., 2005).
The present study is aimed at the assessment of hepato-protective and curative effects of leaf extracts of *Andrographis paniculata* in carbon tetrachloride-induced damage in male rats.

Materials and methods

Collection of plant: The leaves of *A. paniculate* were collected from a farm in Ekosodin village, Benin City, Edo State, Nigeria. The plant was identified and authenticated by Prof. Emmanuel I. Aigbokan in the Department of Plant Biology & Biotechnology, University of Benin, Benin City. For future reference, a herbarium sample with voucher number UBH-A599 was deposited in the Herbarium of the University of Benin, Nigeria.
Preparation of aqueous extract: Fresh leaves of *Andrographis paniculate* were air-dried for two weeks and milled into fine powder. The aqueous extract was prepared by boiling 1000 g of the powdered leaves in 5000 ml of distilled water on medium heat for 15 minutes. The extract was filtered out using a clean muslin cloth and concentrated under low heat until a sticky sample was recovered, which was further air-dried. The ethanol extract was prepared by macerating 1000 g of the milled leaves in 5000 ml of local gin for 72 hours (3 days) with consistent stirring for 30 minutes daily. The mixture was then filtered using a clean muslin cloth and evaporated to dryness under low heat to get the extract.
Animal study: Forty male rats were obtained from the Animal house of the Department of Anatomy, University of Benin, Nigeria. The animals were allowed to acclimatize for seven days in the animal house of the Department of Biochemistry, University of Benin. The animals were allowed free access to food and water *ad libitum*. Good hygiene was maintained by constantly cleaning and removing faeces and spilt feeds from cages daily. The animals were handled following the European Convention for the Protection of Vertebrate Animals guidelines and other scientific purposes (ETS-123, 2005).
Induction of liver damage: After acclimatization, liver damage was induced in the experimental animals by intraperitoneal injection of carbon tetrachloride in olive oil (1:1 v/v) at a dose of 1.25 ml/kg rat body weight.
Experimental design: The forty male rats were divided into eight groups of five animals each and administered as shown below:

S/N	Group	Treatment
1	(Normal control)	-
2	(CCl_4 control)	1.25 ml/kg body weight of carbon tetrachloride once on day 1
3	(Aqueous extract)	300 mg/kg body weight of the aqueous extract of *Andrographis paniculate* once daily for 7 days orally
4	(Ethanol extract)	300 mg/kg body weight of the ethanol extract of *Andrographis paniculate* once daily for 7 days orally
5	(Post-treatment) (CCl_4+Aqueous. Extract)	1.25 ml/kg body weight of carbon tetrachloride once on day 1 and 300 mg/kg body weight of the aqueous extract of *A. paniculate* once daily for 7 days orally
6	(Post-treatment) (CCl_4 +Ethanol Extract)	1.25 ml/kg body weight of carbon tetrachloride once on day 1 and 300 mg/kg body weight of the ethanol extract of *A. paniculate* once daily for 7 days orally
7	(Pre-treatment) (Aqueous extract *A. paniculate* + CCl_4)	300 mg/kg body weight of the aqueous extract of *Andrographis paniculate* once daily for 7 days orally + 1.25 ml/kg body weight of carbon tetrachloride once
8	(Pre-treatment) (Ethanol extract *A. paniculate* + CCl_4)	300 mg/kg body weight of the ethanol extract of *Andrographis paniculate* once daily for 7 days orally + 1.25 ml/kg body weight of Carbon tetrachloride once

At the end of the administration, the animals were fasted overnight and sacrificed under chloroform anaesthesia. Blood was withdrawn through venous puncture into plain bottles and centrifuged at 3000rpm for 15 minutes to obtain the serum. The liver of each animal was excised, blot-dried, weighed and homogenized in Tris-HCl buffer (pH 6.8).

Biochemical assays: The serum levels of total cholesterol, total protein, albumin, total bilirubin, conjugated bilirubin, and the serum activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase were determined by using diagnostic reagent kits. The activities of glutathione peroxidase, glutathione–s-transferase, catalase, and superoxide dismutase were assessed by the methods described by Nyman **(**1959**)**, Habig *et al*. (1974), Cohen *et al*. (1970) and Fridovich (1989) respectively. The levels of MDA and GSH were determined according to the methods of Buege and Aust (1978) and Ellman (1959), respectively.

Data analysis: The data obtained in this study were expressed as Mean ± SEM. Duncan's Multiple Range Test separated the homogeneity of the mean, and the significance level was set at $P<0.05$. All statistical analyses used Statistical Package for Social Sciences (SPSS) version 21.

Results

There was a significant ($P<0.05$) reduction in the body weights of animals pre-administered with CCl_4 before the extracts of *A. paniculata.* In contrast, there was a significant ($P<0.05$) increase in the body weights of animals pre-administered with the extracts of *A. paniculata* when the initial and final body weights were compared (Table 1). The CCl_4 group had reduced liver weight when compared with the control group.

Total cholesterol and bilirubin levels were elevated in the serum of animals administered with CCl_4. In contrast, the total protein and albumin levels were reduced when compared with the control. The animals administered with the extracts of *A. paniculata* and those pre-administered with *A. paniculata* had reduced cholesterol levels compared to the CCl_4 group (Table 2).

Table 1: Effects of the extracts of *A. paniculata* on the body weight and weight of liver

Groups	Initial weight (g)	Final weight (g)	% difference in weight	Weight of liver (g)
Control	188.95 ± 12.53^{d}	196.29 ± 13.63^{d}	3.84 ± 1.70^{b}	6.17 ± 0.57^{c}
CCl_4	145.70 ± 1.55^{a}	146.71 ± 2.77^{a}	0.70 ± 1.80^{b}	5.31 ± 0.14^{a}
Aqueous extract *A. paniculata*	164.88 ± 1.68^{c}	159.90 ± 3.30^{b}	-2.95 ± 2.67^{a}	6.48 ± 0.15^{d}
Ethanol extract *A. paniculata*	155.34 ± 0.98^{b}	163.25 ± 3.32^{b}	5.11 ± 2.28^{b}	5.18 ± 0.13^{a}
CCl_4 + Aqueous extract *A. paniculata*	185.83 ± 1.90^{d}	168.09 ± 6.23^{c}	-9.49 ± 3.74^{a}	7.36 ± 0.36^{e}
CCl_4 + Ethanol extract *A. paniculata*	174.01 ± 1.28^{d}	169.72 ± 7.01^{c}	-2.42 ± 4.29^{a}	6.20 ± 0.31^{c}
Aqueous extract *A. paniculata* + CCl_4	166.14 ± 1.94^{c}	169.25 ± 4.87^{c}	1.89 ± 2.88^{b}	5.74 ± 0.29^{b}
Ethanol extract *A. paniculata* + CCl_4	174.66 ± 0.98^{d}	186.60 ± 3.50^{d}	6.86 ± 2.19^{b}	6.41 ± 0.16^{d}

Values are expressed as Mean ± SEM, n=5. Values with different superscripts in the same column are significantly different from one another.

Table 2: Effects of the Extracts of *Andrographis paniculata* on the levels of total cholesterol, total protein, albumin and bilirubin

Groups	Total cholesterol (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Unconjugated bilirubin (mg/dl)
Control	69.18 ± 6.03^{c}	9.25 ± 0.25^{b}	3.36 ± 0.10^{c}	1.33 ± 0.02^{b}	1.29 ± 0.02^{c}	0.04 ± 0.03^{a}
CCl_4	76.62 ± 1.44^{c}	7.57 ± 0.81^{a}	2.53 ± 0.01^{a}	1.59 ± 0.22^{d}	1.18 ± 0.14^{c}	0.42 ± 0.08^{b}
Aqueous extract *A. paniculata*	41.25 ± 2.46^{a}	11.19 ± 0.97^{c}	2.65 ± 0.10^{b}	1.48 ± 0.22^{c}	0.30 ± 0.05^{a}	1.18 ± 0.26^{d}
Ethanol extract *A. paniculata*	54.56 ± 3.90^{b}	11.84 ± 0.08^{c}	2.68 ± 0.20^{b}	1.54 ± 0.29^{d}	0.60 ± 0.15^{b}	0.95 ± 0.39^{c}
CCl_4 + Aqueous extract *A. paniculata*	85.82 ± 2.87^{d}	6.98 ± 0.29^{a}	2.88 ± 0.49^{b}	1.17 ± 0.02^{a}	0.20 ± 0.00^{a}	0.97 ± 0.03^{c}
CCl_4 + Ethanol extract *A. paniculata*	71.51 ± 2.72^{c}	9.14 ± 1.54^{b}	2.79 ± 0.14^{b}	1.56 ± 0.29^{d}	0.83 ± 0.04^{b}	0.72 ± 0.25^{c}
Aqueous extract *A. paniculata* + CCl_4	53.60 ± 4.79^{b}	15.15 ± 0.04^{d}	3.12 ± 0.20^{c}	1.67 ± 0.08^{d}	1.10 ± 0.15^{c}	0.57 ± 0.13^{b}
Ethanol extract *A. paniculata* + CCl_4	71.10 ± 3.01^{c}	9.06 ± 1.37^{b}	2.54 ± 0.29^{a}	1.20 ± 0.06^{a}	0.74 ± 0.02^{b}	0.45 ± 0.07^{b}

Values are expressed as Mean ± SEM (n=3).

Elevated ALT, AST, ALP, and GGT activities were observed in the CCl_4 group compared to the control group (Table 3). The groups pre- and post-treated with the extracts of *A. paniculata* had reduced activity of ALT, except for the group pre-treated with the ethanol extract, which had a slightly higher activity of ALT than the CCl_4 group. There was a reduction in the activity of AST in the groups pre-treated with the extracts of *A. paniculata*. Except for the aqueous extract pre-treated group, the activity of ALP was reduced in the pre-treated groups. The activity of GGT was elevated in the CCl_4 group when compared with the control. The extracts reduced the activity of GGT in the pre-and post-treatments compared with the CCl_4 group.

Table 3: Effects of the extracts of *A. paniculata* on the activities of ALT, AST, ALP and GGT in the serum

Groups	ALT (u/l)	AST (u/l)	ALP (u/l)	GGT (u/l)
Control	21.09 ± 2.89^a	90.26 ± 2.28^b	53.56 ± 0.34^b	19.69 ± 0.00^a
CCl_4	42.72 ± 4.36^c	149.74 ± 9.27^d	59.39 ± 2.61^b	64.85 ± 0.00^e
Aqueous extract *A. paniculata*	22.03 ± 0.38^a	106.32 ± 2.11^c	55.79 ± 2.53^b	50.95 ± 2.91^d
Ethanol extract *A. paniculata*	28.70 ± 5.66^b	107.37 ± 1.82^c	49.07 ± 2.10^a	48.25 ± 6.35^d
CCl_4 + Aqueous extract *A. paniculata*	30.96 ± 0.00^b	170.70 ± 9.30^e	49.69 ± 2.30^a	28.18 ± 1.68^b
CCl_4 + Ethanol extract *A. paniculata*	39.39 ± 2.02^c	185.26 ± 8.20^e	56.80 ± 2.41^b	56.16 ± 0.00^c
Aqueous extract *A. paniculata* + CCl_4	17.75 ± 0.98^a	81.75 ± 6.03^a	64.46 ± 0.28^c	37.06 ± 2.01^c
Ethanol extract *A. paniculata* + CCl_4	50.65 ± 0.06^d	84.04 ± 0.46^a	57.94 ± 1.48^b	47.09 ± 6.21^d

Values are expressed as Mean ± SEM. Values with different superscripts in a column are significantly different from one another at $p < 0.05$ significance level.

There was an increase in the level of MDA in the liver of the CCl_4 group compared with the control (Table 4). Except for the group pre-treated with the aqueous extract, the level of MDA was reduced in the groups pre- and post-treated with the extracts of *A. paniculata*. *A* significant ($P<0.05$) reduction in the level of reduced glutathione and activities of SOD, CAT and GPx in the liver of the CCl_4 group was observed when compared with the control. Both extracts ameliorated the impact of CCl_4 on the level of GSH in the pre-and post-treatments, with a more significant effect noticed in the groups pre-treated with extracts. The post-treatment with both extracts ameliorated the impact of CCl_4 on the activity of SOD and CAT (Table 4). The pre-and post-treatment with both extracts alleviated the impact of CCl_4 on the activity of GPx, though the effect is significant ($p<0.05$) in the pre-treatment with both extracts only. The activity of GST in the liver of the groups was not significantly different from one another.

Table 4: Effects of the extracts of *A. paniculata* on the levels of GSH and MDA, and the activities of SOD, CAT, GPx and GST in the liver

Groups	MDA 10^{-2} (u/mg protein)	GSH (u/mg protein)	SOD 10^{-4} (u/mg protein)	CAT 10^{-2} (u/mg protein))	GPx 10^{-2} (u/mg protein)	GST 10^{-2} (u/mg protein)
Control	1.06 ± 0.01^a	11.48 ± 0.66^d	7.70 ± 0.86^b	3.58 ± 0.21^c	3.17 ± 0.72^b	5.92 ± 0.19^a
CCl_4	1.16 ± 0.04^a	2.83 ± 0.06^a	1.46 ± 0.02^a	2.39 ± 0.13^b	1.62 ± 0.25^a	5.41 ± 0.02^a
Aqueous extract *A. paniculata*	1.05 ± 0.08^a	8.79 ± 0.89^c	8.44 ± 0.85^b	3.33 ± 0.32^c	3.77 ± 0.03^b	5.79 ± 0.10^a
Ethanol extract *A. paniculata*	1.11 ± 0.03^a	9.19 ± 0.42^c	6.93 ± 0.58^b	2.35 ± 0.25^b	3.73 ± 0.08^b	5.85 ± 0.07^a
CCl_4 + Aqueous extract *A. paniculata*	1.10 ± 0.05^a	4.64 ± 0.50^b	7.91 ± 0.30^b	3.33 ± 0.13^c	1.67 ± 0.54^a	5.88 ± 0.07^a
CCl_4 + Ethanol extract *A. paniculata*	1.11 ± 0.12^a	4.59 ± 0.66^b	7.83 ± 0.88^b	4.38 ± 0.34^d	1.85 ± 0.19^a	5.84 ± 0.01^a
Aqueous extract *A. paniculata* + CCl_4	1.20 ± 0.07^a	10.30 ± 0.46^d	1.49 ± 0.01^a	0.30 ± 0.08^a	3.59 ± 0.05^b	5.22 ± 0.11^a
Ethanol extract *A. paniculata* + CCl_4	1.11 ± 0.02^a	8.17 ± 0.56^c	1.32 ± 0.08^a	3.16 ± 0.10^c	3.57 ± 0.05^b	5.36 ± 0.04^a

Values are expressed as Mean ± SEM. Values with different superscripts in a column are significantly different from one another at $p < 0.05$ significance level.

CCl_4 reduced the level of GSH, and the activities of SOD, CAT, GPx and GST, whereas it increased the level of MDA (Table 5). The concentration of MDA in the serum of the CCl_4 group was significantly elevated compared to the control. The pre-treatment with both extracts better ameliorated the impact of CCl_4 on the level of MDA in the serum than the post-treatment. Only the pre-treatment with the aqueous extract of *A. paniculata* restored the level of GSH in the serum of experimental animals. The post-treatment with both extracts significantly

(p<0.05) restored the activities of SOD and CAT in the serum. The pre-treatment with both extracts significantly (p<0.05) raised the activity of GPx than that of the CCl_4. Except for the pre-treatment with the ethanol extract of *A. paniculata*, the pre-and post-treatment with both extracts significantly elevated the GST activity in the experimental animals' serum.

Table 5: Effects of the extracts of *A. paniculata* on the levels of GSH and MDA, and the activities of SOD, CAT, GPx and GST in the serum

Groups	MDA (u/ml)	GSH (u/ml)	SOD (u/ml)	CAT 10^{-3} (u/ml)	GPx 10^{-2} (u/ml)	GST 10^{-2} (u/ml)
Control	51.65 ± 3.15^{c}	10.17 ± 0.80^{c}	6.07 ± 0.12^{d}	2.37 ± 0.73^{c}	7.59 ± 2.36^{ab}	10.23 ± 0.06^{c}
CCl_4	74.94 ± 10.28^{d}	8.36 ± 0.32^{b}	4.71 ± 0.40^{b}	0.73 ± 0.18^{a}	6.25 ± 1.18^{a}	7.45 ± 0.15^{a}
Aqueous extract *A. paniculata*	41.06 ± 2.88^{b}	21.52 ± 0.04^{e}	6.76 ± 0.05^{d}	2.91 ± 0.66^{c}	8.93 ± 1.18^{b}	8.17 ± 0.38^{b}
Ethanol extract *A. paniculata*	35.40 ± 0.94^{a}	18.18 ± 0.25^{d}	6.91 ± 0.04^{d}	1.09 ± 0.00^{b}	8.93 ± 0.89^{b}	11.13 ± 0.17^{d}
CCl_4 + Aqueous extract *A. paniculata*	60.98 ± 4.16^{d}	5.45 ± 0.46^{a}	6.93 ± 0.18^{d}	3.28 ± 0.63^{c}	6.25 ± 0.44^{a}	8.61 ± 0.46^{b}
CCl_4 + Ethanol extract *A. paniculata*	74.85 ± 1.91^{d}	5.10 ± 0.04^{a}	6.70 ± 0.14^{d}	2.55 ± 0.66^{c}	8.04 ± 0.00^{b}	10.89 ± 0.05^{c}
Aqueous extract *A. paniculata* + CCl_4	53.62 ± 1.84^{c}	17.69 ± 0.52^{d}	5.00 ± 0.26^{c}	1.09 ± 0.00^{b}	14.29 ± 0.45^{c}	13.15 ± 0.29^{e}
Ethanol extract *A. paniculata* + CCl_4	51.24 ± 0.97^{c}	7.69 ± 0.09^{b}	3.17 ± 0.18^{a}	0.73 ± 0.18^{a}	12.06 ± 2.05^{c}	7.01 ± 0.12^{a}

Values are expressed as Mean ± SEM. Values with different superscripts in a column are significantly different from one another at $p < 0.05$ significance level.

Discussion

In the current study, the administration of carbon tetrachloride did not significantly impact the body weight of the experimental animals, which could be because CCl_4 (1.25 ml/kg b.wt i.p) was administered once. Dutta *et al.* (2018) reported a reduction in the body weights of experimental mice administered CCl_4 only. The liver weight of the group administered with only CCl_4 was lower than that of the control group. However, Ouassou *et al*. (2021) reported an increase in the liver weight of the CCl_4 group compared to the control.

The total cholesterol and bilirubin levels significantly ($P < 0.05$) increased in the CCl_4 group compared to the control group. This suggested that CCl_4 could be responsible for the increase. Aqueous and ethanol extracts of *Andrographis paniculata* in the pre-treatment groups reversed the increment in the cholesterol level. The total protein and albumin levels were reduced in the CCl_4 group compared to the control. However, both extracts of *A. paniculata* increased total protein and albumin levels pre- and post-treatment. This observation indicates that both extracts of *A. paniculata* possess curative and prophylactic activities. Ouassou *et al*. (2021) reported increased concentration of total and direct bilirubin and reduced concentration of total protein, while the concentration of total cholesterol was unaffected in the CCl_4 group compared with the control group. The administration of the extract of *Caralluma europaea* reversed the changes (Ouassou *et al*., 2021). The hot aqueous leaf extract of *Justicia secunda*, a plant in the same family as *A. paniculata*, significantly reduced the elevated total bilirubin levels after CCl_4 administration (Anyasor *et al.*, 2020).

ALT, AST, ALP and GGT activities are used to assess liver damage. ALT, AST, ALP, and GGT activities were significantly ($P < 0.05$) elevated in the CCl_4 group compared to the control group in this study. The extracts of *A. paniculata* reduced the activities of these enzymes in the pre- and post-treatments to varying degrees, suggesting hepato-protective and curative activities of the aqueous and ethanol extracts of *A. paniculata*. Anyasor *et al.* (2020) reported that the hot aqueous extract of *Justicia secunda* leaf reduced the elevated activities of ALT and ALT in CCl_4-administered experimental animals (Anyasor *et al.*, 2020). Dutta *et al.* (2018) also reported significant elevations in ALT, AST, ALP, and GGT activities in the CCl_4 group compared to the control group. The extract of Croton bonplandianus reduced the activities of these enzymes in the experimental animals (Dutta *et al.*, 2018).

In the study under consideration, the levels of MDA in the liver and the serum were elevated in the CCl_4 group compared with the control. In contrast, the level of GSH was decreased, and the activities of SOD, CAT, GPx and GST were decreased. Aqueous and ethanol extracts of *A. paniculata* ameliorated the changes in varying degrees in the experimental animals, thus suggesting curative and prophylactic activities of the aqueous and ethanol extracts of *A. paniculata*. GPx is an essential enzyme crucial in detoxifying harmful reactive oxygen species (ROS) within cells (Gills and Tuteja, 2010). Catalase catalyzes the disintegration of hydrogen peroxide

into water and oxygen, thereby, protecting tissues from oxidative damage (Chelikani *et al*., 2004). Thus, a reduction in the activities of these antioxidant enzymes indicates oxidative stress. Khan *et al.* (2012) reported reduced activities of CAT, SOD, GPx and GST the in the CCl_4 group when compared with the control. However, the methanol extract of *Oxalis corniculata* reversed these changes (Khan *et al.*, 2012).

Conclusion

The liver is prone to oxidative damage due to free radicals generated by many processes it houses. Following CCl_4 administration, the antioxidant (reduced glutathione) in the animals was depleted, and the activities of the antioxidant enzymes, SOD, CAT, GPx and GST, were reduced. Thus, regeneration or re-energizing of these antioxidants is necessary. The aqueous and ethanol extracts of *Andrographis paniculata* elevated the activities of antioxidant enzymes and the level of reduced glutathione to varying degrees. This study, therefore, suggests the protective and curative potential of *A. paniculata* extracts in CCl_4-induced damage.

Acknowledgements

The authors are grateful to Dr. K. O. Orumwensodia for assisting with the laboratory equipment.

References

Akbar S: *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Altern Med Rev, 16(1): 66-77. 2011.

Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K: Oxidative stress in liver pathophysiology and disease. Antioxid (Basel), 12: 1653. 2023.

Anyasor GN, Moses N, Kale O: Hepatoprotective and hematological effects of *Justicia secunda* Vahl leaves on carbon tetrachloride induced toxicity in rats. Biotech Histochem, 95(5): 349-359. 2020.

Batkhuu J, Hattori K, Takano F: Suppression of NO production in activated macrophages *in vitro* and *ex vivo* by neoandrographolide isolated from *Andrographis paniculata*. Biol Pharm Bull, 25: 1169-1174. 2002.

Boopathi C: Andrographis spp: a source of bitter compounds for medicinal use. Anc Sci Life, 19(3-4): 164–168. 2000.

Buege JA, Aust SD: Microsomal lipid peroxidation. Meth Enzymol, 52: 306. 1978.

Burgos RA, Hidalgo MA, Monsalve J, Labranche TP, Eyre P, Hancke JL: 14-Deoxyandrographolide as a platelet activating factor antagonist in bovine neutrophils. Planta Med, 71(7): 604-608. 2005.

Chelikani P, Fita I, Loewen PC: Diversity of structures and properties among catalases. CMLS, 61: 192–208. 2004.

Cheung HY, Cheung SH, Li J, Cheung CS, Lai WP, Fong WF, Leung FM: Andrographolide isolated from *Andrographis paniculata* induces cell cycle arrest and mitochondrial-mediated apoptosiss in human leukemia HL-60 cells. Planta Med, 71(12): 1106-1111. 2005.

Cohen D, Dembiec D, Marcus J: Measurement of catalase activity in tissue extracts. Ann Clin Biochem, 34: 30 – 38. 1970.

Dutta S, Chakraborty AK, Dey P, Kar P, Guha P, Sen S, Kumar A, Sen A, Chandhur TK: Amelioration of CCl_4 induced liver injury in swiss albino mice by antioxidant rich leaf extract of *Croton bonplandianus* Baill. PLoS One, 13(4): e0196411. 2018.

El-Haskoury R, Al-Waili N, Kamoun Z, Makni M, Al-Waili A, Lyoussi B: Antioxidant activity and protective effect of propolis against carbon tetrachloride-induced liver and kidney injury by modulation of oxidative parameters. Vet World, 14(12):3076–3083. 2021.

Ellman GC: Tissue Sulfhydryl groups. Arch Biochem Biophys, 82: 70 –77. 1959.

Council of Europe: European convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series. No 123. pp. 1-48. 2005.

Fridovich I: Superoxide dismutases-An adaptation to a paramagnetic gas. J Biol Chem 264(14): 77661-7764. 1989.

Gill SS, Tuteja N: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 48: 909–930. 2010.

Habig WH, Pabst MJ, Jacoby WB: Glutathione-S-transferase, the first enzymatic step in mercaptroic acid formation. J Biol Chem, 249: 7130-7139. 1974.

Hossain S, Urbi Z, Karuniawati H, Mohiuddin RB, Moh Qrimida A, Allzrag AMM, Ming LC, Pagano E, Capasso R: *Andrographis paniculata* (Burm. f.) Wall. ex Nees: an updated review of phytochemistry, antimicrobial pharmacology, and clinical safety and efficacy. Life (Basel), 11(4):348. 2021.

Isunu LE, Omoya FO, Ajayi KO, Akharaiyi FC, Ogundare AO, Babatunde OJ: Evaluation of the antiplasmodial activities of methanol leaf extract of *Andrographis paniculala (*burm.f.): An *in vitro* and *in vivo* study. Microbes Infect, 4(1):296-303. 2022.

Kabir MH, Hasan N, Rahman MM, Rahman MA, Khan JA, Hoque NT, Bhuiyan MRQ, Mou SM, Jahan R, Rahmatullah M: A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh. J Ethnobiol Ethnomed, 10(19): 1-28. 2014.

Khan MR, Marium A, Shabbir M, Saeed N, Bokhari J: Antioxidant and hepatoprotective effects of *Oxalis corniculata* against carbon tetrachloride (CCl4) induced injuries in rat. Afr J Pharm Pharmacol, 6(30): 2255-2267. 2012.

Lin TP, Chen SY, Duh PD, Chang LK, Liu YN: Inhibition of the epstein-barr virus lytic cycle by andrographolide. Biol Pharm Bull, 31: 2018–2023. 2008.

National Center for Biotechnology Information: PubChem Compound Summary for CID 5943, Carbon Tetrachloride. Retrieved October 18, 2023.

Nonso OG, Ejike IG, Obinna EC: Prophylactic and antidiabetic effect of *Andrographis paniculala* and its combination with *Allium sativum* on blood glucose level and some biochemical indices in wistar rats. J Adv Med Pharm Sci, 25(7): 13-26. 2023.

Nyman N: Determination of glutathione peroxidase in tissue. Anal Biochem, 28: 481. 1959.

Ouassou H, Bouhrim M, Daoudi NE, Mekhfi, H, Ziyyat A, Legssyer A, Aziz M, Bnouham M: Evaluation of hepatoprotective activity of *Caralluma europaea* stem extract against CCl_4-induced hepatic damage in Wistar rats. Adv Pharmacol Pharm Sci, Article ID 8883040: 1-8. 2021.

Owoade AO, Alausa AO, Adetutu A, Olorunnisola OS, Owoade AW: Phytochemical characterization and antioxidant Bioactivity of *Andrographis paniculala* (Nees). Pan Afr J Life Sci, 5(2) 246-256, 2021.

Phuong TN, Tai ND, Aloufi, AS, Subramani B, Theivaraj SD: An in-vitro evaluation of antifungal, anti-lungcancer (A549), and anti-hyperglycemic activities potential of Andrographis paniculata (Burm. f.) flower extract. Environ Res, 238(2): 117249. 2023.

Sheeja K, Guruvayoorappan C, Kuttan G: Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. Int Immunopharmacol, 7(2): 211-221. 2007.

Upadhyay J, Tiwari N, Durgapal S, Farzaei, MH: Chapter 5.10 – Antioxidants and liver diseases – In Antioxidants Effects in Health (Eds) Nabavi, S. M. and Silva, A.S. Elsevier, pp. 799-816, 2022.