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Research Paper

Hepatoprotective effect of methanolic extract of *Vernonia amygdalina* leaf

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ABSTRACT

This study investigate the possible antioxidant properties of *Vernonia amygdalin* leaves in preventing damages to the liver and kidney of albino rats. To investigate this, carbon tetrachloride (CCl4) was injected into rats in order to induce liver and kidney damage. The rats were simultaneously given oral doses (20mg/kg and 60mg/kg body weight) of methanolic extract of leaves of *V. amygdalin.* The effects of these treatments on some liver and kidney enzymes were evaluated. Administration of CCl4 alone to rats significantly ($P< 0.05$) reduced the activities of liver ALT and AST by 71% and 60% respectively. The values for kidney AST and ALT are 47% and 80% respectively. Simultaneous treatment of CCl4 injection with oral administration of 20mg/kg and 60mg/kg body weight of the methanolic extract significantly reversed ($P<0.05$) these changes in both the liver and kidney. The activities of liver and kidney GGT were considerably reduced by CCl4 administration and were also reverse by the plant extract. The present findings suggest that the extract may probably possess components that are hepatoprotective.

Keywords: *Vernonia amygdalin*; Antioxidant; Peroxidative damage; Liver; Kidney; Rat.

INTRODUCTION

Products of higher plant origin have been shown to be effective sources of chemotherapeutic agents without underlining side effects (Neetu and Meenakshi, 2003). In recent times, a number of traditional healers claiming the efficacy of medicinal plants are on the increase (Kabir, et al., 2005). Several studies have established the anti-inflammatory, analgesic and anti-pyretic activities of other plant extract (Olajide, et al., 2004). Previous studies have revealed that other plants with polyphenols exhibit clear hepatoprotective properties (Lima, et al., 2006; Yao, et al., 2007), and that flavonoids could protect human hepatocytes against oxidative injury induced by H_2O_2 or CCl_4 *in vitro* (Zhao and Zhang, 2009). Although many other plants have been reported to possess hepatoprotective properties, the scientific

authentication of most of them such as *Vernonia amygdalin* which are used traditionally to treat liver disorder and several other diseases is unavailable.

V. amygdalina is a shrub that grows predominantly in tropical Africa. Leaves from this plant serve as food vegetable and culinary herb in soup. Extracts of this plant are used as tonic, treatment of cough, feverish condition, constipation and hypertension (Kambizi and Afolayan, 2001). The aim of this work is to provide some experimental support for the health benefit of *V. amygdalin.* To achieve this, studies were carried out to investigate the phytochemical component of *V. amygdalina* and to evaluate the hepatoprotective activities of methanolic extract of the leaves of *V. amygdalina* against oxidative damage induced by CCl_4 in rats.

MATERIALS AND METHODS

Animals: The fifteen albino rats (*Rattus novergicus*) used for this study were obtained from Ayo Ola farms Ilorin,Kwara state, Nigeria. The animals were fed with standard laboratory feeds (Bendel Feeds Ltd,Ewu, Nigeria) and tap water (*ad libitum*). This research was carried out in Joseph Ayo Babalola University, Ikeji-Arakeji,Osun State, Nigeria according to the rules in Nigeria governing the use of laboratory animals (Revised Helsinki Declaration, 2008) as acceptable internationally.

Chemicals: All reagent kits used for enzyme assay were obtained from Randox Company, United Kingdom. All other reagents used were of analytical grade and were prepared in all glass distilled water.

Plant material and extraction: The leaves of *Vernonia amygdalina* were obtained from Agricultural farm of Joseph Ayo Babalola University, Ikeji-Arakeji,Osun State, Nigeria and were identified at the Herbarium section (No. DBS/H/487) of the Department of Biological Sciences of the same University. 50g of shade-dried leaves pulverized to powder was percolated in distilled water. The mixture was allowed to stand for 24 h and then filtered by a suction pump and then evaporated by a rotary evaporator to obtain the methanolic extract. The filtrate was evaporated to dryness in a water bath at 60^oC.

Experimental design: The rats were randomly grouped into four (n = 4) as follows:

(i) Control received orally 1 ml of distilled water daily.

(ii) Group A received 1.5ml/kg body weight (i.p.) CCl4 and 20 mg/kg body weight of methanolic extract of *Vernonia amygdalina* leaves (orally) simultaneously daily.

(iii) Group B received 1.5ml/kg body weight (i.p.) CCl4 and 60 mg/kg body weight of methanolic extract of *Vernonia amygdalina* leaves (orally) simultaneously.

(iv) Group C received 1.5ml/kg body weight (i.p.) CCl4

The dosage of the extracts was determined from preliminary studies in our laboratory. Higher doses >80mg/kg of the plant extract was toxic the rats.

Biochemical studies: The activities of liver and kidney aminotransferases (ALT, AST and GGT) were assayed basically by the method of Reitman and Frankel (1957).

Statistical analysis: The statistical analysis was carried out by one way Analysis of variance and Duncan Multiple Range test. $P < 0.05$ was considered significant.

RESULTS

Table 1 shows the results obtained for the phytochemical screening of methanolic extracts *of V. amygdalina* leaf. Tables 2 shows the effect of the methanolic extract of

V. amygdalina leaves on the activities of liver and kidney AST in CCl_4-induced liver damage in rats. The activities of liver and kidney AST were significantly reduced ($P< 0.05$) by CCl4 administration alone on the rats when compared with the control. It was observed that simultaneous administration of 20mg/kg and 60mg/kg methanolic extract with CCl4 to rats significantly increased ($P< 0.05$) the activities of the enzymes when compared with administration of CCl4 alone to rats. The activities of the liver and kidney ALT as shown in Table 3 indicates that the treatment of CCl4 injection with oral administration of 20mg/kg and 60mg/kg body weight of the methanolic extract significantly reversed ($P< 0.05$) the enzyme activity in the kidney when compared with the administration of CCl4 alone to rats.. The activities of liver GGT were considerably reduced by CCl4 administration when compared with the control and were significantly reversed ($P< 0.05$) by the administration of 20mg/kg and 60mg/kg methanolic extract (Table 4).

DISCUSSION

The effects of methanolic extracts of *V. amygdalina* leaves on CCl_4-induced hepatotoxicity in albino rats were evaluated. The significant reduction ($P< 0.05$) in activities of liver and kidney AST and ALT in the group treated with only CCl_4 when compared with controls suggests hepatotoxicity. This may be as a result of leakage from the cells through peroxidative damage of the membrane (Li, et al., 2010). The hepatotoxic effects are due to its active metabolite, trichloromethyl radical (Johnson, et al., 2002). CCl_4 has been known to be toxic on cells and are often used to induce oxidative stress in cell systems. It has also been reported that treatment of rats with CCl_4 is capable of generating free radicals that trigger a cascade of events resulting in hepatic fibrosis (Obi, et al., 2003). The mechanism by which CCl_4 causes cell oxidative injury involves that cytochrome P-450 system transforms CCl_4 into CCl_3 and then CCl_3 is transformed into a more reactive CCl_3O_2, CCl_3O_2 causes lipid peroxidation, disturbs Ca^{2+} homeostasis and eventually kills cells (Wang, et al., 2004). Previous work has revealed that compounds such kaempferol, quercetin and myricetin showed protective effects against oxidative injury of human hepatocytes induced by CCl_4 (Zhao and Zhang, 2009). Also extract such as bilberry displayed protective effect against primary rat hepatocytes oxidative stress induced by tert-butylhydroperoxide or allyl alcohol by protecting the leakage of the organ (Valentová, et al., 2007). The monitoring of the leakage of liver and kidney enzymes into the serum has proven to be very useful tool in assessing liver (Nelson and Cox, 2000). The leakage of the organ leads to the decrease in the activity of the enzyme. The significant increase showed in liver and kidney AST and ALT activities after administration of 20mg/kg and 60mg/kg body weight of methanolic extract (Tables 2,3 and 4) when compared with that administered with only CCl_4 suggest that the extract must have reverse the injurious effect of CCL_4 on the organs. This property might be as a result of antioxidant activity of the extracts which might be attributed to the presence of phenolics and flavonoids (Table1). The antioxidant activity of some extracts has been attributed to the presence of phenolics and flavornoids (Iniaghe, et al., 2008). The protective effect of the extract on liver especially after administration of 20mg/kg was further supported in the significant increases showed in the liver GGT (Table 4). Thus methanolic extract of *V. amygdalina* leaf has hepatoprotective

properties against CCl_4 induced liver damage. Hence it is possible that a probable mechanism of hepatoprotection of *V. amygdalina* leaf extract against CCl_4 induced damage is the antioxidant activity.

CONCLUSION

This study reveals that *V. amygdalina* leaf contains phenolics and flavonoids. Based on the result obtained from this study, the methanolic extract of *V. amygdalina* leaves possess hepatoprotection effect against rat organ damage.

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Table- 1: Phytochemical screening of *Vernonia amygdalina* leaf extract.

Phytochemical component	*Vernonia amygdalina*
Alkaloids	+
Tannins	+
Phenolics	+
Glycosides	-
Saponins	+
Flavonoids	+
Steroids	+

Table- 2: Effects of methanolic extract of *Vernonia amygdalina* leaf on the activities of liver and kidney AST in CCl_4-induced liver damage in rats.

Groups	Asparte Aminotransferase Activities	
	Liver	Kidney
CONTROL	30.90±0.01 [a]	38.50±0.07 [a]
A	10.80±0.02 [b]	22.00±0.09 [b]
B	15.40±0.07 [c]	24.00±0.02 [b]
C	10.25±0.01 [d]	17.34±0.01 [d]

- Each value is the mean of three different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05)

Table- 3: Effects of methanolic extract of *Vernonia amygdalina* leaf on the activities of liver and kidney ALT in CCl_4-induced liver damage in rats.

Groups	Alanine Aminotransferase Activities	
	Liver	Kidney
CONTROL	50.90±0.73 [a]	44.80±1.95 [a]
A	21.91±0.55 [b]	4..90±0.06 [b]
B	27.10±0.71 [c]	11.50±0.57 [c]
C	2.25±1.04 [d]	3.50.50±.82 [d]

- Each value is the mean of three different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05)

Table- 4: Effects of methanolic extract of *Vernonia amygdalina* leaf on the activities of liver GGT in CCl_4-induced liver damage in rats.

Groups	Gamma Glutamyl Transferase Activities
	Liver
CONTROL	1.18±0.02 [a]
A	1.14±0.01 [b]
B	1.16±0.02 [c]
C	1.17±0.08 [c]

- Each value is the mean of three different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05)