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Effect of Methanol Extract of *Abrus precatorius* Leaves on Male Wistar Albino Rats Induced Liver Damage using Carbon Tetrachloride (CCl₄)

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ABSTRACT

This study evaluates qualitative and quantitative phytochemical properties of *Abrus precatorius* leaves. The vitamins and therapeutic effect of methanol extract against tetrachloride (CCl₄) induced liver damage in male Wistar albino rats. The results of phytochemical and vitamins indicates richness in saponins, tannins, alkaloids and flavonoids with value 30.05±0.22%, while vitamins such as thiamine, riboflavin, niacin, antioxidant and vitamins A, C and E was quantified. The liver damage was observed in the animals with significant increase in ALT, AST and ALP following the in traperitoneal administration of CCl₄. The methanol extract caused significant decrease (p<0.05) in the activities of these enzymes in the treated animals relative to positive control that received CCl₄ but were not treated. The extract showed potentials to mopping-up of free radicals generated by CCl₄. The extract reduced lipid peroxidation in concentration dependent manner. The findings suggests that the extract have the capacity to improving liver functions and liver regeneration at high concentration. The extracts are safe for consumption, abundant phytochemicals and antioxidant vitamins could offer reasonable protections against oxidative stress.

Key words: *Abrus precatorius*, phytochemicals, vitamins, CCl₄, liver enzymes, superoxide dismutase, lipid peroxidation

INTRODUCTION

The liver disorders are in the increase due to high morbidity and high mortality as its medical management is inadequate at present. Currently, there is no reliable therapy to successfully prevent the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs were not without significant side effects. Liver damage due to chemicals and infectious agents may lead to liver failure (Willett *et al.*, 2009). However, effective agents that delay progression to liver failure and complications are yet to be found. Though studies suggested that traditional herbs and micronutrients such as carotenoids and selenium may be useful for this purpose (Willett *et al.*, 2009). Administration of carbon tetrachloride (CCl₄) is

an established experimental model for severe liver injury involving generation of oxidative stress is frequently used for the screening of antihepatotoxic and/or hepatoprotective activities of drugs (Tunon *et al.*, 2009). Antioxidants and anti-inflammatory agents can play a role in liver protection by scavenging active oxygen and free radicals and neutralizing lipid peroxides. The major causes of carbon tetrachloride (CCl₄) induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals (Castro *et al.*, 1974). Liver injury caused by CCl₄ depends on its metabolite to the highly reactive trichloromethyl (CCl₃) radical, which initiates lipid peroxidation. This leads to CCl₄ hepatotoxicity by starting lipid peroxidation in membranes (Saukkonen *et al.*, 2006).

Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Saukkonen *et al.*, 2006; Deng *et al.*, 2009). The hepatotoxic response elicited by chemical agent depends on toxicant concentration which may be parent compound, toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Kedderis, 1996). Major symptoms commonly associated with hepatotoxicity include jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light coloured stool (Bleibel *et al.*, 2007; Chang and Schaino, 2007).

The potential of antioxidant plants to contribute to human health and to protect against heart diseases and cancer has attracted considerable interest among scientists, food manufacturers and consumers and it has led to the formulation of functional foods with specific health effects (Loliger, 1991). Flavonoids are phenolic compounds known for its characteristic red, blue and purple anthocyanin pigments (Winkel-Shirley, 2001). Flavonoids occur in food either as free monomers (quercetin, catechin) or oligomers (procyanidins), they are bound to saccharides as glycosides. Consumption of food rich in flavonoid is associated with lower incidence of coronary heart disease, myocardial infarction, cancer, neurodegenerative psychic diseases and other chronic diseases. In the pathology of these diseases, oxidative stress was assumed to play a role and flavonoids have been suggested to exert health benefits through antioxidant mechanisms. In addition to their antioxidant properties, flavonoids have been reported to exhibit other multiple biological effects, antiviral, anti-bacterial, anti-inflammatory, vasodilatory, anti-cancer and anti-ischemic (Prochazkova *et al.*, 2011).

Abrus precatorius is a slender perennial climber that twines around trees, shrubs and hedges. It has slender branches with cylindrical wrinkled stem with smooth textured brown bark (Fernando, 1988). Its root is deep and tenaciously difficult to eradicate and increase population size following fire outbreak (Holm, 1991). *Abrus precatorius* locally called "IdonZakara" in Hausa is a species of the family Fabaceae. *Abrus precatorius* leaves possess medicinal properties and taste sweet with bitter taste that lasts long upon ingestion. The leaves can be masticated as medicine and does not contain as much of the deadly component abrin (a deadly poison) as in the seed (Adedapo *et al.*, 2007; Reedman *et al.*, 2008). It is commonly chewed or sucked to obtain its sweet taste (Kennelly *et al.*, 1996) and reports suggests it can be

consumed by boiling with food for example, cereal pulp, as a sweetener and as vegetable. In addition, fresh leaves have been reportedly used for treating sores in the mouth by compression (Adedapo *et al.*, 2007). *Abrus precatorius* leaves have been used in Nigeria for the treatment of myriad of diseases including malaria, typhoid, cough, respiratory tract infections and hepatitis (Saganuwan and Onyeyili, 2010). The aim of this work is to investigate the hepatoprotective effect of the Methanol extract of *A. precatorius* leaves on male Wistar rats treated with carbon tetrachloride (CCl₄).

MATERIALS AND METHODS

Materials

Sample collection and preparation: The leaves and stems of mature *A. precatorius* were collected from Bassawa, Zaria metropolis in Kaduna State, Nigeria and identified at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, with Voucher No. 932.

Preparation of plant material: The leaves (green in colour) were hand-picked from the stems and air dried under shade until they were fully dried. The dry leaves were ground into powdered form with ceramic mortar and pestle. Powdered sample was packed into clean and dry sample containers for analysis.

Chemicals and equipment: The chemicals and reagents used for this study were of analytical grades. Some of the chemicals and equipment were obtained from the National Research Institute for Chemical Technology, Basawa Zaria, Kaduna State, Nigeria, Department of Chemistry and Department of Biochemistry, Faculty of Sciences, Ahmadu Bello University, Zaria.

Extraction procedure: A quantity, 500 g of finely ground sample was dissolved in 2.5 L absolute methanol for 24 h. After that, the resulting extract was filtered using Wattman No. 1 filter paper. The resulting filtrate was concentrated to dryness using rotary evaporator at 40°C. The concentrated extract was stored in the refrigerator and used for the study.

Methods

Experimental procedure for animal study: Thirty male Wistar albino rats weighing 170-215 g and 18 mice weighing 20-26 g were purchased from the National Animal Production Research Institute, Ahmadu Bello University, Shika, Zaria for the study and were housed for 12 h light/dark cycle at 25 with free access to food and water *ad libitum* during acclimatization which lasted for 7 days. The mice were divided into six groups of three mice each and used for the phase I and phase II of the lethal dose (LD₅₀) determination. The rats were divided into six groups of five rats each:

Group 1: Served as control administered with normal saline solution (0.9%) in 14 days

Group 2: Positive control administered CCl₄+olive oil (1:1, v/v) 10 mL kg⁻¹ b.wt. given normal saline solution (0.9%) in 14 days

Group 3: CCl₄+olive oil (1:1, v/v) 10 mL kg⁻¹ b.wt. treated with 200 mg kg⁻¹ b.wt. vitamin E in 14 days

Group 4: CCl₄+olive oil (1:1, v/v) 10 mL kg⁻¹ b.wt. and treated with 100 mg kg⁻¹ b.wt. of the methanol extract in 14 days

Group 5: CCl₄+olive oil (1:1, v/v) 10 mL kg⁻¹ b.wt. and treated with 200 mg kg⁻¹ b.wt. of the methanol extract in 14 days

Group 6: CCl₄+olive oil (1:1, v/v) 10 mL kg⁻¹ b.wt. and treated with 400 mg kg⁻¹ body weight of the methanol extract in 14 days

The dose was based on LD₅₀ result of methanol extract of *A. precatorius* leaves. All animals were sacrificed on the 15th day and blood samples collected based on standard laboratory procedures for the determination of biochemical parameters.

Phytochemical analysis

Qualitative phytochemical analysis of *A. precatorius* leaves:

The phytochemical analysis of the leaves of *A. precatorius* was carried out according to the method of Harborne (1984) while Trease and Evans (1983) was used to identifying phytochemical constituents.

Quantitative phytochemical analysis of *A. precatorius* leaves:

Alkaloid, saponin and flavonoid contents were quantitatively determined by the methods of Harborne (1998). Total phenolics were determined using Folin Ciocalteu Reagent (FCR) as described by Velioglu *et al.* (1998). Tannin content was determined according to the method of Pearson (1976).

Quantitative determination of some vitamins in the *A. precatorius* leaves:

The quantitative riboflavin, thiamine and niacin were determined by the method of Scalar (2000) while vitamins A, C and E were estimated using the methods described by Pearson (1976).

Lethal dose (LD₅₀) determination: The LD₅₀ of the methanol extract was determined using the method described by Lorke (1983).

Serum enzymes assay

Alanine Amino Transferase (ALT) activity: The ALT activity was assayed using the method of Reitman and Frankel (1957) as outlined in Randox test kit (USA).

Principle: The ALT activity was assayed by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 540 nm.

Aspartate Amino Transferase (AST) activity: The *in-vivo* activity of AST was assayed by Reitman and Frankel (1957) using Randox test kit (USA).

Principle: The AST activity was generally assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 546 nm.

Alkaline phosphatase (ALP) activity: The ALP activity was assayed using the method described by Klein *et al.* (1960).

Principle: The principle of this assay is based on the reaction involving serum ALP and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein at alkaline pH values, turns pink that can be determined photometrically.

Determination of lipid peroxidation product:

The concentration of lipid peroxidation product, malondialdehyde (MDA) was determined by the method described by Wallin *et al.* (1993).

Principle: The principle for the estimation is based on the fact that thiobarbituric acid (TBARS) reacts with MDA to give a red or pink colour, which absorbs maximally at 532 nm.

Assay of superoxide dismutase (SOD) activity: The SOD activity was assayed using the method described by Martinez *et al.* (2001).

Statistical analysis: Data were expressed as Mean±standard deviation statistically using one-way analysis of variance (ANOVA). Acceptable value of p<0.05 was considered to be statistically significant. The Statistical Products and Service Solutions (SPSS) software version 20 was used for this analysis.

RESULTS

The result of phytochemical screening of *A. precatorius* leaves shows that flavonoids and saponin are present in high concentration while alkaloids and tannins were present in moderate concentration. The leaves contain also phytate, hydrogen cyanide and oxalate in low concentration as shown in Table 1.

Table 2 shows the quantitative phytochemical contents of leaves of *A. precatorius* leaves with flavonoid having the highest value of 30.05±0.22% followed by saponin with a value of 8.25±0.90%.

The result of vitamins analysis show that vitamin C has the highest value compared to other vitamins in the *A. precatorius* leaves followed by niacin, vitamin E, thiamine, vitamin A and riboflavin, respectively (Table 3).

Percentage yield of the extract: The extraction 500 g of the finely ground *A. precatorius* leaves in methanol gave 36.05 g amounting to 7.21% yield of the extract which was used for the experiment.

Table 1: Phytochemical screening of *Abru precatorius* leaves

Constituents	Bioassay	Interpretation
Alkaloids	++	Moderate concentration
Flavonoids	+++	High concentration
Phytate	+	Low concentration
Hydrogen cyanide	+	Low concentration
Oxalate	+	Low concentration
Phenols	+	Low concentration
Saponin	+++	High concentration
Tannins	++	Moderate concentration

Table 2: Quantitative phytochemicals properties of *Abru precatorius* leaves

Constituents	Quantity
Alkaloids (%)	6.00±0.00
Flavonoids (%)	30.05±0.22
Phytate (mg/100 g)	643.05±1.87
Hydrogen cyanide (mg/100 g)	424.08±0.30
Oxalate (mg g ⁻¹)	22.22±0.31
Phenols (% w/v)	2.23±0.05
Saponin (% w/v)	8.25±0.90
Tannins (mg/100 g)	5.56±0.03

The lethal dose (LD₅₀) of methanol extracts of in Table 4 showed no death or adverse reaction up to 5000 mg kg⁻¹ b.wt.

There was a significant (p<0.05) increase in ALT activity from group 1-5 when compared to the control. The group 3 that received vitamin E had the least ALT activity relative to that of the control (group 1). However, a concentration dependent decrease in ALT activity was observed in group 4, 5 and 6 compared to positive control (CCl₄ induced liver damage without treatment), though they were higher than that of the control Fig. 1.

The result in Fig. 2 shows that group CCl₄ induced liver damage untreated has the highest AST activity when compared to that of the control (group 1). Although, group 2-6 shows significant (p<0.05) increase in AST activity when

Table 3: Vitamins analysis of the *Abru precatorius* leaves

Vitamin	Quantity (mg/100 g)
Vitamin E	1.84±0.02
Vitamin A	0.98±0.00
Vitamin C	15.25±1.03
Niacin	5.02±0.08
Riboflavin	0.26±0.00
Thiamine	1.65±0.02

Values are presented as Mean±standard deviation of triplicate determination (n = 3)

Table 4: Lethal dose of methanol extract of *Abru precatorius* leaves

Parameters	Dosage (mg kg ⁻¹ b.wt.)	Seeds mortality
Phase 1		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase 2		
Group 1	1600	0/1
Group 2	2900	0/1
Group 3	5000	0/1

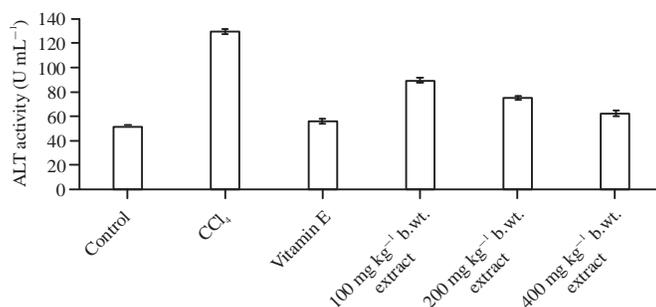


Fig. 1: ALT activities of different experimental animal groups

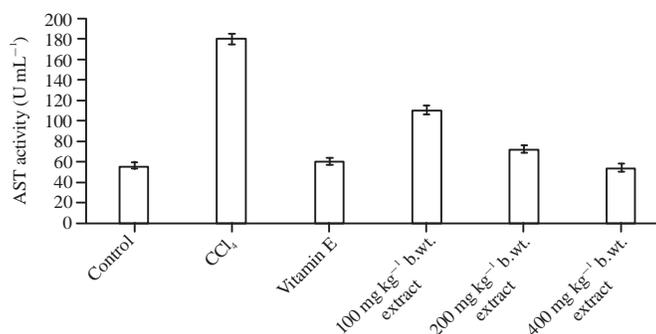


Fig. 2: AST activities of different experimental animal groups

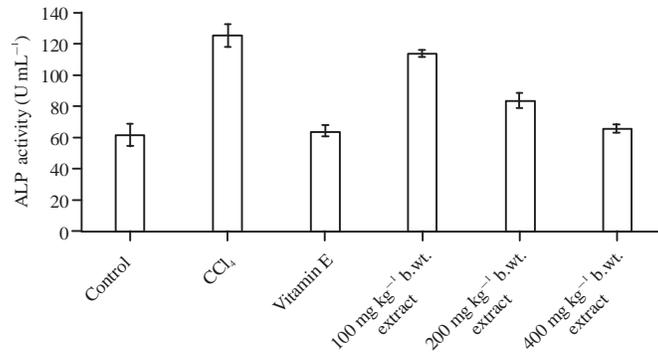


Fig. 3: ALP activities of different experimental animal groups

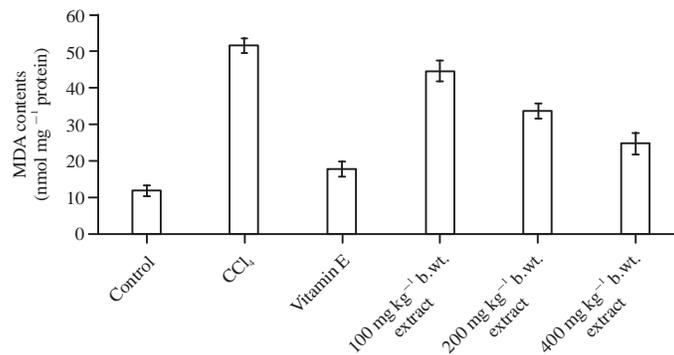


Fig. 4: MDA contents of different experimental animal groups

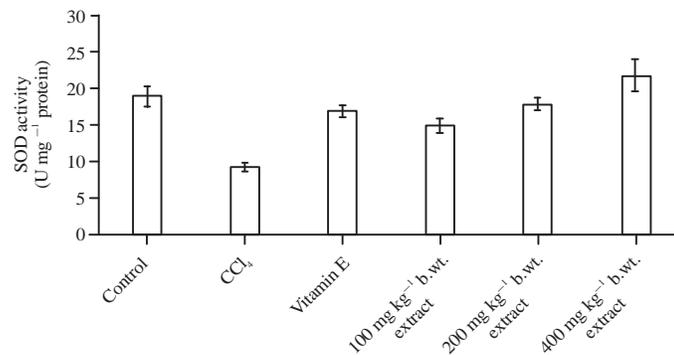


Fig. 5: Activity of hepatic SOD in different test animal groups

compared to that of control, group 4-6 had significant ($p < 0.05$) decrease in AST in concentration dependent manner when compared to that of positive control (group 2) but significantly ($p < 0.05$) higher than that of group 3 that received 200 mg kg⁻¹ b.wt. vitamin E.

In Fig. 3, group 2-6 indicate significant ($p < 0.05$) increase in ALP activity relative to that control while group 3 that received 200 mg kg⁻¹ b.wt. vitamin E showed no significant ($p < 0.05$) increase in ALP activity compared to control. It was observed that group 4-6 showed concentration dependent significant ($p < 0.05$) decrease in ALP activity compared to the positive control.

The MDA content increased significantly ($p < 0.05$) from group 2-6 when compared to control. The group 2 CCl₄ induced liver damage untreated and group 3 CCl₄ induced liver damage treated with vitamin E had the highest and least MDA content, respectively compared to control. However, group 4-6 administered with CCl₄ induced liver damage. These groups treated with varying doses of methanol extract showed concentration dependent decrease in MDA compared to positive control (group 2) (Fig. 4).

Figure 5 shows that positive control (group 2) and group 4 had significant ($p < 0.05$) decrease in SOD activity compared to control. The group 3 treated with vitamin E

(200 mg kg⁻¹ b.wt.) and group 5 treated with 200 mg kg⁻¹ b.wt. of the extract show no significant (p>0.05) decrease in SOD activity compared to control. However, group that received 400 mg kg⁻¹ b.wt. of the extract showed significant (p<0.0) increase in SOD activity relative to control and also had the highest SOD activity compared to other groups tested.

DISCUSSION

This study evaluated phytochemical and vitamin contents of *A. precatorius* leaves. The therapeutic effect of methanol extract of *A. precatorius* leaves on Wistar male albino rats. The CCl₄ was used to induced liver damage in male Wistar albino rats, whereas the methanol extract of *A. precatorius* leaves was administered as treatment to ascertain the toxicity of the extract and its capacity or potential for liver functions. The significant amount of phytochemicals such as alkaloids, saponins and flavonoids in the leaves of *A. precatorius* shows that the leaves have phytochemicals that could confer significant health benefits to individual that consume the vegetable in right proportion. For instance, flavonoids constitute the largest portion phytochemicals in the leaves. Phenolic compounds known for their characteristic red, blue and purple anthocyanin pigments in plant tissues (Winkel-Shirley, 2001). Flavonoids occur in food either as free monomers (quercetin, catechin) or oligomers (procyanidins) and they are bound to saccharides as glycosides. Consumption of flavonoid-rich food is associated with low incidence of coronary heart disease, myocardial infarction, cancer, neurodegenerative psychic diseases and other chronic diseases. In the pathology of these diseases, oxidative stress has been assumed to play a role while flavonoids have been suggested to exert health benefits through antioxidant mechanisms. In addition to their antioxidant properties, flavonoids alkaloids and saponins have been reported to exhibit other multiple biological effects, antiviral, anti-bacterial, anti-inflammatory, vasodilatory, anti-cancer and anti-ischemic (Prochazkova *et al.*, 2011).

The high levels of vitamins observed in the leaves shows that the plant is rich source of vitamins most especially antioxidant vitamins such as vitamin A, C and E. Therefore consumption of these leaves could offer protection against free radicals that could cause cellular damage and prevention of lipid peroxidation. The antioxidant vitamins may play a significant role in the reduction of liver damage and lipid peroxidation caused by free radicals generated by CCl₄.

The lethal dosage showed that the methanol extract of *A. precatorius* leaves are safe for consumption as no signs of toxicity or death was recorded even at high concentration (500 mg kg⁻¹ b.wt.) of the extract was given to the animals.

The liver damage caused by CCl₄ was evident by the alteration in serum marker enzymes concentration. The enzyme activities assayed include alanine aminotransferase

(ALT), aspartate aminotransferase (AST) alkaline phosphatase (ALP) and superoxide dismutase (SOD). The significant (p<0.05) increase in ALT, AST and ALP activities following the administration of carbon tetrachloride (CCl₄) may be due to liver damage resulting from free radicals generated in response to the effect of the CCl₄ which could lead to the leakage of these enzymes to the extrahepatic tissues raising the extrahepatic levels and activities of these enzymes. The leakage of these enzymes may be attributed to the compromised integrity of the plasma membrane following the administration of the CCl₄. The AST is one of liver enzymes that aids in production of proteins. It catalyses the reductive transfer of an amino group from aspartate to α -ketoglutarate to yield oxaloacetate and glutamate. Besides liver, it is also found in other organs such as heart, muscle, brain and kidney. Injury to any of these tissues may cause elevation of AST in the blood level (Nathwani *et al.*, 2005). It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury (Ozer *et al.*, 2008) as it can also signify abnormalities in heart, muscle, brain or kidney (Fernandez and Kidney, 2007). The ALP cleaves phosphate monoesters making the excessive leakage of the enzyme to the extrahepatic tissues a threat to the cells of these tissues that rely on phosphate ester for survival. This could lead to excessive hydrolysis of phosphate ester.

The studies on liver have shown that liver damage are associated with increase in serum levels of ALT, AST and ALP. The increased serum ALT, AST and ALP activities are not specific to liver damage as their increase may be attributed to contribution of other organs in the body. Serum enzyme levels are not static rather fluctuate widely from normal to moderately abnormal values (Ozer *et al.*, 2008). Marked elevation of aminotransferases in the appropriate clinical context indicates acute cell necrosis caused by viral infection, drugs, toxins, alcohol, or Ischemia (Teschke, 2009). The aminotransferases occupy a central position in the amino acid metabolism as they help in retaining amino groups during degradation of amino acid. They are involved in the biochemical regulation of intracellular amino acid pool (Amacher, 2002).

The CCl₄ induced hepatotoxicity in rats leading to hepatic injury triggers the generation of toxic free radicals which are masked by using correct antioxidant in adequate concentration (Rabeh and Aboraya, 2014). The methanol extract of *A. precatorius* leaves protects the liver from damage by CCl₄, as shown by improved biochemical markers of liver damage and SOD activity. The mechanism of the hepatoprotective action of the plant may be related to the ability of the plant to inhibit lipid peroxidation in the liver due to abundant flavonoids in the leave which serve as antioxidants. It is attributed to the induction of SOD activity by the extract that mopped free radicals generated by carbon tetrachloride. The presence of flavonoids, tannins, saponins and phenols in *A. precatorius* leaves explain its role in hepatoprotection by inhibiting the free radicals mediated damage

(Yakubu *et al.*, 2001). Rabeih and Aboraya (2014) suggested that flavonoids and tannins are antioxidant agents and may exhibit hepatoprotective activities by interfering with free radicals formation.

Oxidative stress is a state of redox imbalance caused by increased Reactive Oxygen Species (ROS) generation and decreased antioxidant capacity (Yacout *et al.*, 2012). During lipid peroxidation malonaldehyde (MDA) is produced and has been mostly used as index of lipid peroxidation and marker of oxidative stress (Njayou *et al.*, 2013). A significant ($p < 0.05$) decrease in the level of superoxide dismutase, an antioxidant enzyme and increase in lipid peroxidation resulting to high levels of MDA recorded after CCl_4 administration. This study is in accordance with the finding of Campo *et al.* (2004) and Yacout *et al.* (2012) who independently reported similar result. The present findings demonstrate the effectiveness of methanol extract of *A. precatorius* leaves in the reduction of CCl_4 hepatotoxicity. This was via observed enhancing activity of liver SOD and reducing liver MDA contents which may have occurred by prevention of cell membrane lipids. This is attributed to the presence of numerous compounds in the leaves with high antioxidant content that scavenge the produced superoxide anion and hydroxyl radicals (Dasgupta *et al.*, 2004). High flavonoid contents of this plant leaves may have contributed significantly in line with Kostic *et al.* (2013) that flavonoids have high antioxidant activities.

CONCLUSION

The results of this study suggests that methanol extract *A. precatorius* leaves possess hepatoprotective properties against carbon tetrachloride induced liver damage in male Wistar albino rats with pronounce activity at higher concentration. It is rich in important phytochemicals and vitamins that could offer health benefits to individuals who may consume the leaves in right proportion.

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