



Research Article



In vivo antioxidant potential of *Anogeissus latifolia* bark in Ethanol-induced Oxidative stress in Rats

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ABSTRACT

Substances that have a hepatoprotective activity are those that can inhibit oxidation to protect the cells of the body from the damaging effects of oxidation. It can bind to free oxygen radicals preventing these

radicals from damaging healthy cells. The aim of the present investigation is to evaluate the *in vivo* antioxidant potential of the bark of *Anogeissus latifolia* in ethanol-induced hepatotoxicity. Animals were treated with the methanol extract of *Anogeissus latifolia* (MEAL) for 15 days and oxidative stress was induced with a single dose of ethanol (36mg/kg, p.o). The activity was determined by measuring the levels of oxidative stress markers such as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels in hepatotoxic rats. Administration of MEAL at the dose of 100 and 200mg/kg b.w., markedly decreased ethanol-induced elevation levels of oxidative stress markers and liver in a dose dependent manner. The effects of extract was compared with Silymarin, standard, at 100mg/kg b.w. In methanol extract treated animals, the toxic effect of ethanol was controlled significantly (P<0.05) by restoration of the levels of enzymes of enzymes as compared to the normal and standard treated groups. Based on the results, it was concluded that the methanol extract of *Anogeissus latifolia* bark possesses significant *in vivo* antioxidant activity and can be employed in protecting hepatic tissue from oxidative stress.

Key words: *Anogeissus latifolia*, ethanol, oxidative stress markers, *in vivo* antioxidant activity.

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ABSTRACT

Substances that have a hepatoprotective activity are those that can inhibit oxidation to protect the cells of the body from the damaging effects of oxidation. It can bind to free oxygen radicals preventing these radicals from damaging healthy cells. The aim of the present investigation is to evaluate the *in vivo* antioxidant potential of the bark of *Anogeissus latifolia* in ethanol-induced hepatotoxicity. Animals were treated with the methanol extract of *Anogeissus latifolia* (MEAL) for 15 days and oxidative stress was induced with a single dose of ethanol (36mg/kg., p.o). The activity was determined by measuring the levels of oxidative stress markers such as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels in hepatotoxic rats. Administration of MEAL at the dose of 100 and 200mg/kg b.w., markedly decreased ethanol-induced elevation levels of oxidative stress markers and liver in a dose dependent manner. The effects of extract was compared with Silymarin, standard, at 100mg/kg b.w. In methanol extract treated animals, the toxic effect of ethanol was controlled significantly ($P < 0.05$) by restoration of the levels of enzymes of enzymes as compared to the normal and standard treated groups. Based on the results, it was concluded that the methanol extract of *Anogeissus latifolia* bark possesses significant *in vivo* antioxidant activity and can be employed in protecting hepatic tissue from oxidative stress.

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1. INTRODUCTION

Alcoholism is a major public health problem and causes disease and toxicity. High alcohol consumption results in critical problems in the body alcoholic liver disease [1 & 2]. Liver injuries may be viral or caused by drugs, chemical and alcohol. One of the factors that play a central role in many pathways of alcohol induced damage in oxidative stress. Oxidative stress in the cells or tissues refers to the enhanced generation of reactive oxygen species (ROS) and/or depletion in the antioxidant defense system, causing an imbalance between pro-oxidants and antioxidants. ROS are regulated by endogenous superoxide dismutase, reduced glutathione, and catalase as a result of over-production of ROS, due to

the exposure to external oxidant substances or a failure of enzyme regulatory mechanisms leading to damage of cell structures, DNA, lipids and proteins [3].

Although, alcohol is undoubtedly a hepatotoxin, it induces a number of deleterious metabolic changes in the liver. Its excessive use for a long-time leads to development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes [4]. Recent studies in animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis and impaired liver functions and increased apoptosis [5].

In the traditional system of medicine, there are a number of plants which are used in the treatment of liver diseases. Their extract, fractions, and active constituents exhibit marked hepatoprotective action, which has been related to their antioxidant properties [6]. Many wild plants that grow in deserts, mountains and other different environments have been used traditionally to treat various problems. Traditionally, *Anogeissus latifolia*, family: Combretaceae, commonly known as Axlewood has been used as a remedy for fever [7], anemia and urinary discharges, piles [8], diarrhea, dysuria, cough [9], wound healing [10], antiulcer [11], hypolipidemic [12], hepatoprotective activity [13], colic, liver complaints, snake bite [14], and skin diseases.

We have already reported hepatoprotective activity of *Anogeissus latifolia* in ethanol intoxicated rats from our laboratory [13]. As no reports are available on the hepatoprotective activity of the *Anogeissus latifolia* bark in ethanol-induced hepatotoxicity, the present work was undertaken to investigate on ethanol-induced hepatotoxicity by estimating the oxidative stress markers in ethanol-induced hepatotoxic experimental animals.

2. MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used for the current investigation ethanol, thiobarbituric acid (TBA), trichloroacetic acid (TCA), ethylenediamine tetraacetic acid (EDTA), nitroblue tetrazoleum (NBT), 5,5-dithio bis-2-nitro benzoic acid (DTNB), reduced glutathione (GSH), phenazone methosulfate (PMS), nicotinamide adenine dinucleotide (NADH), and hydrogen peroxide (H₂O₂) were purchased from Sigma Aldrich, USA. All other chemicals and reagents were procured commercially from local sources and were of analytical grade.

Plant material and extraction

The bark of *Anogeissus latifolia* was collected from Tamilnadu State of India. The plant material was identified and authenticated by the standard literature and different extracts were obtained by hot continuous extraction method to obtain its respective extracts. The extracts were filtered and concentrated in vacuum under reduced pressure using Rotary flash evaporator. The extracts were kept in desiccator for further analysis. Methanol extract was

selected for the present investigation as it is found to contain most of its active constituents.

Experimental animals

Wistar albino rats of either sex weighing between 150-200g were used and were kept in polypropylene cages and maintained 25±5°C under 12 h light/dark cycle. The animals were acclimatized for seven days and fed with commercially available rat pelleted diet. Water was allowed *ad libitum* during the experiment.

Evaluation of Hepatoprotective activity

A total of 30 rats (6 normal; 24 ethanol induced hepatotoxic rats) were used in the present experiment. The animals were divided into five groups of 6 animals each (n=6) and were treated orally for 15 days. Group-I: served as Normal control and treated with distilled water only; Group-II: served as Hepatotoxic control, treated with ethanol in a single dose (3.76mg/kg) to produce acute hepatotoxicity; Group-III and IV animals were treated with daily doses of 100mg and 200mg/kg, p.o., respectively, of MEAL for 15 days and Group-V: served as a standard group, and were administered Silymarin at the dose of 100mg/kg. The animals of Groups III to V were given single dose of ethanol, 3.76mg/kg, and 6 h after the last treatment. The effect of MEAL on ethanol-induced hepatotoxic rats was determined by measuring the physical parameters (such as liver weight and volume), biomarker enzymes and histopathology [13].

Assessment of oxidative stress markers

There is an increasing evidence that oxidative stress plays an important etiologic role in the development of alcoholic liver disease. Alcohol administration causes accumulation of reactive oxygen species (ROS), including superoxide and hydrogen peroxide. Livers of experimental animals were homogenized with ice-chilled 10% KCl solution and centrifuged at 3000rpm for 10 minutes. Then the supernatant obtained was used for the oxidative stress markers such as lipid peroxidation (LPO) [15], reduced glutathione (GSH) [16], superoxide dismutase (SOD) [17], and catalase (CAT) [18] in liver tissue.

Statistical Analysis

The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of analysis of ANOVA followed by Student's *t*-test. P

value of < 0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

Estimation of oxidative stress markers

The oxidative stress in the liver tissue was assessed by measuring the levels of TBARS, and antioxidant defense enzymes viz., GSH, SOD, and CAT in ethanol administered and plant extract treated groups.

Determination of TBARS levels

Thiobarbituric acid reactive substances (TBARS) levels were measured as a marker of LPO and malondialdehyde (MDA) production. MDA is an endogenous genotoxic product of enzymatic and ROS-induced LPO whose adducts are known to exist in DNA isolated from healthy human being [19]. The alcohol intoxication increases lipid peroxide production (LPO) in various tissues, and is indicative of tissue oxidative stress. Nearly 60-80% ingested alcohol is metabolized in the liver, and is makes it more vulnerable than other organs to alcohol-induced oxidative stress [20].

In our study, a significant increase in TBARS level was noted on ethanol exposure in liver compared to normal animals (Table 1). Increased lipid peroxidation in ethanol intoxication group showed imbalance in redox status of liver.

MEAL was able to prevent induction of lipid peroxidation in a dose dependent manner by significantly ($P<0.05$) depleting TBARS formation. The extract proved to protect liver from peroxidation injury (decreasing TBARS content).

The significant depletion of levels of TBARS and lipid peroxides in the liver tissue of the plant extract administered animal group might be due to reduced lipid peroxidation and/or elevation of tissue antioxidant defense enzymes activity levels, indicating that the plant extract could reduce the generation of free radicals and increase free radicals scavenging mechanism.

Determination of reduced GSH levels

Reduced GSH is the main antioxidant found in cells that plays a critical protective role in the metabolism of a large number of toxic agents, including ethanol. Several studies have reported that acute ethanol administration decreases hepatic GSH content [21,

22]. In addition, a decline in hepatic GSH has also been reported in patients chronically dependent on alcohol [23]. Restoration of GSH has been shown to inhibit ethanol-induced liver injury [24].

In the current study, it is confirmed that ethanol administration significantly decreased the hepatic GSH level. The decreased level of GSH in ethanol control group may be due to its use by the excessive amount of free radicals. The reduced GSH levels were increased to near normal in drug treated rats (MEAL at 100mg and 200mg/kg), which may be due to the antioxidant activity of MEAL (Table 1).

Determination of SOD levels

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering steady-state level of O_2 .

Administration of MEAL increased the reduced levels SOD at the dose of 100mg (Group III) and 200mg (Group IV) when compared to the levels in ethanol-induced control group. Silymarin at the dose of 100mg/kg (Group V) significantly increased the SOD when compared to the ethanol-induced control rats (Table 1).

Determination of CAT activity

CAT is a key component of the antioxidant defense system. It acts as a preventive antioxidant and plays an important role in the protection against the deleterious effects of LPO (lipid hydroperoxide). Reports have shown that there is a significant decrease in the activities of catalase in alcoholic subjects [25]. A decreased activity of CAT is due to exhaustion of the enzyme as a result of oxidative stress induced by the alcohol. Presumably, a decrease in CAT activity could be attributed to cross-linking and in activation of the enzyme protein in the lipid peroxides.

Feeding with MEAL increased the activity of CAT in ethanol induced liver damaged rats in a dose dependent manner to prevent the accumulation of excessive free radicals and protects the liver from ethanol induced toxication (Table 1).

Table 1: Effect of MEAL on TBARS, reduced GSH, SOD and CAT levels

Group	Treatment	TBARS levels (nM MDA/g of protein)	Reduced GSH levels (µm/mg of protein)	SOD levels (Units/mg of protein)	CAT levels (Units/mg of protein)
I	Normal control	4.50 ± 0.29	17.32 ± 0.09	12.10 ± 0.08	94.02 ± 4.08
II	Ethanol control (3.76mg/kg)	9.01 ± 2.13*	6.21 ± 0.92*	4.01 ± 0.77*	28.59 ± 3.06*
III	Ethanol + MEAL (100mg)	7.30 ± 1.72 [#]	7.88 ± 0.06 [#]	5.09 ± 0.84 [#]	38.12 ± 1.46 [#]
IV	Ethanol + MEAL (200mg)	7.01 ± 2.23 [#]	10.51 ± 0.18 [#]	7.03 ± 0.23 [#]	55.08 ± 4.36 [#]
V	Ethanol + Silymarin (100mg)	6.15 ± 1.20	15.86 ± 1.71	8.04 ± 0.55	81.05 ± 5.17

Values are mean ± S.E.M., n=6; *Values are statistically significant at P<0.01, compared to normal control; [#]Values are statistically significant at P<0.05, compared to ethanol control.

4. CONCLUSION

The current research work was focused on *in vivo* antioxidant status of methanol extract of *Anogeissus latifolia* bark in ethanol induced hepatotoxic rats. From the results, it can be concluded that the methanol extract of *Anogeissus latifolia* bark was found to play an important role in the protection against alcohol induced hepatotoxicity and oxidative stress. Further clinical studies are required to assess the benefits and safety of *Anogeissus latifolia* bark before use in human beings.

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