

Protective Effect of Abrus Precatorius Seed Extract following Alcohol Induced Renal Damage

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Abstract

Acute alcohol intoxication and chronic alcoholism are common medical conditions that are difficult to treat. Abrus Precatorius is a leguminous plant of the fabacea family whose seed, leave and roots are widely used for medicinal purposes in tropical and subtropical regions of the world. This study investigated the renal protective activities of the seed extract of abrus precatorius following alcohol induced renal damage in adult male Sprague dawley wister rats. Experimental rats were divided into six groups of five rats per group. Renal damage was induced with alcohol (1.6g/kg) orally. The treated group received the crude extract (200mg/kg) orally in addition to alcohol for six weeks, with normal feeds and water ad libitum. Histological studies, biochemical indicators of renal function and thiobarbituric acid-reactive substances, as markers of lipid peroxidation, were thereafter determined. Oral administration of alcohol caused significant elevation of serum potassium and sodium levels as well as creatinine and malondialdehyde levels. There were structural alterations in renal tubules, glomerular infiltration by chronic inflammatory cells. Concurrent administration of same doses of alcohol and seed extract of abrus precatorius resulted in a suppression of alcohol- induced renal injury. Measurement of malondialdehyde level indicated that this effect is related to the attenuation of alcohol induced lipid peroxidation by the seed extract ($p < 0.05$). We conclude that the seed extract of abrus precatorius could protect the kidney against alcohol- induced parenchymal injury.

Keywords: Abrus precatorius, Alcohol, Anti oxidants, Ethnopharmacology

Introduction

The kidney is central to total body homeostasis, regulating water and electrolyte balance and acid base maintenance, among other critical functions (Kumar and Clark, 2002). Renal damage may occur as a result of acute intoxication or chronic alcoholism and this has been well established (Heidland et al, 1985; Vamvalas et al, 1998, Cecchin and Demarch, 1996; Epstein, 1997). As much as sixty five percent of chronic alcoholics may have IgA nephropathy at autopsy (Gonzalez- Quitela et al, 2008). The mechanism by which alcohol induces renal damage is uncertain. Nevertheless, a role has been postulated for free radical induced lipid peroxidation (Nordman et al, 1992; Toykuni, 1999; Kera et al, 1985)

In the field of ethnopharmacology, there is an ongoing search for medicinal plants that may have protective effects against toxin induced tissue damage. This study focused on the seed extract of *abrus precatorius*. *Abrus precatorius* is a leguminous plant of the fabacea family that is also called Indian liquorice, Jequirity, Crab eye, Glycyrrhizin glabra, among others. The plant grows widely in fairly dry climates of tropical and subtropical regions, such as India, Sri Lanka, Nigeria and the West Indies. The leaves, roots and seeds of *abrus precatorius* are used for medicinal purposes, a practice most probably dating back to antiquity (Ivan, 2003).

This study was designed to investigate the following hypothesis: that the aqueous extract of the seed of *abrus precatorius* has protective effect against alcohol- induced renal damage; and that this effect is related to a reduction in alcohol- induced lipid peroxidation.

Materials and Methods

Materials

The plant material, *Abrus precatorius* seeds, were obtained from a local market in Lagos and authenticated by Professor Dele Olowokudeji of the Department of Botany, University of Lagos.

The seeds were ground into powder and then soxhlet extracted with distilled water in the Department of Pharmacognosy, University of Lagos. The yield was concentrated into a solid paste *in vacuo* at 50°C using a rotary evaporator and then stored at 00C until ready for use. 200mg/kg of the extract was administered to rats orally.

50% Ethanol (NAAFCO, London) was obtained from the Department of Biochemistry, University of Lagos.

For this study, thirty male Sprague-Dawley rats with age range of 12- 14 weeks and weighing 216 – 234g were utilized. The rats were acclimatized in well ventilated metal cages at room temperature of 29-30⁰C in the Department of Anatomy, University of Lagos for two weeks. They were fed on rat pellet and water *ad libitum* and weighed weekly. The rats were randomly sorted into six groups of five rats per group and the experimental protocol set up as follows

Control (Negative control): was administered pellet feeds and water.

Group A: (Positive control): was given alcohol (1.6g/kg/rat) for a period of 6 weeks.

Group B: was treated with Alcohol (1.6g/kg/rat) for six weeks and then alcohol withdrawn for the next four weeks.

Group C: was treated with Alcohol (1.6g/kg/rat) and *Abrus precatorius* seed extract at a daily dose of 200mg/kg/body weight for a period of 6 weeks.

Group D: was treated with Alcohol 1.6g/kg/rat and vitamin E at a daily dose of 400mg/kg/rat for a period of 6 weeks.

Group E: received alcohol, vitamin E and *Abrus precatorius* extract at same doses as above for a period of 6weeks.

Retrieval of tissue

The rats were subsequently anaesthetized with intramuscular ketamine 1mg/kg, the chest opened and blood samples collected by heart puncture. Plasma was separated and stored at 0°C. Serum sodium and

potassium concentration were estimated by the Sompler flame photometric method (Fortes and Starkey, 1977). Serum creatinine was estimated by the alkaline picrate method of Hare (1950).

The experimental rats were sacrificed; the kidneys harvested, sliced and homogenized with 1.15% KCl solution according to the method of Mihara and Uchiyama (1978).

Determination of Lipid Peroxidation by Measuring Thiobarbituric Acid Reactive Substance (MDA)

Malondialdehyde (MDA) level was determined in the supernatant of the renal homogenates by the modified method (Buege and Aust, 1978). The concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56×100000 M.

Tissue Preparation

Harvested kidneys were weighed on an electronic weighing scale and thereafter prepared and processed according to routine procedures for Haematoxylin and Eosin (H & E) staining. Sections of 0.5 micron were taken for examination under the light microscope. Qualitative differences were evaluated among the six experimental groups. Renal damage was evaluated as tubular epithelial cell necrosis, tubular dilation, protein casts, infiltration by inflammatory cells and medullary congestion. The alterations were semi-quantitatively graded by a pathologist blinded to the nature of the experiments. The grading was performed by the following criteria: - = absent, + = barely present, ++ = moderate, +++ = severe.

Statistical Analysis

Data of biochemical analysis are reported as means \pm SEM and analyzed statistically by one-way analysis of variance and the Student-Neumann-Keuls test. The level of significance was set at $P < 0.05$.

Results

The changes in body weight of experimental animals during the study period are as presented in figure 1. There were no significant changes in body weight during the initial two weeks of the study. Animals in the control group had the highest increase in body weight whereas those in group B showed the lowest weight change in the first 6 weeks.

The relative weights of the kidneys of the different groups of experimental rats are presented in figure 2. The result showed a significant increase in organ weight in groups B, C and D animals when compared to group A ($P < 0.05$). However, experimental animals in Group E had no significant change in the weight of the kidney as compared to group A ($P > 0.05$).

Electrolytes and Creatinine levels

Results presented in Table 1 represent electrolyte and creatinine concentration of the different experimental groups. There was significant alteration in renal function in groups A and E in comparison to control group as indicated by electrolytes (sodium and potassium) and creatinine levels. Creatinine levels were also significantly increased in Group A, B and E. Sodium and potassium levels in groups B, C and D showed significant reduction ($P < 0.05$) in comparison to levels in group A (positive control).

Malondialdehyde (MDA) Levels

Malondialdehyde concentration, an index of lipid peroxidation, was significantly increased in the kidneys of group A animals in comparison to control ($P < 0.05$). Groups B, C and D showed significant reduction in the levels of MDA as compared to group A ($P < 0.05$) (table 2 and figure 3).

Histopathological examination of the specimens showed severe tubular dilatation in group A. Renal parenchymal alterations were minimal in group C rats which were treated with alcohol and abrus precatorius for six weeks (Table 3; Figure 4).

Figure 1: Body weight changes in rats challenged with alcohol and treated with *A. precatorius*. Group A, treated with 1.6g/kg of alcohol for 6weeks. Group B, treated with alcohol 1.6g/kg for 6weeks and then withdrawn for 4weeks. Group C, treated with alcohol and *A.precatorius* for 6weeks. Group D, treated with alcohol and vitamin E. Group E, treated with alcohol, *A. precatorius* and vitamin C. Control group, saline treated. The columns and bars represents mean \pm SEM values ($n=5$) and differences were analyzed by student's t test relative to group A. * $P > 0.05$ indicates no significant difference and # $P < 0.05$ indicates significant difference in average weight

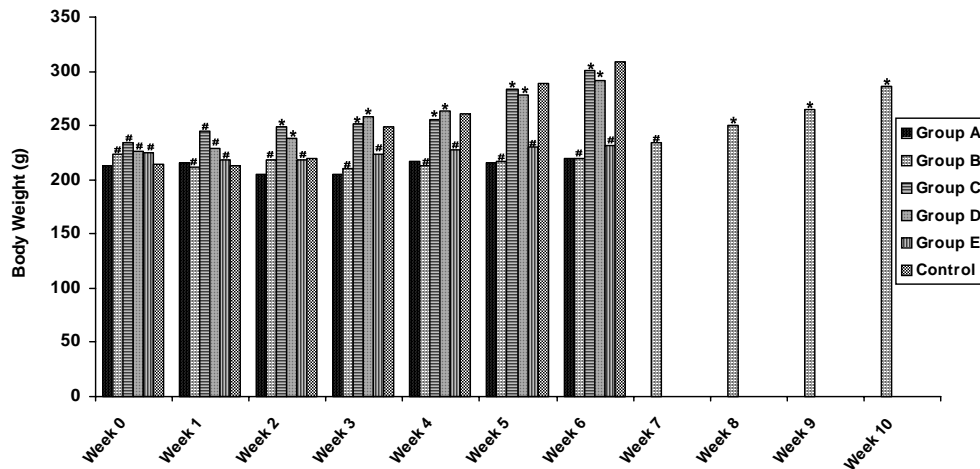


Figure 2: Kidney weight changes in control and treated rats. Groups are as follows: A; treated with alcohol for 6weeks, B; treated with alcohol for 6weeks and then withdrawn for 4weeks, C; treated with alcohol and A.precatorius, D; treated with alcohol and vitamin E, E; treated with alcohol, A. precatorius and vitamin E and Control; saline-treated. All values are expressed as Mean±SEM (n=5) # =P>0.05; * =P<0.05

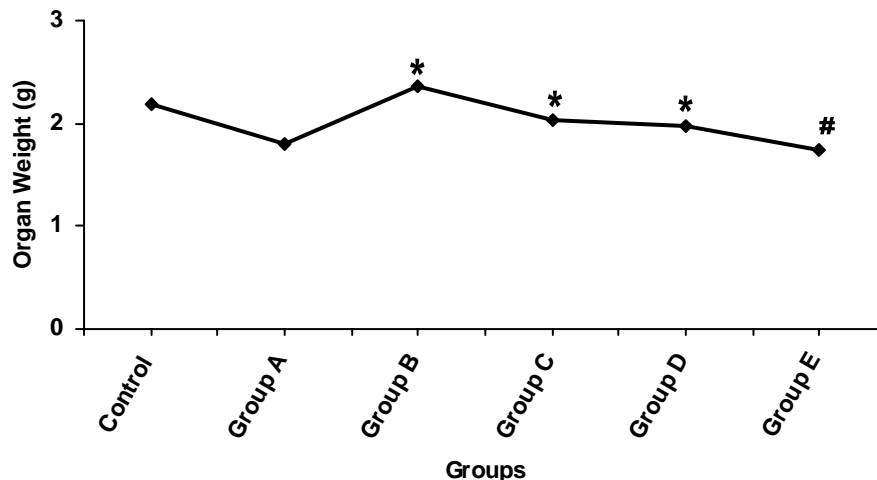


Table 1: Electrolytes and creatinine of control and treated rats

| GROUP | SODIUM (mmol/L) | POTTASIUM (mmol/L) | CREATININE (mmol/L) |
|---------|-----------------|--------------------|---------------------|
| Control | 132.2±0.64 | 4.36±0.12 | 53.0±0.32 |
| Group A | 159.0±0.77 | 7.28±0.15 | 62.07±0.91 |
| Group B | 134.0±2.42* | 5.90±0.16* | 62.4±0.97# |
| Group C | 141.0±0.057* | 4.60±0.15* | 58.06±0.59* |
| Group D | 139.0±2.59* | 4.64±0.10* | 55.1±0.81* |
| Group E | 157.8±3.11# | 6.70±0.14# | 80.58±1.64# |

All values are expressed as Mean±SEM (n=5). Groups are as follows: A; treated with alcohol for 6weeks, B; treated with alcohol for 6weeks and then withdrawn for 4weeks, C; treated with alcohol and A.precatorius, D; treated with alcohol and vitamin E, E; treated with alcohol, A. precatorius and vitamin E and Control; saline-treated.# =P>0.05 * =P<0.05

Table 2: Malondialdehyde Concentration in kidneys of control, treated rats and lipid peroxidation inhibition rate of seed extract of abrus precatorius.

| Groups | MDA (umol/mg) | Inhibition rate (%) |
|---------|---------------|---------------------|
| Control | 0.43±0.015 | - |
| A | 0.72±0.012 | - |
| B | 0.54±0.015 | 62.1 |
| C | 0.52±0.05 | 69.0 |
| D | 0.56±0.07 | 55.2 |
| E | 0.69±0.05 | 10.3 |

All values are expressed as Mean±SEM (n=5). Groups are as follows: A; treated with alcohol for 6weeks, B; treated with alcohol for 6weeks and then withdrawn for 4weeks, C; treated with alcohol and A.precatorius, D; treated with alcohol and vitamin E, E; treated with alcohol, A. precatorius and vitamin E and Control; saline-treated.# =P>0.05 * =P<0.05

Figure 3: Peroxidation Inhibition rate expressed in percentage. All values are expressed as Mean±SEM (n=5) the groups are: A; treated with alcohol for 6weeks, B; treated with alcohol for 6weeks and then withdrawn for 4weeks, C; treated with alcohol and A.precatorius for 6weeks, D; treated with alcohol and vitamin E for 6weeks, E; treated with alcohol, A. precatorius and vitamin E for 6week and Control; saline-treated. # = P>0.05; * =P<0.05

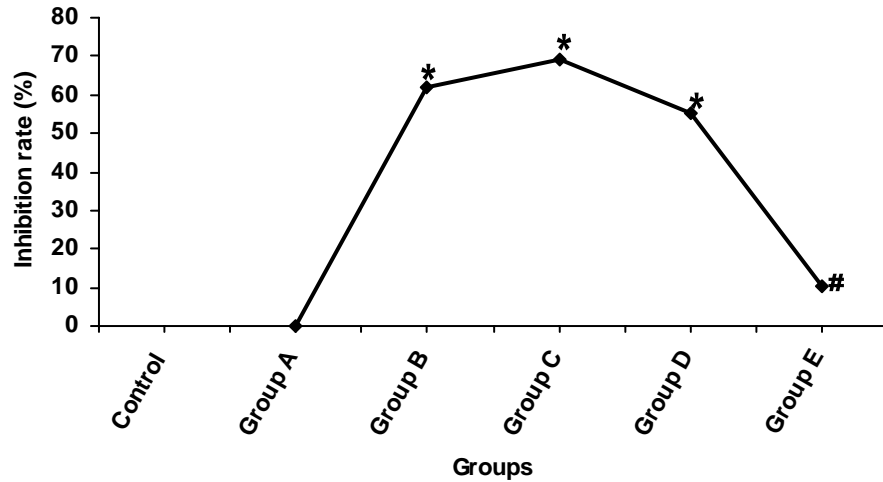


Figure 4: Micrographs of kidneys from control and treated rats

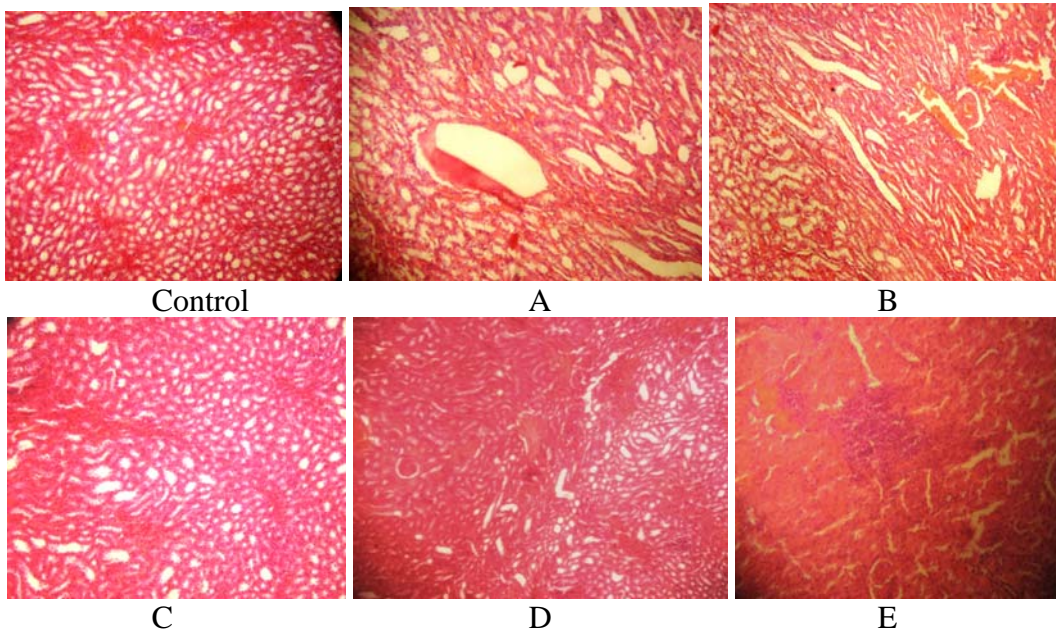


Table 3: Histopathological findings in kidneys of control and treated adult male wister rats

| GROUPS | TUBULAR DILATATION | PROTEIN CAST | MEDULLARY & CORTICAL CONGESTION | INFLAMMATORY CELLS | HERMORRHAGE AND NECROSIS |
|--|--------------------|--------------|---------------------------------|--------------------|--------------------------|
| CONTROL-saline treated | - | - | - | - | - |
| A-treated with alcohol for 6weeks | +++ | ++ | - | ++ | - |
| B-alcohol withdrawn for 4weeks after 6weeks of treatment | ++ | - | ++ | + | - |
| C-treated with alcohol and <i>A.precatorius</i> for 6weeks | + | - | - | - | - |
| D-treated with alcohol and vitamin E for 6weeks | + | - | - | - | - |
| E-treated with alcohol, <i>Abrus precatorius</i> and vitamin E | - | - | - | +++ | ++ |

- Absent
+ Barely present
++ Mild
+++ Moderate
++++ Severe

Discussion

Alcohol is widely consumed. It is regarded as the most commonly abused drug in the world with profound consequences, both societal and medical (Masters, 2004). In this study, alcohol administration induced severe renal injury evident as derangement of serum electrolyte, elevation of creatinine levels and structural alterations of tubules, glomeruli as well as parenchymal infiltration by chronic inflammatory cells. The attendant elevation of malondialdehyde level indicates that the damage is related to increased lipid peroxidation. This is consistent with the findings of previous studies which have implicated the generation of reactive oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals in alcohol- induced tissue injury (Kera et al., 1985). Alcohol may also produce tissue damage by causing depletion of glutathione, mitochondrial damage, dysregulation of growth factor signalling and the potentiation of cytokine- induced cellular injury (Master, 2004). Renal damage that occurs as a result of alcohol consumption may be reversible with abstinence (Cecchin and Demarch, 1996); as was also the case in this study.

Rats administered with alcohol and *Abrus precatorius* seed extract exhibited significant attenuation of both structural and functional derangement with concomitant reduction in malondialdehyde level. This finding is supportive evidence that the seed extract of *Abrus precatorius* has protective effect against alcohol induced renal injury and that this effect may be related to a reduction in alcohol- induced lipid peroxidation. The active metabolites in the seed of *abrus precatorius* include abrin, abrus agglutinin, glycyrrhizin gallic acid, trigonelline, precatorine and lipolytic enzymes. Glucine, Coumestrans, resin asparagines and sterols, among others, have also been demonstrated (Rajavam and Janard, 1992; Ivan, 2003). Gallic acid, glycyrrhizin and trigonelline are potent antioxidants (Lakshmi et al, 2006). These metabolites may account for the ability of the seed extract of *abrus precatorius* to attenuate alcohol induced lipid peroxidation of renal cell membrane vivo.

The seed extract of *abrus precatorius* have also been shown to possess other pharmacologic properties. It was shown to have antifertility effect by Rao (1987). Nwodo also demonstrated ureterotonic effect (1991a) and antidiarrhoeal effect (1991b). More recently, Adelowotan et al. (2008) demonstrated antimicrobial activities with the aqueous extract of the seed of *abrus precatorius*. Although *abrus precatorius* has been shown to be stable in the gastrointestinal tract, the presence of toxic lectins in its seed limits its pharmacologic utility. Abrin and Abrin agglutinin are type IV ribosome inactivating proteins that inhibit protein synthesis in eukaryotes and induce apoptosis (Bagaria et al., 2006).

Concurrent administration of vitamin E, a potent antioxidant, with alcohol and *abrus precatorius* seed extract did not produce enhanced antioxidant effect in this study. This can be explained by the diversity of the mechanisms by which antioxidants restrict lipid peroxidation by free

radicals. Denisov and Azatyam (2000) explained that the co-administration of two inhibitors of free radicals to an oxidized hydrocarbon or other substances may exhibit a net additive, synergistic or antagonistic effect. It is not unlikely therefore that a net antagonistic effect was the outcome following concurrent administration of the seed extract of abrus precatorius, alcohol and vitamin E.

Conclusion

The results of this study strongly indicate that the aqueous extract of the seed of abrus precatorius has protective effect on alcohol- induced renal injury and that this effect is related to the attenuation of alcohol- mediated lipid peroxidation of renal parenchymal cells.

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