PHARMACOLOGY AND CELL METABOLISM

Methanolic Extract of Cnidoscolus aconitifolius Attenuates Renal Dysfunction Induced by Chronic Ethanol Administration in Wistar Rats

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Abstract — Aims: The present work studied the modulatory role of methanolic extract of Cnidoscolus aconitifolius leaf (MECA) in rat model of renal dysfunction induced by chronic ethanol administration. Methods: Forty-two male Wistar albino rats weighing between 170 and 180 g were distributed into seven groups of six animals each. Some groups were pretreated with MECA (100 and 200 mg/kg) or kolaviron (KV) (200 mg/kg) for 2 weeks before simultaneous administration of MECA or KV and 20% ethanol (7.9 g/kg) for eight consecutive weeks. Others were given ethanol or MECA (200 mg/kg) or KV alone, and the control received corn oil (Vehicle). KV served as the standard antioxidant. Results: In ethanol-treated rats, serum urea, creatinine, urinary glucose, gamma-glutamyltransferase and protein increased by 59, 81, 70, 148 and 63%, respectively, while creatinine clearance significantly (P<0.05) decreased by 79%. MECA significantly (P<0.05) attenuated the above biochemical indices to near normal. Also, the levels of serum and kidney malondialdehyde (MDA) (Index of lipid peroxidation) increased by 102 and 143%, respectively, in ethanol-treated rats. Ethanol intake was significantly (P<0.05) decreases in the levels of catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) in kidney of the rats. MECA attenuated the ethanol-induced increases in serum and kidney MDA, and also enhanced the antioxidant status of the rats by increasing the levels of CAT, SOD and GSH. The activity of MECA was comparable with KV at 200 mg/kg. The biochemical findings were corroborated by histopathological examination of the kidney. Conclusion: The results suggest that the renal protective effect of C. aconitifolius leaf extract is by attenuating oxidative stress induced by chronic ethanol administration.

INTRODUCTION

It is known that excessive alcohol consumption may induce dramatic changes in the physiological and biochemical processes of the whole organism (Clemens and Jerrells, 2004; Oba et al., 2005; Poschl and Seitz, 2004). Ethanol, the type of alcohol considered in this study, is absorbed mainly in the intestine, where it is channelled through the portal vein directly towards the liver before passing through the circulatory system to the whole body. Three enzymatic systems are involved in ethanol oxidation: alcohol dehydrogenase (ADH), the microsomal ethanol-oxidizing system (MEOS) and catalase (CAT) (Riveros-Rosas et al., 1997). Ethanol is metabolized mostly by ADH, an enzyme that couples the oxidation of ethanol into acetaldehyde with the reduction of nicotinamide adenine dinucleotide (NAD⁷). The MEOS system connects ethanol and NAD⁺ phosphate oxidation to the reduction of molecular oxygen to hydrogen peroxide, and requires the participation of the P-450 cytochrome (Lieber, 2005). In the third system, the oxidation of ethanol molecule into acetaldehyde is linked to the simultaneous decomposition of hydrogen peroxide in a reaction catalyzed by CAT. It is a fact that chronic ethanol ingestion leads to the formation of reactive oxygen species (ROSs) (Mantel and Preedy, 1999), and may accelerate oxidative mechanism directly or indirectly, which eventually produces cell death and tissue damage (Sun et al., 2001). The liver is the primary organ responsible for the oxidation of ingested ethanol, but other tissues, including the kidney, may contribute to ethanol metabolism (Lieber, 1988). The biochemical alterations caused by ethanol consumption on the kidney are not well understood. Some clinical and experimental studies have been focused on the effects of ethanol feeding on renal function, gross and microscopic morphology of the kidney (Assadi and Zajac, 1992; Brzoska et al., 2003; Rodrigo et al., 1998).
protective effect of *C. aconitifolius* leaf extract in alcohol-treated rats.

**MATERIALS AND METHODS**

**Chemicals**

Trichloroacetic acid and thiobarbituric acid (TBA) were purchased from BDH Chemical Ltd., Poole, UK. Absolute ethanol and hydrogen peroxide were procured from Sigma Chemical Co., Saint Louis, MO, USA. Other reagents were of analytical grade and purest quality available.

**Animals**

Inbred 6-week-old male Wistar albino rats weighing between 170 and 180 g were purchased from the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. The animals were kept in well-ventilated cages at room temperature (28–30°C) and under controlled light cycles (12 h light/12 h dark). They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. Handling and treatments of rats conform to the guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use, and also approved by the University of Ibadan Animal Ethical Committee (UI-00213-2009).

**Preparation of plant extract**

Fresh leaves from *C. aconitifolius* were collected from the premises of University of Ibadan, Nigeria. The botanical identification was carried out at the herbarium in the Department of Botany, University of Ibadan, Nigeria, where a voucher specimen already exists. About 3 kg of the air-dried leaf was extracted in a soxhlet extractor at 30°C for 5 h using methanol. The extract was concentrated under reduced pressure in vacuum at 35°C for 30 min using a rotary evaporator. The resulting residue, called methanolic extract of *C. aconitifolius* (MECA), was transferred to a hot air oven, where it was dried to a constant weight at 40°C. The yield of the preparation was 10.5% (w/w). Prior to the experiments, MECA was dissolved in corn oil overnight and was administered to the animals according to their weights.

**Study design**

Forty-two male albino rats (Wistar strain) were randomly distributed into seven groups of six animals each. Animals were given a period of 2 weeks for acclimatization before the experiment. The first group served as the control and was given corn oil (Vehicle for drugs). The second group received 20% ethanol only (7.9 g/kg) for 8 weeks. The third and fourth groups were pretreated for 2 weeks with MECA at doses of 100 and 200 mg/kg, respectively, before treatment with 20% ethanol (7.9 g/kg) for the next 8 weeks. The fifth and sixth groups were treated with MECA and kolaviron (KV), respectively, at a dose of 200 mg/kg throughout the duration of the study. The seventh group was pretreated for 2 weeks with KV at a dose of 200 mg/kg before treatment with 20% ethanol (7.9 g/kg) for the next 8 weeks. Previous studies showed that KV was very effective at 200 mg/kg body weight and thus informed the choice of this dosage for the present study (Adaramoye et al., 2005; Farombi et al., 2005). KV was prepared with corn oil, and absolute ethanol (95%) was diluted with distilled water to 20% and given at a dose of 7.9 g/kg according to Rajakrishnan et al. (1997) and Arulmozhi et al. (2010). All drugs were administered daily to the animals by oral gavage.

**Preparation of samples**

Twenty-four hours following the last treatment, 24-h urine free of food and faeces was collected into ice-cold glass tubes for the determination of protein, glucose and the activity of gamma-glutamyltransferase (GGT). Rats were killed after an overnight fast by cervical decapitation under light aether anaesthesia. Kidney samples were quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. A section of kidney sample was fixed in 10% formalin. Other parts were homogenized in four volumes of 5 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 min to obtain post-mitochondrial supernatant fraction (PMF). The samples were stored at −80°C until used. All procedures were carried out at temperature of 0–4°C. The antioxidant profile of the animals was investigated in the PMF.

Blood was collected from the inferior vena cava of heart of the animals into plain centrifuge tubes and was allowed to stand for 1 h. Serum was prepared by centrifugation at 3000g for 15 min in an MSC bench centrifuge. The clear supernatant was used for the estimation of serum urea, creatinine and extent of lipid peroxidation.

**Biochemical assays**

Urinary, serum and PMF protein levels were determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard. Serum urea and creatinine and urinary glucose were measured using Randox diagnostic kits. GGT was determined according to the method of Fossati et al. (1986). Serum and PMF lipid peroxidation levels were assayed by the reaction between 2-TBA and malondialdehyde (MDA), an end product of lipid peroxides as described by Walls et al. (1976). PMF reduced glutathione (GSH) level was assayed by measuring the rate of formation of chromphoric product in a reaction between 5,5-dinitrobenzoic acid and free sulphydryl groups (such as GSH) at 412 nm as described by Moron et al. (1979). PMF superoxide dismutase (SOD) activity was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich (1969). PMF CAT activity was assayed by measuring the rate of decomposition of H₂O₂ as described by Aebi (1974).

**Histological assessment**

Kidneys from rats were fixed in 10% formaldehyde, dehydrated in ethanol and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter stained with haematoxylin–eosin for light microscopic analysis. The slides were examined by a Histopathologist who was ignorant of the treatment groups. Renal histological damage was assessed on a score previously described by Teixeria et al. (1982).
Statistical analysis

All values were expressed as the mean ± SD of six animals per group. Data were analysed using one-way ANOVA followed by the post hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0). Values were considered statistically significant at $P < 0.05$.

RESULTS

Figures 1 and 2 depict the effect of MECA or KV on serum creatinine and urea levels in rats treated with ethanol for eight consecutive weeks. Serum creatinine and urea levels were significantly ($P < 0.05$) increased in rats treated with ethanol when compared with controls. Treatment of rats with MECA or KV at 200 mg/kg before and simultaneously with ethanol prevented the ethanol-induced increase in serum creatinine and urea. Furthermore, rats treated with ethanol showed high levels of urinary protein, glucose and significantly ($P < 0.05$) increase in fractional excretion of GGT than the control (Figs 3 and 4). Also, ethanol-intoxication significantly ($P < 0.05$) decreased creatinine clearance in these animals (Fig. 5). Precisely, urinary protein, glucose and GGT levels in ethanol-treated rats increased by 63, 70 and 148%, respectively. However, treatment with MECA or KV, especially at 200 mg/kg, provided marked protective effect and improvement on these biochemical indices. Figure 6 shows that chronic ethanol-intoxication caused marked
elevation of serum and PMF lipid peroxidation as assessed by measuring MDA levels in the samples. Precisely, serum and PMF MDA levels were elevated by 102 and 143%, respectively, when compared with controls. The enhanced serum and PMF MDA levels in ethanol-treated rats were significantly (*P* < 0.05) attenuated following treatment with MECA or KV at 200 mg/kg. Specifically, MECA and KV (200 mg/kg) decreased the serum MDA levels by 39 and 47%, whereas PMF MDA levels were decreased by 35 and 56%, respectively, when compared with ethanol-treated group.

In Table 1, chronic ethanol administration to rats caused a significant (*P* < 0.05) decrease in the levels of PMF SOD, CAT and GSH by 33, 42 and 55%, respectively, when compared with controls. However, treatment with MECA or KV (200 mg/kg) restored the ethanol-mediated decrease in the levels of SOD, CAT and GSH. Precisely, MECA (200 mg/kg) treatment of ethanol-intoxicated rats increased PMF SOD, CAT and GSH levels by 41, 47 and 77%, respectively, whereas KV treatment caused 52, 63 and 115%, elevation of PMF SOD, CAT and GSH levels, respectively, relative to ethanol-treated rats. Furthermore, there were no significant differences (*P* > 0.05) in the levels of PMF protein in ethanol-treated rats when compared with others. In Fig. 7, some of the histopathological observations in slide from ethanol-treated rats include tubular necrosis, presence of protein casts in the tubular lumen and interstitial congestion.
These histopathological alterations were significantly reduced in slides obtained from rats treated simultaneously with ethanol and MECA (100 and 200 mg/kg) or KV (200 mg/kg). No visible lesions were observed in rats treated with MECA or KV alone. The results of the histopathology correlated with the biochemical changes in the serum and urinary indices of the animals.

**DISCUSSION**

In the present study, we found that chronic ethanol administration to rats for eight consecutive weeks caused significant elevation of urinary glucose, protein, GGT activity and drastic fall in creatinine clearance. The apparent increase in urinary protein and glucosuria in these animals indicates proximal tubular dysfunction. Furthermore, the presence of tubular damage was confirmed by increased urinary excretion of brush border marker GGT, suggesting a direct toxic injury caused by chronic ethanol administration in these animals. Renal morphologic examination, which revealed the presence of tubular necrosis and proteinous casts in the kidney slides of ethanol-treated rats, further confirmed renal tubular damage. Our data indicate that MECA or KV at a dose of 200 mg/kg reduced the levels of urinary glucose, protein, GGT and improved creatinine clearance. These results demonstrate the protective effect of MECA and KV against ethanol-induced renal dysfunction in these animals. This effect may be related to the antioxidant properties of MECA and KV since ROS has been implicated in the impairment of glomerular filtration rate (Hughes et al., 1996).

Serum creatinine and urea levels are the sensitive and reliable biochemical indices for evaluation of renal function in animal models (El Daly, 1996). The increased serum urea levels indicate impairment to the kidney function such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis (Jaramillo-Juarez et al., 2008). In the present study, it is noteworthy that chronic ethanol intoxication for eight consecutive weeks caused significant elevation of serum urea and creatinine. This observation has also been reported in several models (Cigremis et al., 2004). These increases could be linked to adverse effect of ethanol, which results in decline of glomerular filtration rate of renal tissue. The fact that these parameters were reversed to near normal following MECA or KV treatment further confirmed the protective effect of these natural products against ethanol-induced renal dysfunction in these rats.

Increased ROS, partly generated from acetaldehyde oxidation during ethanol metabolism, may contribute to the occurrence of oxidative stress in kidney tissues (Rodrigo et al., 1998). Our results show that ethanol-intoxication-induced oxidative stress as demonstrated by significant elevation in lipid peroxidation, and decreases in SOD and CAT activities. Previous results concerning lipid peroxidation induction by ethanol feeding in rat kidneys are controversial. Significant increase of MDA level in kidney after chronic ethanol ingestion was reported by Cigremis et al. (2004), which is in line with our study. In contrast, other studies have shown that there were no significant modifications in the kidney MDA levels of rats subjected to chronic or acute ethanol exposure (Jurczuk et al., 2004; Rodrigo et al., 2002a). The depletion in GSH status in ethanol-treated rats in this study, which is in agreement with the findings of Rodrigo et al. (2002b) and Kode et al. (2004), may be due to increase in ROS generated during ethanol metabolism in the rat kidneys (Balasubramaniyan et al., 2003). However, with MECA and KV supplementation to ethanol-treated rats, the MDA levels were drastically reduced, which may account for the observed increase in renal GSH contents of the treated rats. Chronic alcohol consumption has been linked to decrease in SOD and CAT activities in the liver, heart, brain, kidney, muscle and blood of rats (Dinu et al., 2005; Husain et al., 2001; Ozaras et al., 2003). In this study, we also observed that the activities of SOD and CAT in kidneys of ethanol-treated rats decreased significantly when compared with controls. The reduction in the levels of these enzymes may cause accumulation of superoxide radicals and hydrogen peroxide or other decomposition products. It was observed that the SOD and CAT activities in ethanol-treated rats dosed with MECA or KV were elevated. This elevation further confirmed the protective effect of MECA and KV in the kidney of these ethanol-treated rats. The elevation of SOD and CAT activities in the co-treated rats may be due to the antioxidant properties of the biflavonoid complex (KV) (Adaramoye et al., 2005) or high content of polyphenolic compounds especially flavonoids in MECA (Kuti and Konuru, 2004), which may be involved in the scavenging of superoxide radicals or hydrogen peroxide. From these findings, it can be inferred that MECA positively modulates the antioxidant status and regenerates the kidney of ethanol-treated rats to near normal.

In conclusion, this study confirms that co-administration of C. aconitifolius leaf extract with ethanol attenuates the increase in lipid peroxidative damage, restores antioxidant status, markers of renal injury and urinary excretory indices in these animals. These findings show that C. aconitifolius is a promising candidate for chemoprevention of ethanol-induced renal dysfunction. However, further detailed studies are required to establish the toxicity and protective effect of this plant on ethanol-induced liver disorders before it can be recommended for clinical trials.

**REFERENCES**


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