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Evaluation of Antioxidant potential of *Costus igneus* in ethanol induced peroxidative damage in albino rats

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ABSTRACT

Improved antioxidant status helps to minimize the oxidative damage and thus can delay the risk of developing many chronic age related, free radical induced damage in diseases like diabetes, cancer, neurodegenerative diseases. We tried to evaluate the antioxidant potential of *Costus igneus* (*CI)* leaves in ethanol induced peroxidative damage in albino rats. Wistar albino rats of either sex were divided into four groups. Group I was control group and received normal saline, Group II received ethanol, Group III received test drug *CI* at 300 mg/kg and Group IV received *CI* at dose of 600 mg/kg. Study duration was 30 days. Antioxidants estimated at the end of 30 days. The levels of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) significantly reduced and (malondialdehyde) MDA levels raised in ethanol treated group compared to control group. The levels of reduced glutathione, SOD and catalase enzyme recovered completely in a dose dependent manner. Results demonstrate *CI* significantly reversed the reduction of GSH, SOD and CAT and reduced significantly the levels of MDA, a biomarker of lipid peroxidation in a dose dependent manner, suggesting its ability to enhance the antioxidant defense to prevent alcohol induced oxidative stress injury.

INTRODUCTION

Oxidative stress is well recognized in the pathogenesis of numerous diseases like diabetes, cancer, neurodegenerative diseases and respiratory tract disorders and plays an important contributory role in the process of aging. Alcohol consumption for long period decreases the endogenous antioxidants and enhances the lipid peroxidation process in tissues (Sadrzadeh *et al.,* 1994; Anderson *et al.,* 2000). Studies have shown free radical generation during ethanol metabolism. The raised level of oxidative stress markers observed in ethanol treated rats, confirms extensive generation of free radicals (Nordmann *et al.,* 1992).The *Costus igneus* (*CI*) in Sanskrit is called as *katar kata* and its common name is *Fiery Costus* or Spiral Flag belonging to the Costaceae family found in tropical Africa, Asia, and Australia. In India, it is cultivated in coastal areas like Uttar Kannada district of Karnataka state. In this area, people traditionally take 2-3 leaves of this plant twice a day for the management of diabetes. Preliminary phytochemical screening of this plant extract revealed the presence of carbohydrate, protein, steroids, alkaloids, tannins, glycosides, saponins, fixed oils and flavonoids (Nandhakumar *et al.,* 2007). Studies have shown the antioxidant activity of plant flavonoids (Malomo *et al.,* 2011).

Earlier studies suggests that *CI* leaf extract exerts antidiabetic and hypolipidemic effects in diabetic rats (Akhila Shetty *et al.,* 2010; Vishalakshi Devi and Asna Urooj, 2008). Administration of the aqueous and ethanolic stem extract of *Costus igneus* to rats with experimentally induced urolithiasis by ethylene glycol has been found to reduce the growth of urinary stones (Manjula *et al.,* 2012). Contents of *CI* plant preparation indicate its potential to reduce the oxidative stress. This study is intended to evaluate the antioxidant potential of CI on ethanol induced free radical injury in rats.

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MATERIAL AND METHODS

Preparation of *Costus igneus* leaves extract

The leaves of *CI* were collected from the plants grown in Mangalore district, Karnataka state, India. The leaves then were shade-dried and finely powdered; the ethanolic extract is obtained by soxhlet extraction (20 g in 100 ml of 95% ethanol at 55 °C). The extract is then concentrated to 10 ml on a water bath and dried at room temperature. From 170 grams of *Costus igneus* leaf powder, the ethanolic extract yield obtained after soxhlet extraction was 46 grams. The plant was identified and authenticated by Prof. Valsaladevi, Department of Botany, Malaparamba, Calicut, Kerala where the voucher specimen (#KOBH 5791) is deposited.

Chemicals

Absolute alcohol (99.9%) was purchased from Sigma Aldrich Chemicals Private Limited, Bommasandra Jigani Link Road, Bangalore. All the chemicals used were of analytical grade.

Animals

Wistar albino rats of either sex weighing 150-250 gram inbred in institutional central animal house were used for the study. Rats were housed in clean polypropylene cages, in a controlled environment (26° - 28 °C) with a 12 hour light and dark cycle with standard rat chow (supplied by Amruth laboratory animal feed, manufactured by Pranav Agro industries ltd., Sangli) and water *ad libitum*. The rats were allowed to acclimatize for these conditions for one week prior to study. The study was carried out after obtaining the approval from the Institutional Animal Ethics Committee.

Experimental Design

The rats were divided into four groups comprising of six rats in each group as follows:

Group I: Normal Control rats received 0.9% normal saline

Group II: Ethanol (20% w/v of 2g/kg body weight)

Group III: 20% w/v of 2g/kg ethanol + ethanolic extract of *Costus igneus* 300 mg/kg body weight.

Group IV: 20% w/v of 2g/kg ethanol + ethanolic extract of *Costus igneus* 600 mg/kg body weight.

All the study groups received treatment through oral route for thirty days at a constant volume of 10 ml/kg. After the study period, three ml blood was collected by cardiac puncture in tubes containing potassium oxalate and sodium fluoride. The samples were centrifuged at 3000 rpm for 10 min to obtain the plasma and RBC, for further estimation of reduced GSH (glutathione), superoxide dismutase (SOD), catalase (CAT) antioxidant enzymes and lipid peroxidation.

Biochemical analysis

Reduced glutathione in erythrocytes was estimated by Beutler method (Beutler *et al.,* 1963). The extent of lipid peroxidation in plasma was determined by estimating malondialdehyde (MDA) which is a thiobarbituric acid reactive substances (TBARs) (Poornima K*et al.,* 2003). Enzyme assay of CAT and SOD in erythrocytes were estimated by the methods of Brannan (Brannan *et al.,* 1981) and Anuradha Nandi (Nandi and Chatterjee, 1988) respectively.

RESULTS

There were no significant changes in the body weight of rats before and after 30 days of study period in all the groups (Table 1).

Table. 1: changes in body weight of rats before and at the end of study.

Group	Weight (gm) on day one	Weight (gm) after 30days	P
I	205.50±24.18	223.00±22.40	0.22
II	228.50±22.59	201.83±22.01	0.064
III	221.75±18.28	212.25±17.20	0.34
IV	226.00±28.08	217.75±28.19	0.63
I	205.50±24.18	223.00±22.40	0.22

Group I: Normal control; Group II: Ethanol treated; Group III: *Costus igneous* extract at 300 mg/kg; Group IV: *Costus igneous* extract at 600 mg/kg. Values are expressed as mean ± SD. Data were analyzed by unpaired student t- test. P≤0.05 considered significant.

The level of MDA which is an index of lipid peroxidation is increased in ethanol group compared to control group. There is dose dependent decrease in the levels of MDA in *CI* groups. The levels of MDA is less in group 3 and 4 compared to group 2 implying enhanced antioxidant activity in test groups (Table 2).

Reduced glutathione decreased in ethanol treated group compared to control group and increased in group 3 and 4 compared to ethanol group. Its level in the test group recovered to the levels of the control group at the highest dose. There was dose dependent recovery of cellular GSH with the test drug (Table 2).

Antioxidant enzyme SOD levels reduced significantly in ethanol group compared to control group indicating increased SOD consumption for scavenging enhanced free radicals produced by ethanol. Levels of SOD in test groups shows increasing trend compared to ethanol group and recovered to control values at the highest dose (Table 2)

Catalase enzymes reduced significantly in ethanol group compared to control group indicating increased consumption of the enzyme to scavenge ethanol induced free radicals. There is dose dependent increase in the catalase enzymes in test groups. Catalase levels increased in group 3 and 4 compared to group 2 and at the highest dose there is complete recovery of the catalase enzyme (Table 2).

Statistical analysis

The results were expressed as mean ± SD and analyzed by student t-test and one-way Analysis of variance (ANOVA) followed by Tukeys post hoc test wherever applicable. SPSS version 17.0 was used for the analysis. Probability P value ≤0.05 was considered as statistically significant.

Table. 2: effect of *Costus igneus* extract on the levels of SOD, CAT, GSH and MDA.

Groups	GSH mg/dl	SOD units/dl	CAT units/dl	MDA µ mol/L
I	97.638±17.97	59130.42±21749.57	109833.33±14885.85	0.534±0.992
II	$46.206\pm 3.623^{\alpha C\delta}$	27826.06 ± 5820.26^{AD}	$71358.33\pm4757.56^{\alpha\delta}$	$1.557\pm0.205^{\alpha\gamma\delta}$
III	70.700 ± 2.966^{ABD}	38260.84±7906.18	84732.50 ± 8756.11^{AD}	$0.682\pm0.236^{\beta D}$
IV	$104.98\pm10.513^{\beta C}$	52956.08 ± 2700.00^{B}	$120700.00\pm14759.065^{\beta C}$	$0.274\pm0.077^{\beta C}$

Group I: Normal control; group II: ethanol treated; group III: costus ingeors extract at 300mg/kg; group IV: costus igneous extract at 600mg/kg. GSH=raduced glutathione; SOD=superoxide dismutase; CAT=catalase; MDA=malondialdehyde. Values are expressed as mean ± SD. α,β,γ,δ represents P≤0.001 compared to groups I, II, III, and IV respectively; A, B, C, D represents P≤0.05 compared to groups I, II, III, and IV respectively.

DISCUSSION

Lipid peroxidation (LPO) is a free radical related process, whose main end product is MDA which induces cellular damage by cross-linking cellular macromolecules such as proteins or DNA. In the present study MDA was used as a biomarker of lipid peroxidation. In our study we found that blood MDA levels were elevated in ethanol treated rats compared to the control rats, indicating increased lipid peroxidation and enhanced oxidative stress by ethanol. The results were similar to earlier reports that ethanol induces extensive generation of free radicals by tissue injury (Nordmann *et al.,* 1992).*CI* significantly inhibited alcohol induced excessive amounts of MDA as shown in Table 2. MDA levels reduced significantly in groups 3 and 4 in a dose dependent manner indicating this herb can prevent alcohol induced LPO injury.

In order to overcome the oxidative stress, organisms possess a host of antioxidant systems, including the non-enzymatic system mainly GSH.GSH related antioxidant system plays crucial role as an antioxidant, in nutrient metabolism (Wu *et al.,* 2004). GSH can scavenge free radicals and other reactive oxygen species through non-enzymatic and enzymatic process in which GSH is oxidized to glutathione disulfide (GSSG). GSSG can be reduced to GSH by glutathione reductase (GR) with the consumption of NADPH (Fang *et al.,* 2002). Previous studies report that depletion of cellular GSH is related to oxidative damage (Han *et al.,* 2006). From the present study it was observed that GSH reduced significantly in ethanol treated group indicating increased exhaustion of cellular GSH by ethanol. Results also showed that *CI* significantly increased the levels of cellular GSH in a dose dependent manner with complete reversal at highest dose, implying this herb reversed alcohol induced excessive exhaustion of GSH (Table 2). This suggests protective effects of *CI* against alcohol induced cellular injury. Results were similar to another study done in streptozotocin induced diabetic rats by a single dose of *CI* (Kripa Krishnan *et al.,* 2011).

SOD, CAT and GPx (glutathione peroxidase) are important enzymatic antioxidants that protect cell and tissue from lipid peroxidation, by scavenging the reactive oxygen species. Sabitha and Shyamaladevi have suggested that lack of antioxidant defense is responsible for the elevated lipid peroxidation in erythrocytes (Sabitha *et al.,* 1999). SOD is one of the chief cellular defense enzymes that dismutate superoxide radicals to water and oxygen. Catalases are heme-containing proteins that protect the cells from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen. Present study showed the levels of SOD and catalase enzymes reduced significantly in ethanol group compared to control indicating increased utilization of these antioxidant enzymes for scavenging alcohol induced free radical production. There was increasing trend in the levels of SOD enzyme with *CI* groups and complete recovery is seen at the highest dose.

In test groups there was significant recovery of catalase enzyme. Quantity of catalase enzyme in group 4 exceeded the quantity in control group indicating complete reversal of alcohol caused exhaustion of this enzyme (Table 2).The results were similar to earlier study (Kripa Krishnan *et al.,* 2011).

Flavonoids have been reported to be involved in antioxidant (Malomo *et al.,* 2011), anti-inflammatory (Funakoshi-Tago *et al.,* 2011), anti-tumor (Sun *et al.,* 2007) activities. Flavonoids are abundant in *CI* (Nandhakumar *et al.,* 2007). This may be partially responsible for its antioxidant activity. Further studies are required for complete understanding of intracellular mechanism of *CI* in enhancing the antioxidant defense.

CONCLUSION

Costus igneus significantly reversed the reduced GSH, SOD and CAT activities in a dose-dependent manner that was raisedby ethanol treatment and reduced significantly the levels of MDA, a biomarker of lipid peroxidation in dose-dependent manner suggesting its ability to enhance the antioxidant defense to prevent alcohol induced oxidative stress injury.

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CONFLICT OF INTEREST: No conflict of interest

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