How to Cite:
Dhone, P. G., Manish, G., Sahu, Y. P., & Mubeen, M. F. (2022). To evaluate of antioxidant activity of Costus igneus in ethanol induced peroxidative damage in albino rats. *International Journal of Health Sciences*, *6*(S5), 4506–4513. https://doi.org/10.53730/ijhs.v6nS5.9858

To evaluate of antioxidant activity of *Costus igneus* in ethanol induced peroxidative damage in albino rats

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***Abstract*---**The free radical oxidants such as reactive oxygen species, reactive nitrogen species, and reactive sulphur species are produced inside cells through various metabolic processes. The body is equipped with an antioxidant defence system that guards against oxidative damage caused by these reactive oxidants and plays a major role in protecting cells from oxidative stress and damage. *Costus igneus* possess various pharmacological activities like hypolipidemic, diuretic, antioxidant, anti-microbial, anti-cancerous. Preparation of *Costus igneus* leaves extract. The leaves of CI were collected from the plants grown. 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 rats were divided into four groups: 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. For further estimation of reduced GSH (glutathione), superoxide dismutase (SOD), catalase (CAT) antioxidant enzymes and lipid peroxidation. 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. 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. 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. *C. 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.

***Keywords*---**Oxidant, Antioxidant activity, *Costus igneus.*

Introduction

The free radical oxidants such as reactive oxygen species, reactive nitrogen species, and reactive sulfur species are produced inside cells through various metabolic processes [1]. The body is equipped with an antioxidant defense system that guards against oxidative damage caused by these reactive oxidants and plays a major role in protecting cells from oxidative stress and damage [2]. Antioxidants such as glutathione (GSH), thioredoxin, ascorbic acid and enzymes, for example, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) counter the oxidative stress and protect lipids, proteins, and DNA [3]. Antioxidants such as tocopherols, ascorbic acid, carotenoids, flavonoids, amino acids are also natural antioxidants present in foods [4]. There is increasing demand and availability of designer foods fortified with antioxidants and probiotics that may be important in human health [5].

Costus igneus Nak commonly known as Spiral flag, is a member of Costaceae and a newly introduced plant in India from South and Central America. It is a perennial, upright, spreading plant reaching about two feet tall, with spirally arranged leaves and attractive flowers [6]. In southern India, it usually grows as an ornamental plant and its leaves are used as a dietary supplement in the treatment of diabetes mellitus [7]. Recently, a number of researches have been carried out to evaluate the anti-diabetic potential of this plant. Besides, it has been proven to possess various pharmacological activities like hypolipidemic, diuretic, antioxidant, anti-microbial, anti-cancerous [8]. Further, various phytochemical investigations reveal the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, steroid, and appreciable amounts of trace elements [9].

Material and Methods

Preparation of *Costus igneus* leaves extract. The leaves of CI were collected from the plants grown. 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.

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.

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 Ket 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

Table 1: Effect of *Costus igneus* extract on the levels of GSH

Group	GSH mg/dl
I	97.638±17.97
II	46.206± 3.623αCδ
III	70.700±2.966ABD
IV	104.98±10.513βC

Group I: Normal control; group II: ethanol treated; group III: *C. igneus* extract at 300mg/kg; group IV: *C. igneus* extract at 600mg/kg. GSH=reduced glutathione; 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

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 1).

Table 2: Effect of *Costus igneus* extract on the levels of SOD

Group	SOD units/dl
I	59130.42±21749.57
II	27826.06±5820.26AD
III	38260.84±7906.18
IV	52956.08±2700.00B

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).

Table 3: Effect of *Costus igneus* extract on the levels of CAT

Group	CAT units/dl
I	109833.33±14885.85
II	71358.33±4757.56αδ
III	84732.50±8756.11AD
IV	120700.00±14759.065 βC

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 3).

Table 4: Effect of *Costus igneus* extract on the levels of MDA

Group	MDA μ mol/L
I	0.534±0.992
II	1.557±0.205αγδ
III	0.682±0.236βD
IV	0.274±0.077βC

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 are less in group 3 and 4 compared to group 2 implying enhanced antioxidant activity in test groups (Table 4).

Discussion

Sequential screening for phytochemicals of *C. igneus* leaves revealed that it is rich in protein, iron, and antioxidant components such as ascorbic acid, α-tocopherol, β-carotene, terpinoids, steroids, and flavonoids.[10] It was revealed in another study that methanolic extract was found to contain the highest number of phytochemicals such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids.[11] Preliminary phytochemical evaluation of Insulin plant (*C. pictus*) revealed that the leaves contain 21.2% fibers. Successive extracts gave 5.2% extractives in petroleum ether, 1.06% in cyclohexane, 1.33% in acetone, and 2.95% in ethanol. Analysis of successive extracts showed presence of steroids in all extracts. The ethanol extract contained alkaloid also. The major component of the ether fraction was bis (2'-ethylhexyl)-1,2-benzenedicarboxylate (59.04%) apart from α-tocopherol and a steroid, ergastanol. [12]

Stem showed the presence of a terpenoid compound lupeol and a steroid compound stigmasterol.[13] Bioactive compounds quercetin and diosgenin, a steroidal sapogenin, were isolated from *C. igneus* rhizome.[14] Trace elemental analysis showed that the leaves and rhizomes of *C. pictus* contains appreciable amounts of the elements K, Ca, Cr, Mn, Cu, and Zn.[15] Steam distillation of stems, leaves, and rhizomes of *C. pictus* D. Don yielded clear and yellowish essential oils.

An *in vitro* study of alcoholic extract of leaves of *C. igneus* showed moderate antioxidant activity. [16] The antioxidant activities of leaves and rhizomes in methanol, aqueous, ethanol, and ethyl acetate extracts were assessed using different models like DPPH, β-carotene, Deoxyribose, superoxide anion, reducing power, and metal chelating assay at different concentrations. Leaves and rhizomes of *C. igneus* showed good antioxidant activity of about 89.5% and 90.0% when compared with standard BHT (Butylated Hydroxy Toulene) (85%) at a concentration of 400 μg/ml. Results obtained revealed that methanolic extracts of

both leaves and rhizomes of *C. igneus* possess higher antioxidant activity when compared with other extracts. [17]

In another study, methanolic leaf extract of *C. igneus* caused significant increase in superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, vitamin A, vitamin C, vitamin E and reduced glutathione, and thus, could be effective in reducing oxidative stress and free radical-mediated diseases. The antioxidant property of this plant may be due to the presence of phenolic substances. [18, 19] Methanolic extracts of flower and stem of *C. igneus* possess *in vitro* antioxidant activity against oxidative protein damage. [20] Among the extracts tested for, chloroform extract of *C. igneus* bark possessed high antioxidant activity. [21] Oral administration of ethanolic extract of *C. igneus* rhizome at 200 mg/kg body weight to diabetic rats for 30 days induced a significant antioxidant effect. The bioactive compound quercetin and diosgenin present in the plant exhibited antioxidant activity, which was sufficient to reverse oxidative stress in liver, pancreas, and kidney of diabetic rats as well as to stimulate glycolytic enzymes and control gluconeogenesis in diabetic animals. [22]

Conclusion

C. 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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