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Evaluation of the antioxidant potential of methanol extract of *Cyclea peltata* in DAL Model

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Abstract
The dynamics of reactive oxygen species in the human body have been found to be the recurrent origin to various unrelated and transitional reactions that lead to cancer. Although these oxygen species are usually disposed by homeostasis by the human body, elevated environmental and food intake based stress reactions shift the balanced protective function. In recent years, an upsurge in counter acting these reactions has been targeted using synergistic ethnobotanical approaches. *Cyclea peltata* which has repeatedly been termed as a medicinal plant in the folk medicine is used in this study to analyse its antioxidant potential. Healthy Swiss albino mice were subjected to toxicity analysis on treatment with methanolic extract of the plant after inducing them with DAL cells. The antioxidant levels on the liver and kidney tissues revealed lower ranges in control group when compared to the treated groups. These results indicate administration of methanolic extract showed a reversed value towards the normal thus clearly validating its potential to be used in ethnobotanical based pharmaceutical research.

Keywords: *Cyclea peltata,* antioxidant, DAL, ethnobotany, methanol.

1. Introduction

Biochemical reactions in the cells and organelles are the driving force in maintaining life. But these reactions also result in the formation of free radicals or reactive oxygen species (ROS). ROS includes superoxide anion (O_2^-), hydroxyl radical (OH), singlet oxygen (O_2), reactive nitrogen species (RNS) such as NO^-, NO_2 and ONOO [1]. The uninhibited production of oxygen derived free radicals contributes to oxidative stress [2, 3]. These free radicals cause membrane lipid peroxidation resulting in membrane fluidity, loss of enzyme receptor activity and damage to membrane proteins leading to cell machinery inactivation.

Human beings have well developed antioxidant defense systems that generally maintain a homeostasis by disposal of these oxidative products. Under conditions of elevated oxidative stress like low antioxidant intake, the balance will shift in favor of pro oxidants, destroying the homeostasis. Oxidative stress is caused by both exogenous (eg: smoking) as well as endogenous processes during normal cell metabolism. Excess oxidative stress can lead to oxidative damage of DNA causing significant base damage, strand breaks, altered gene expressions and ultimately mutagenesis [4, 5]. Continuous oxidative damage to DNA is believed to be the main contributor to the development of breast, colon and prostate cancer [6, 7].

Antioxidants play an important role in alleviating the deleterious effects induced by free radicals by blocking the initiation or propagation of oxidizing chain reactions [8]. Both synthetic and natural antioxidants have the potency to prevent free radical formation. The natural antioxidants also have the added benefit of posing no side effects while the synthetic counterpart can be toxic in long term use. The global interest has currently been shifted towards the use of natural antioxidants mostly present in herbs for health benefits.

According to WHO around 80% of the world population is resorting towards traditional medicine. It has been reported that most of the cancers can be prevented through appropriate dietary modification [9]. An inverse correlation between consumption of fruits, vegetables and spices and the risk of cancer suggest that phytochemicals are potent anti-cancer agents. These phytochemicals are found to exert their anti-cancer action through induction of cellular defense systems including detoxifying and antioxidant enzymes as well as through inhibition of cell proliferation and inflammatory pathways leading to cell cycle arrest and /or apoptosis. Polyphenols and flavanoids are the major plant antioxidants shown to possess anticancer property. Antioxidants are also used as adjuvants with anti-cancer drugs to achieve synergistic therapy [10].

Cyclea peltata (Lam) Hook. F and Thoms is a slender twining shrub belonging to the family Menispermaceae and commonly called Patakizhangu or Malaithangi. Due to the high

medicinal value of this plant, National Medicinal Plant Board of India identified this plant as "medicinal plant species in high trade sourced from tropical forests." In spite of so many claims this remains a poorly investigated plant. Therefore the present study was undertaken to analyze the antioxidant property of Cyclea peltata.

2. Materials and Methods

2.1 Plant material and extraction: The plant Cyclea peltata used for the present study was collected from Alappuzha district of Kerala, India. The plant was authenticated by Department of Botany, Sanatana Dharma College, Alappuzha. A voucher specimen (10002) is prepared as herbarium and submitted to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The whole plant Cyclea peltata was washed in running tap water to remove soil and adhered debris. The plant was then washed using sterile distilled water and shade dried at room temperature. The dried plant material were ground into a fine powder in an electric grinder and subsequently sieved for obtaining fine powder. The sieved powder was subjected to methanol extraction.

2.2 Cell lines: The DAL cell line was obtained from Amala Cancer Research Institute, Thrissur, India and was propagated into transplantable tumors in the peritoneal cavity of mice. The freshly aspirated cells from the mouse peritoneum were washed with Phosphate buffer saline (PBS) under sterile conditions and their concentration was determined using a hemocytometer before transplantation. Animals were inoculated with 1x106 cells / mouse.

2.3 Experimental animals: Healthy Swiss albino mice (20±5 gm) were used for the study. The animals were obtained from Amala Cancer Institute, Thrissur, Kerala and brought to the laboratory. Animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided ad libitum. The animals were acclimatized to laboratory condition for about one week before commencement of the experiment. The experiments were performed after the approval from the Institutional Animal Ethical Committee and in accordance with the recommendation for the proper care and use of the laboratory animals.

2.4 Acute toxicity study: Acute oral toxicity of MECP was performed in swiss albino mice as per OECD 423 guidelines. The animals were divided into 3 groups. Group 1 and 2 received 3000mg/kg body weight of MECP. Group 3 served as control. After oral administration of these extracts, the animals were continuously observed for behavioral changes for the first 2 and 4 h and then observed for mortality if any, after 24 hours.

2.5 Treatment procedure: Animals were divided in to five groups each comprising six animals. One group served as the control while the remaining four groups were injected with Dalton's ascites lymphoma (1 x 106 cells/ mouse) to induce tumor. The treatments were given intraperitoneally at 24 h after the tumor inoculation and continued for 14 consecutive days.

The designation of the animal groups and treatment details were as follows:

Group I → Normal control
Group II → DAL control
Group III → DAL + 5-FU (10 mg/kg)
Group IV → DAL + MECP 100 mg /kg
Group V → DAL+MECP 200 mg /kg

2.6 Antioxidant studies: The antioxidant assay was performed with liver and kidney tissues. All the tissue preparations were frozen on dry ice and then transferred to a -80 °C freezer. The isolated organs were divided in to two parts for the preparation of homogenates. One part was used for the preparation of 10% w/v homogenate in potassium chloride (0.15 M). It was centrifuged at 8000 rpm for 10 min and the supernatant thus obtained was used for the estimation of catalase (CAT) and malondialdehyde (MDA). The second part was used for the preparation of 10% w/v homogenate in 0.25% w/v sucrose in phosphate buffer (5M, pH 7.4) and was centrifuged at 8000 rpm for 10 min. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD) and glutathione peroxidase (GSH). All the other estimations were done according to the manufacturer manual of standard enzymatic kits procured from Piramal Healthcare limited, Lab Diagnostic division, Mumbai, India by using semi auto analyzer (Photometer 5010 v5+).

2.7 Statistical analysis: All the grouped data were statistically evaluated with one way analysis of variance (ANOVA) followed by Dunnett's test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD.

3. Results

3.1 Antioxidant parameters in liver: SOD activity was decreased significantly in DAL control mice (4.35 ± 0.24) when compared to normal (8.58 ± 0.15). Positive control showed a value of 8.48 ± 0.15. MECP 100 mg increased SOD to (8.42 ± 0.06) whereas MECP 200 mg showed more significant result with a value of 9.81 ± 0.18. Catalase in normal mice was 9.69 ± 0.48 whereas it decreased to 3.28 ± 0.31 in DAL induced mice. Treatment with 100 and 200 mg of MECP helped to increase catalase to 5.75 ± 0.28 and 5.58 ± 0.47 respectively. In positive control catalase was increased to 6.70 ± 0.24. Both MECP treatment groups (100 & 200 mg) showed a significant increase in GPx (2.69 ± 0.29 & 3.57 ± 0.25) when compared with DAL control group (0.57 ± 0.52). In DAL control group, GSH showed significant reduction of 0.48 ± 0.27 as compared to normal 2.76 ± 0.15. MECP treated groups (100 and 200 mg) showed a significant increase in GSH activity (1.24 ± 0.22 and 1.66 ± 0.02). Positive control increased GSH to 2.16 ± 0.02. In DAL control group there was a slight increase in LPx (0.48 ± 0.27) when compared to normal (0.29 ± 0.08). MECP (100 and 200 mg) helped to restore LPx to near normal (0.28 ± 0.01 and 0.26 ± 0.01). Positive control was 0.33 ± 0.02. A decrease of vitamin C was observed (4.67 ± 0.22) whereas the normal was 6.31 ± 0.15. But the administration of MECP in 100 mg increased the vitamin C level to 7.20 ± 0.14. MECP 200 mg showed no significance. A tremendous and significant decrease in the level of vitamin E (10.44 ± 0.22) was observed when compared to normal (38.63 ± 0.10). Administration of MECP 100 mg showed a reversed value towards the normal (37.24 ± 3.10). MECP 200 mg showed a value of 23.31 ± 0.27 with no significance in positive control (Figure 1).

3.2 Antioxidant activity of Kidney: The SOD activity was found to be significantly increased in all treatment groups in comparison with DAL control mice. Of the two doses of MECP treatment MECP 200 mg showed more increase (20.79 ± 0.18; p< 0.01) than MECP 100 mg (16.25 ± 0.79) when compared to the DAL control (11.76 ± 0.39). With regard to Catalase level, treatment with MECP 100 & 200 mg significantly increased the level to 4.36 ± 0.37 and 5.69 ±0.78 respectively when compared with DAL control (3.21 ± 0.59). The reduced level of GPx in DAL bearing mice (0.45 ± 0.10), was found to be significantly increased in MECP 100 mg and 200 mg treated animals (4.12 ± 0.67 and 1.82 ± 0.43). When compared with DAL control group, both the MECP treated groups (100 and 200 mg) showed an increase in GSH activity (1.45 ± 0.46 and 3.73 ± 0.51) and the higher dose was found to be significantly different from DAL control group. The level of lipid peroxidation in kidney tissue was increased in DAL control mice (0.59 ± 0.10) when compared with normal control group (0.49 ± 0.05). The MECP 200 mg treated animals (0.31 ± 0.06) was found to be statistically significant when compared with DAL control mice. The level of Vitamin C was significantly (p < 0.01) decreased in DAL control mice (2.73 ± 0.19) when compared with normal control group (6.24 ± 0.49). Among the MECP treated groups (100 & 200 mg), lower dose showed significant increase (8.00 ± 0.51) than higher dose (6.74 ± 0.23) when compared to the DAL control. The present study showed a significant (p < 0.01) increase in all treatment groups when compared with DAL control mice. A significant reduction in Vitamin E was observed in DAL control mice (8.42 ± 0.53) when compared with normal control (33.97 ± 2.13) (Figure 2).

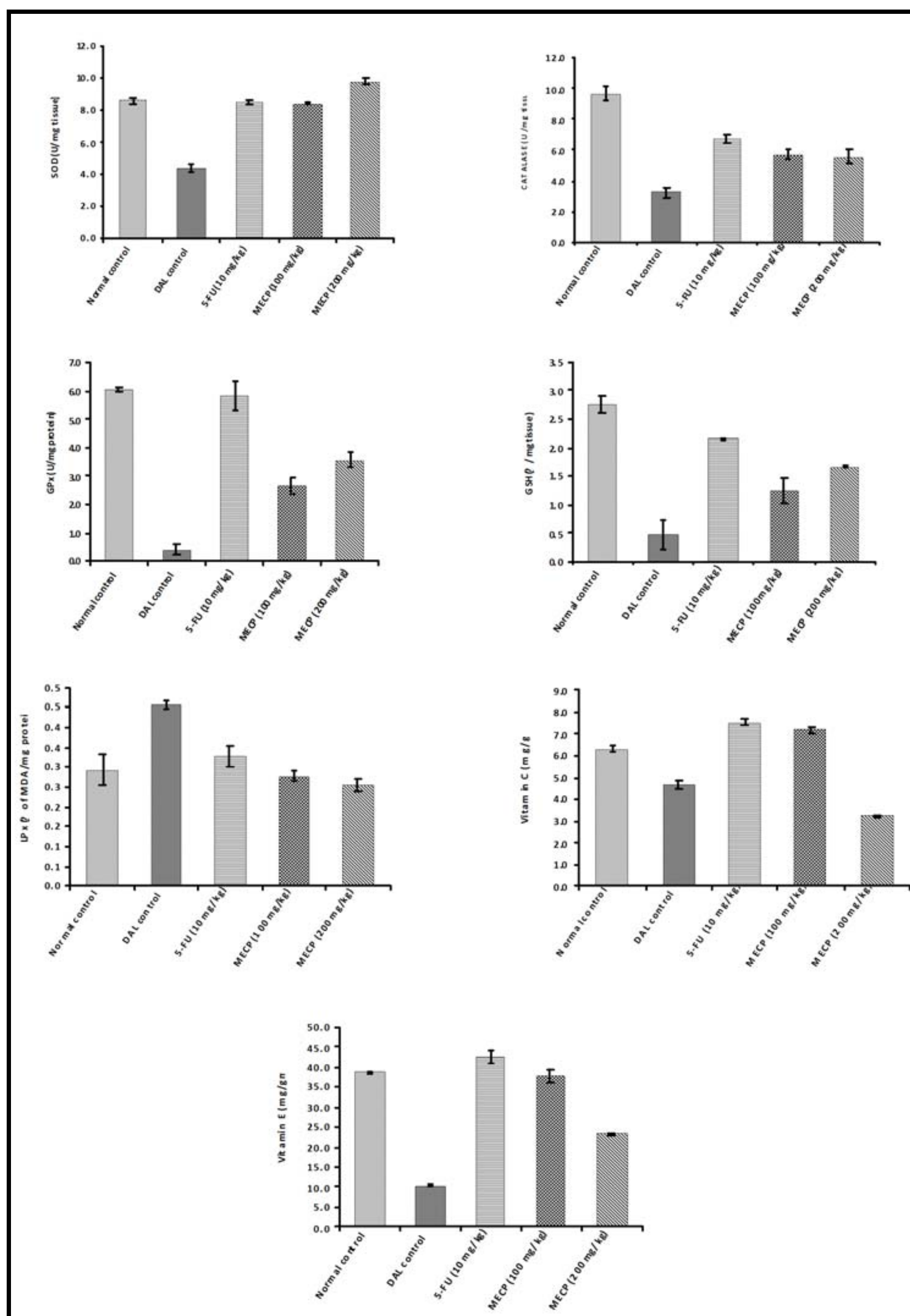

Fig 1: Cumulative antioxidant tests performed on liver tissues using C. peltata extract

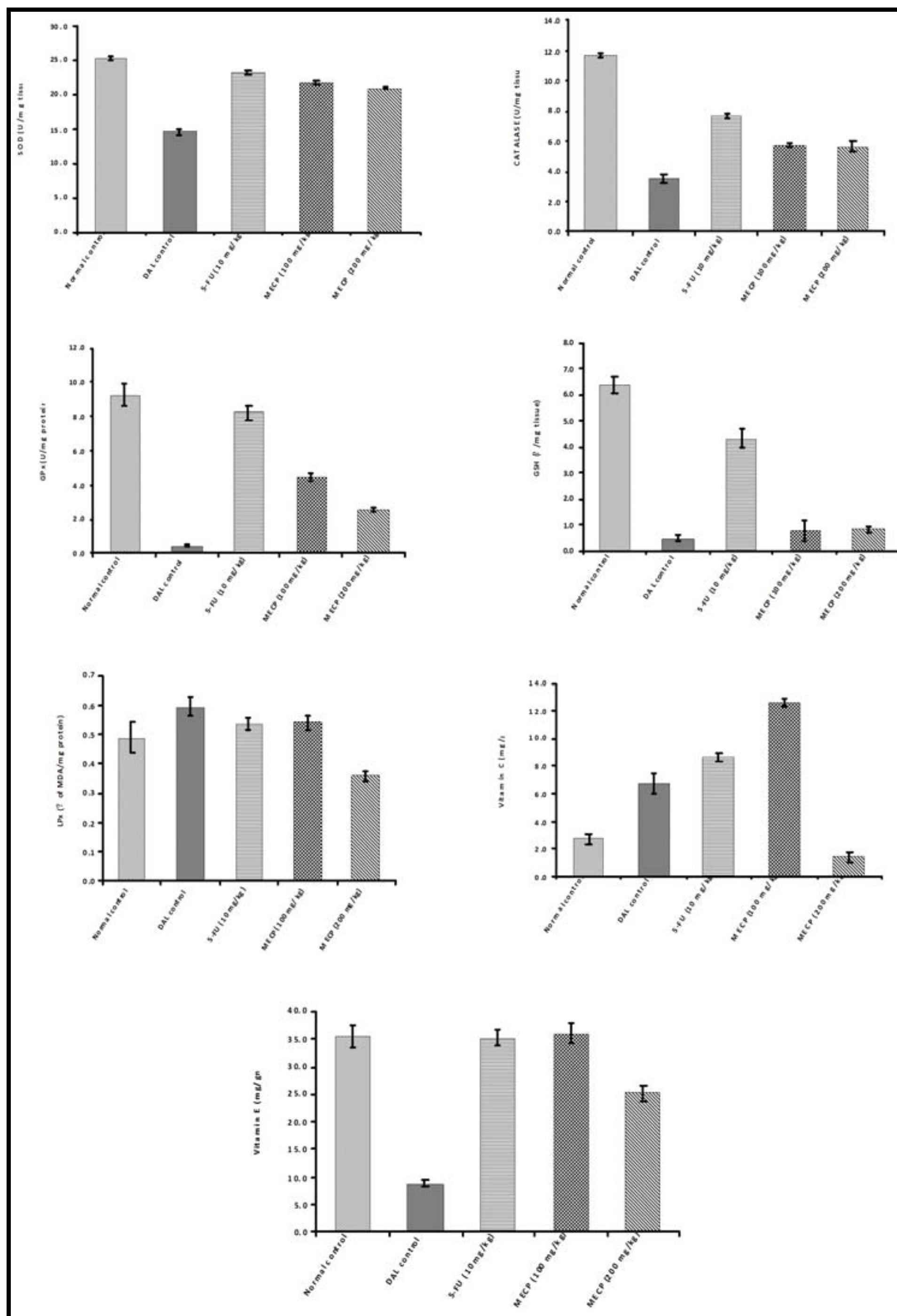

Fig 2: Statistical results of the antioxidant tests performed using kidney tissues

4. Discussion

Excessive generation of reactive oxygen species has been considered as an indicator in several cancers including DAL model [11]. Antioxidants are capable of scavenging free radicals at different stages of their action. Preventive antioxidants like SOD stop the release of ROS by catalyzing the dismutation of superoxide to H2O2 and CAT that breaks it down to water. Interception of free radicals is by radical scavenging, while at the secondary level scavenging of peroxyl radicals are affected. The effectors include antioxidants like glutathione, vitamin C and E, carotenoids, flavanoids etc. at the repair and reconstitution level many repair enzymes are involved [12].

All aerobic cells contain SOD and catalase, which as a free radical scavenging system by providing defense against the potentially damaging reactivates of superoxide and hydrogen peroxide. Loss of mitochondria and loss of Mn SOD activity in DAL cells might lead to decrease in SOD acivity in DAL bearing mice. Several investigators have reported reduced activities of SOD, CAT and GPx in tumor bearing mice due to down regulation of SOD and CAT genes which may be caused by hormones or the free radicals itself [13]. The present study also reports marked decrease in SOD and CAT in DAL bearing mice. Treatment with increasing concentrations of MECP helped to increase SOD and CAT.

Lipid peroxidation mediated by free radicals is considered as the main cause of cell membrane destruction and DNA damage. The oxidation of unsaturated fatty acids in biological membrane leads to a reduction in membrane fluidity and disruption of membrane structure and function [14]. Lipid peroxide formed in the primary site is transferred through circulatory system and provokes damage by lipid peroxidation. Malondialdehyde, the end product of lipid peroxidation has

been found to be higher in carcinomatous tissues than in non-diseased organs. This correlates with the present study which shows increased lipid peroxidase in DAL bearing mice. Glutathione a potent inhibitor of neoplasm is seen in higher concentrations in liver and is known to have key functions in antioxidant system. The lowering of lipid peroxidation, GST, GPx and the increase in SOD and CAT in MECP treated mice indicates its potential as inhibitor of DAL induced antioxidative stress.
Reduced glutathione is present in high concentration in cells for protection from free radical attack. In the present study the decreased level of GSH in DAL injected mice may have been due to excess utilization of this antioxidant by resident cells against ROS. When there is reduction in levels of GSH, cellular levels of ascorbic acid and tocopherol are also lowered [15]. Similar results were reported in *C. indica* against DAL model. In the present study same pattern was observed in liver tissues whereas in kidney the level of ascorbic acid was found to be increased [16].

5. Conclusion
C. peltata has been largely used as a respite against snake bites in tribal folk medicine. The present study suggests that the basis of folklore medicine used in treatment of life threatening snakebites is at large due to the antioxidant properties. Similarly various other pharmacological studies are to be undertaken to utilize this plant for its ethanobotanical and pharmaceutical benefit.

6. Conflict of Interest: None declared

7. Reference

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