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EVALUATION OF ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF *MOLLUGO CERVIANA(L.) SER.* USING PORCINE LIVER SLICES AGAINST HYDROGEN PEROXIDE INDUCED OXIDATIVE STRESS

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ABSTRACT

Plants produce large number of antioxidants and these natural products play a main role in radical scavenging capacity and prevents biological system from oxidative stress by terminating the chain function. Therefore, the present study was to evaluate the antioxidant potential of ethanolic extract of Mollugo cerviana(L.)Ser.(EEMC) using porcine liver slices. The porcine liver slices were induced for oxidative stress using Hydrogen peroxide and treated with EEMC was evaluated for its antioxidant activity using standard procedures. Results obtained in the present study shows the significant difference in the levels of antioxidants among all the groups were used. It is suggested that the EEMC serve as a free radical scavenger and acting as antioxidants in preventing many ailments.

KEY WORDS

Mollugo cerviana(L.)Ser., Porcine liver slices, Free radicals, Oxidative stress, Antioxidants

INTRODUCTION

Excessive generation of free radicals and reactive oxygen species (ROS) are due to the exposure to external oxidant substances or the biological system loss its defense mechanisms against these oxidants causes oxidative stress. Further, it leads to various deteriorating diseases such as aging, cancer, cardiovascular disease, cataracts and also decline in the immune system and dysfunction of brain [1,2].

Lipids present in the membrane are more prone to the attack of free radicals and subsequently breakdown the chain reaction and causes lipid peroxidation. The metabolites produced during this process are toxic to the cells and tissues [3,4]. Mostly oxidative stress caused due to an imbalance in the free radical production and antioxidant scavenger mechanisms with the damage to molecular species like proteins, nucleic acids and lipids [5].

The primary substances that possess an ability to act against free radicals induced oxidative stress are called antioxidants. Sometimes, endogenous antioxidants might not have the ability to protect cells from oxidative stress and thereby, necessitate the supplement from exogenous antioxidants. Due to this requirement, many synthetic antioxidants developed to mitigate oxidative damage but it's one of the disadvantages is these substances are of high cost, less availability and also has health risk. To overcome this complication, synthetic antioxidants were replaced by natural antioxidants and plays an effective role in protection from ROS and curing diseases [6]. These natural antioxidants derived from plant sources contains bioactive compounds like carotenoids, flavonoids, tocopherols, cinnamic acid, folic acid and ascorbic acid etc,. [7]. These compounds from herbal structure shown to possess the capability to inhibit the generation of ROS and scavenge against free radicals [8].

The chosen medicinal plant *Mollugo cerviana(L.)Ser.* belongs to the family Molluginaceae, commonly known as threadstem carpet weed. *Mollugo cerviana(L.)Ser.* is a species of flowering plant known by the common name threadstem carpetweed. It can be found on most

continents growing as a weed in many types of dry sandy habitat types. *Mollugo cerviana(L.)Ser.* known to cure diseases like blood impurity, burns, Gonorrhea, Hangover, jaundice, Pleurisy and effective in antifungal, antimicrobial, Diaphoretic, febrifuge, stomachic and laxative.

However, as far as we know there are no reports on the analysis of antioxidant activity of EEMC. using porcine liver slices. The present study was investigated to evaluate an antioxidant activity of EEMC using porcine liver slice technique t for the first time. The pig is considered the best donor of hepatocytes for bioartificial liver devices, but little is known about the metabolic capability of pig hepatocytes. Therefore, using slaughter house specimen minimizes unnecessary sacrifice of animals and this regard no ethical issues on using this liver tissue.

MATERIALS AND METHODS

Collection of the plant material

The whole plant of *Mollugo cerviana(L.)Ser.* was collected from Theni District, Tamil Nadu,India. The collected plant material was identified abd authenticated at Botanical Survey of India, Tamilnadu Agricultural university, Coimbatore, Tamil Nadu, India. A voucher specimen of the plant was deposited at the herbarium of the BSI (Register Number: BSI/SRC/5/23/2016/Tech/1203).

Preparation of extract

Whole plant material were collected freshly and washed in distilled water and allowed for shadedry and the dried sample were powdered. The powdered material (10 g) was extracted with 100 mL of ethanol using soxhlet apparatus and filtered. The filtrate was concentrated and dried under reduced pressure and controlled temperature.

Preparation of porcine liver slices

The porcine liver was selected as the mammalian tissue to evaluate the antioxidant effect of EEMC in the presence and absence of the standard oxidizing agent(H_2O_2). The dose of H_2O_2 used was the same as the level used in vivo studies by intraperitonial administration (2 mL/kg tissue). The liver was collected freshly from the local slaughter house immediately after the sacrifice of the animal. The tissue was quickly plunged into cold sterile Hanks balanced salt solution (HBSS) buffer and maintained at 4°C. The tissues were cut into very thin (≈1mm) slices using sterile scalpel and 250mg of tissue was taken in 1.0ml of sterile HBSS in broad, flat bottomed flasks.

Induction of oxidative stress and treatment with the plant extract

The following groups were set up for antioxidant assay. Group 1 served as normal control, group 2 considered as H_2O_2 induced free radicals, group 3 were treated with ethanolic extract of *Mollugo cerviana(L.)Ser.* at 20 mg per mL of HBSS, group 4 represented as positive control (Quercetin at 70 mg/kg tissue) and group 5were treated with EEMC alone. The oxidant required for induction of oxidative damage is H_2O_2 and EEMC used for the treatment were added to the tissues and incubated at 37 °C for one hour with mild shaking. Appropriate control groups were also set up. The standard oxidant H_2O_2 was used at a concentration of 2 mL/kg tissue. After the incubation period, the tissues were homogenized in the same aliquot of the HBSS buffer using a Teflon homogenizer and centrifuged to remove the debris. The supernatant was then used for the estimation of various parameters to assess the antioxidant potential.

Determination of antioxidant status

The supernatant obtained from homogenized liver tissues were used for the analysis of enzymic antioxidants such as super oxide dismutase (SOD)[9], catalase (CAT)[10], glutathione peroxidase (GPX)[11], glutathione -S-transferase (GST)[12] , glucose-6-phosphate dehydrogenase (G6PD)[13], glutathione reductase [14], and non-enzymic antioxidants such as glutathione (GSH)[15], vitamin C (Vit-C)[16], vitamin E(Vit-E)[17] and also lipid peroxidation (LPO)[18], protein[19] using standard procedures.

Statistical analysis

The values of antioxidant status were expressed as mean±SD. Statistical difference

in mean was analyzed using one-way ANOVA and followed by least square mean deviation comparison tests (LSD). $P<0.05$ was considered as statistically significant.

RESULTS

The results of the present study reveal that, there was a decline in the levels of enzymic and non-enzymic antioxidants in H_2O_2 induced oxidative stress group when compared with positive control group. Elevated levels of enzymic and non-enzymic antioxidants were

observed in the EEMC treated group when compared with toxic group.

In the H_2O_2 instigated group the levels of LPO were elevated and the levels were brought to nearby control group in *Mollugo cerviana(L.)Ser.* treated liver slices. The levels of protein were also decreased in the H_2O_2 toxicated group and its levels were rised up in *Mollugo cerviana(L.)Ser.* treated group (Table-1).

Table-1- Determination of antioxidant effect of ethanolic extract of *Mollugo cerviana(L.)Ser.* using porcine liver slices

Parameters	Group 1	Group 2	Group3	Group 4	Group5
SOD	10.05±0.15	7.85±0.08[a]	9.24±0.46[b]	9.55±0.24[b]	10.04±0.21
CAT	31.13±2.15	10.16±2.60[a]	28.25±0.89[b]	28.34±1.60[b]	29.90±1.90
GPX	16.65±1.21	18.01±0.48[a]	15.31±0.50[b]	16.26±0.10[b]	16.21±0.06
GST	6.49±0.04	3.37±0.30[a]	6.05±0.08[b]	6.33±0.05[b]	6.41±0.09
GR	6.55±0.15	3.74±0.33[a]	5.29±0.40[b]	6.04±0.29[b]	6.08±0.0
G6PD	2.28±0.09	0.92±0.07[a]	1.78±0.03[b]	1.85±0.01[b]	1.92±0.06
GSH	40.74±0.44	26.34±0.56[a]	38.48±0.02[b]	39.26±0.10[b]	39.54±0.08
Vit-C	16.82±0.31	11.87±0.69[a]	15.35±0.48[b]	16.11±0.28[b]	16.31±0.06
Vit-E	23.42±0.56	16.52±0.31[a]	21.16±0.09[b]	22.06±0.15[b]	22.68±0.51
LPO	21.16±0.71	35.30±0.74[a]	23.18±0.62[b]	21.25±0.21[b]	21.01±0.34
Protein	0.61±0.02	0.07±0.02[a]	0.12±0.01[b]	0.16±0.01[b]	0.17±0.08

The values are represented in triplicates of mean ± SD.
a. Statistically significant (P<0.05) compared with normal control
b. Statistically significant (P<0.05) compared with H_2O_2 induced group

H_2O_2 intoxicated liver slices were also treated with the standard Quercetin and compared with the EEMC treated group to find out the efficacy of plant extract.

DISCUSSION

Pig liver mimics most of the metabolic activities similar to human liver. Using this pig liver slice model, the efficacy of antioxidant potential of *Mollugo cerviana(L.)Ser.* were evaluated and its levels were compared between different groups. Free radical scavengers derived from plant sources are tremendously important chemical compounds which possess an ability to prevent the biomolecules from the oxidation.

SOD uses free radicals as a substrate and inactivates superoxide radicals into non-toxic form. SOD shows its effective scavenging role when there is increased levels of catalase and peroxidases were observed [20]. Catalase enzyme present in all living organisms and its main function is breakdown of hydrogen peroxide to water and oxygen [21]. Through this way cells quickly decomposes this harmful hydrogen peroxide to non-toxic forms [22]. In comparision with other organs catalase occurs at higher concentration in the liver [23]. Glutathione peroxidase and glutathione-S-transferase are very effective scavenger of hydrogen peroxide and they exhibit its maximum activity with lipid peroxides and are involved in detoxification metabolism [24,25]. An important role of glucose-6-phosphate dehydrogenase is to maintain GSH in reduced state.

Chinthamony Arul raj *et al* [26] reported that the leaf extract of *Alpinia purpurata* shown the presence of enzymic antioxidants such as peroxidases 173.12±9.40 µmol/g tissue, SOD 58.03±2.11U/mg protein and CAT 46.70±2.35µmol of H_2O_2 consumed/min/mg protein in higher amount.

The plant extract chosen for our investigation have been detoxified the damages caused by H_2O_2 radical in liver tissue and through its potential scavenging activity brought up the levels of enzymic antioxidants to normal limits after the treatment.

Non-enzymatically GSH acts in the first line of defense for the body to protect against ROS [27]. During Oxidative stress GSH oxidized to glutathione disulfide and causes decline in the level of GSH by inhibiting glutamate cystine antiporter [28]. There is depletion in the levels of GSH in toxic group and its levels were increased to normal limit after treatment with the ethanolic extract of *Mollugo cerviana(L.)Ser.* Ascorbic acid is a strong reducing agent and thereby shown its potential activity by neutralizing ROS such as hydrogen peroxide and other radicals [29]. Adaikpoh *et al* [30] observed the antioxidant properties in the aqueous extract of *Scoparia dulcis* and the report showed significant

antioxidant activity were analyzed and it is sufficient to scavenge against cadmium induced oxidative stress in experimental rats.

Vitamin – E protects cell membranes from oxidative damage by reacting with lipid radicals during lipid peroxidation reaction [31,32]. It plays a vital role in the formation and function of RBC and muscles [33]. In this study the levels of Vit-C and Vit- E were drastically increased in EEMC treated group in comparison with toxic group.

Basically, erythrocytes are more prone to peroxidation due to the deposition of PUFA and Haemoglobin. When erythrocytes are frequently exposed to more tension of oxygen it causes oxidative stress during respiration [34]. Malondialdehyde is an important indicator of LPO, owing to this reason the levels of LPO were noticed to be elevated in toxic group and its levels were decreased and brought up to normal limit after treatment with EEMC. It represents that the free radical scavengers present in the plant extract reacts with the malondialdehyde and decreases its levels by its defensive action. EEMC had a greater potential of the inhibitory effect on lipid peroxidation and improved antioxidant enzymes in pig liver homogenate.

CONCLUSION:

From the results discussed above, it is concluded that the ethanolic extract of

Mollugo cerviana(L.)Ser. whole plant constitute that there was preferment in the levels of both enzymic and non-enzymic antioxidant antioxidants in comparison with H_2O_2 induced toxic group and this plant also possess an anti-lipid peroxidative effect by its detoxifying nature. The antioxidant activity of this plant might be due to the synergistic action of bioactive compounds present in them. Further this plant extract may be effective as a protective source in many diseases.

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