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ANTIOXIDANT ACTIVITY OF GOAT LIVER SLICES TREATED WITH *CUCUMIS MELO* (L). FRUIT EXTRACT

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ABSTRACT: Plant derived natural products such as flavonoids, terpenes, alkaloids and polysaccharides have received considerable attention in recent years due to diverse pharmacological properties including cytotoxic and cancer chemo preventive effects. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases. Oxidative stress results from an excessive production of reactive oxygen species beyond the body's antioxidant capacity. The over production of reactive oxygen species can lead to damage to cellular biomolecules, which is implicated in the development of many diseases including cell death. The reactive oxygen species can be eliminated /deactivated by a number of antioxidants which include enzymic and non-enzymic antioxidants. The plant selected for the present study is *Cucumis melo* (Family: Cucurbitaceae) commonly known as Musk melon. *Cucumis melo* helps in increasing appetite, weight loss, urinary tract infections, constipation, acidity and ulcers. The goat liver was selected as the mammalian tissue to determine the antioxidant effect of ethanol extract in the presence and absence of the standard oxidizing compound (H_2O_2). This work suggested the antioxidant effects of *Cucumis melo* fruit extract on oxidatively stressed precision-cut liver slices is potentially exhibited.

INTRODUCTION: Antioxidants are compounds which act as inhibitors of the oxidative process. They are quite large and diverse and their main function is to oppose the process of oxidation largely by neutralizing free radicals [1]. Antioxidant compounds scavenge free radicals such as peroxide, hyproperoxide or lipid peroxyl and thus reduce the level of oxidative stress and prevent the development of complications associated with oxidative stress related disease [2].

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Lipid peroxidation (LPO) is a free radical mediated process leading to oxidative deterioration of poly unsaturated lipids. LPO is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. It has been found to be connected with various disease processes, such as carcinogenesis, atherosclerosis and hypertension. To control LPO, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging reactive oxygen species [3].

Oxidative stress is a harmful condition that occurs when there is an excess of ROS and a decrease in antioxidant levels. It can cause damage to all molecular targets: DNA, lipids and protein. Oxidative stress initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl,

nitric oxide and peroxynitrite play a vital role in damaging various cellular macromolecules. This damage may result in many diseases, including diabetes mellitus, atherosclerosis, myocardial infarction, arthritis and neurodegenerative diseases[4].

The applicability of these liver slices to study the H_2O_2 induced oxidative stress and to test the antioxidant effect of *C. melo fruit extract* has not previously been evaluated. The present investigation was undertaken to *in vitro* assess the effect of ethanol extract of *C. melo* in inhibiting lipid peroxidation and to analyze the enzymic and non-enzymic antioxidant property.

MATERIALS AND METHODS:

Preparation of the extracts

Cucumis melo (L) fruits were collected from local farmers at Coimbatore district, Tamilnadu. The specimen sample was authenticated by Dr. V.S. Ramachandran, Associate Professor, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India. The voucher specimen was deposited in the herbarium center, Department of Botany, Bharathiar University, Coimbatore. The pulp of fresh fruits of *C. melo* was chopped into pieces and dried at room temperature for 24 hours. The air dried pulps were kept at 400C in hot air oven for 24 hours to remove moisture content. The completely dried fruits were taken and powdered for extraction. Soxhlet extraction was used to isolate the active drug principle.

Preparation of mammalian liver slices

The goat liver was selected as the mammalian tissue to determine the antioxidant effect of ethanol extract in the presence and absence of the standard oxidizing compound (H_2O_2). The dose of H_2O_2 used was the same as the level used *invivo* studies by intraperitonial administration (2ml/kg) tissue). The fresh liver was collected from 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. very thin (≈ 1mm) slices of the tissues were cut by using the sterile scalpel and tissue (250 mg) was taken in 1.0 ml of sterile HBBS, in broad, flat bottomed flasks. The necessary compounds (H_2O_2 and ethanol extract) were added 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.

Experimental Design

The experiment was planned to further probe the antioxidant property of *C. melo* fruit extract in goat liver slices. The extracts of the fruits were administered to the oxidatively stressed goat liver slices and the antioxidant status was analyzed in them.

The following groups were set up for antioxidant assay. Group I served as normal control, Group II corresponded to H_2O_2 induced free radicals, Group III were treated with ethanol extract of *C. melo* at 20 mg (20 µL), Group IV represented as positive control (rutin at 70 mg/kg tissue) and Group V were treated with *C. melo* extract. After the incubation time, the estimations of various parameters indicative of antioxidant potential were carried out in the homogenate.

Analysis of antioxidant activity

The homogenized liver tissues were used for the analysis of antioxidant enzymes such as superoxide dismutase (SOD) [5], catalase (CAT) [6], glutathione peroxidase (GPx) [7], glutathione transferase (GST) [8], glutathione reductase (GR) [9], glucose -6 – phosphate dehydrogenase (G6PD) [10], glutathione (GSH) [11], Vitamin C (vit – C) [12], vitamin E (vit – E) [13], lipid perooxidation (LPO) [14], protein[15].

STATISTICAL ANALYSIS

Values were expressed as mean ± SD. Statistical difference in mean was analyzed using one way ANOVA and followed by least significant difference (LSD). $P < 0.05$ was considered statistically significant.

RESULTS:

In the present study, enzymic and non-enzymic antioxidants status were compared between Group I, Group II, Group III ,Group IV and Group V; Goat liver slices were used for the assessment of

enzymic and non-enzymic antioxidants. The obtained results are summarized and represented in **Table 1**.

TABLE1: EFFECT OF *CUCUMIS MELO* FRUIT EXTRACT ON THE ANTIOXIDANT STATUS IN GOAT LIVER SLICES EXPOSED *INVITRO* TO HYDROGEN PEROXIDE

Parameters	Group I	Group II	Group III	Group IV	Group V
SOD	6.38±0.02	3.67±0.32 [a]	5.07±0.32 [b]	7.43±0.50 [b]	6.18±0.21
CAT	31.36±0.47	21.01±0.41 [a]	26.73±0.29 [b]	28.25±0.16 [b]	30.10±0.75
GPX	7.30±0.03	4.56±0.48 [a]	6.07±0.42 [b]	6.73±0.39 [b]	7.12±0.26
GST	2.66±0.07	1.68±0.10 [a]	2.12±0.11 [b]	2.33±0.08 [b]	2.64±0.05
G6PD	1.01±0.01	0.57±0.02 [a]	0.82±0.02 [b]	0.92±0.02 [b]	0.98±0.01
GSH	38.93±0.69	26.41±0.69 [a]	37.07±1.04 [b]	37.54±0.69 [b]	38.11±1.83
VIT- C	3.12±0.04	2.42±0.06 [a]	2.95±0.03 [b]	3.03±0.04 [b]	3.05±0.06
VIT- E	17.22±0.39	12.84±0.54 [a]	14.12±0.49 [b]	16.47±0.10 [b]	16.96±0.28
LPO	28.99±0.59	87.29±1.35 [a]	42.03±2.87 [b]	34.61±1.14 [b]	27.25±0.53
PROTEIN	1.95±0.12	0.65±0.12 [a]	1.25±0.11 [b]	1.60±0.11 [b]	1.75±0.06

The values are Mean ± S.D. of triplicates.
a: statistically significant ($P<0.05$) compared with normal control;
b: statistically significant ($P<0.05$) compared with H_2O_2 induced group.

The activities of enzymic and non-enxymic antioxidants in the liver slices exposed to H_2O_2 showed a significant decrease when compared to untreated control. Hydrogen peroxide caused a considerable decrease in antioxidant activity. Treatment with *C. melo* extract resulted in a significant increase compared with toxic group. The antioxidant effect was also compared to that of rutin, the known standard antioxidant. *C. melo* alone treated group showed no significant changes in the levels of antioxidants when compared with Control (group I) at $P < 0.05$.

In this report, the increased level of LPO on H_2O_2-induced tissue homogenate was observed. Exposure of ethanol extract of *C. melo* to group III maintained the level of lipid peroxidation to near normal. However, the protein content in liver tissues showed a significant decline in group II. Pretreatment with *C. melo* and rutin, offered significant increase in the protein level as compared with toxic group.

Units
SOD- 50 % inhibition of nitrite formation / min / mg protein, CAT- µmoles of H_2O_2 decomposed / min / mg protein, GPx- µg of glutathione utilized / min / mg protein, GST- µmoles of CDNB conjucated / min / mg protein, G6PD- Units/ mg protein, GSH-µg/ g tissue, VIT-C- µg/ g tissue, VIT-E- µg/ g tissue, LPO- nMoles of MDA formed/min/mg protein.

DISCUSSION: Precision-cut liver slices are described as a valuable tool for *in vitro* metabolism studies of potential drug candidates. In the current study, by employing the precision-cut liver slices generated from goat liver as a tool, the protective effects of the *Cucumis melo fruit in vitro* against hydrogen peroxide-induced oxidative stress was evaluated. Enzymic and non-enzymic antioxidants were assessed in the liver slices subjected to oxidative stress in the presence and the absence of the fruit extracts.

Antioxidants are substances that protect other chemicals of the body from damaging oxidation reaction by reacting with free radicals and they sacrifice it by becoming oxidized. It possesses several defense systems which are constituted to enzymes and radical scavengers. There are called as 'first line antioxidant defense system'[16].

Superoxide Dismutase, as an antioxidant , help to protect against cell destruction by superoxide radical anion (O2-), which is generated by the transfer of one electron to molecular oxygen. It is responsible for both the direct damage of biological macromolecules and for generating other reactive oxygen species. SOD keeps the concentration of

superoxide radicals at low levels and therefore play an important role in the defense against oxidative stress [17].The level of SOD was found to be increased in treatment group when compared with toxic group.

Catalase is a heme containing enzyme, which is present in most cells and catalyses the decomposition of hydrogen peroxide to water and oxygen. Cytosolic catalase is found to be important in the inactivation of many environmental mutagens [18]. The level of Catalase was increased to near normal range as in positive control group.

Glutathione Peroxidase is a well-known first line of defense against oxidative stress, which in turn requires glutathione as cofactor. It catalyses the oxidation of GSH to GSSG at the expense of H_2O_2. It is found in both cytosol (70%) and mitochondria (30%) of various tissues [19]. The level of this enzyme was decreased in toxic group compared to control. From the table 1, it was observed that the levels of enzymatic antioxidants were increased in treatment group compared with standard antioxidant rutin.

Yousef *et al*., [20] have reported that grape seed proanthocyanidin extract significantly elevated the hepatic GST activity during cisplatin intoxication. Kundu *et al*., [21] demonstrated that the methanolaqueous fraction of *Cajanus cajan* leaf extract could prevent the chronically treated alcoholinduced rat liver damage by augmenting the antioxidant enzyme activities. The administration of *Cucumis melo fruit* extracts in the present study, improved the GST activities from the effect of the oxidant assault. This observation shows that the fruit extracts are effective in ensuring GSH homeostasis in the cell, as GR replenishes GSH (reduced) from GSSG (oxidized). GSH, apart from being a strong antioxidant by itself also acts as a substrate for antioxidant enzymes like GPx and GST.

The ethanol extract of the *Piper trioicum Roxb.* and *Physalis minima L.* has been shown to suppress lipid peroxidation in goat liver homogenate reported by Dinakaran *et al*.,[22]. The methanol extract of *Borreria hispida* have significant *in vivo* antioxidant and lipid peroxidation activity when compared with petroleum ether and ethyl acetate extracts were demonstrated by Shajiselvin *et al*., [23]. In the present study too, the extracts of *Cucumis melo* fruits inhibited lipid peroxidation.

Vitamin-C or L-ascorbate is an essential nutrient for a large number of higher primate species, a small number of other mammalian species, a few species of birds and some fish. The presence of ascorbate is required for a range of essential metabolic reactions in all animals and plants. In living organism ascorbate is an antioxidant, since it protects the body against oxidative stress and it is a cofactor in several vital enzymatic reactions[24]. The *Casearia esculenta* root extract improved the levels of Vitamin C, vitamin E and GSH of the liver and kidney of streptozotocin diabetic rats[25]. The recovery towards normalization of these enzyme caused by plant treatment was almost similar to that caused by rutin.These supportive studies are in accordance with our reports indicating the protective effect of *C. melo fruit* extract against H_2O_2 induced oxidative stress.

CONCLUSION: Plants are widely acclaimed as rich sources of antioxidants. Several studies have analyzed the antioxidant potential of a variety of herbs. The present study showed that ethanol extract of *Cucumis melo* fruits possesses inhibition of lipid peroxidation and potent antioxidant activity. It improves the antioxidant status in the goat liver slices exposed *in vitro* to oxidative stress. Since the model was carefully planned to simulate *in vivo* conditions, it is perceivable that the effects also occur in the intact system. Increased activity of antioxidant enzymes observed in this study may be due to the increased availability of antioxidants from *Cucumis melo.* This study strongly warrants closer attention to this traditional plant for the development of drugs to treat various complications initiated by free radicals. Further studies can be carried out to identify the active components in *C. melo* and to investigate the antioxidant defense mechanisms in *in vivo* condition.

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