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SHORT COMMUNICATION

PROBING THE ANTI-HYPERLIPIDEMIC EFFICACY OF THE ALLSPICE (*PIMENTA OFFICINALIS* LINDL.) IN RATS FED WITH HIGH FAT DIET

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Abstract : In this study, the anti-hyperlipidemic effect of aqueous extract of *Pimenta officinalis* (APO) was investigated in experimental rats fed with high fat diet (HFD). Hyperlipidemia in experimental rats was evidenced by a significant enhancement in the level of glycerol, triglycerides and phopholipids in serum, and also in liver and kidney tissues. HFD caused oxidative stress in these animals as shown by marked increment in the levels of thiobarbituric acid reactive substances (TBARS) and diene conjugates (CD), and a distinct diminution in reduced glutathione (GSH) content in liver and kidneys. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) showed reduced activity in hyperlipidemic rats. All these biochemical parameters showed reliable signs of retrieving towards near-normalcy in APO-administered HFD fed rats. This study unveiled the anti-hyperlipidemic as well as antioxidant activity of APO.

Key words : *Pimenta officinalis* high fat diet lipid peroxidation antioxidant enzymes anti-hyperlipidemic activity

INTRODUCTION

Traditionally, spices have been employed as flavouring agents and they are well known for their antioxidant, anti-microbial, anti-carcinogenic and anti-inflammatory properties. *Pimenta officinalis* Lindl. of family Myrtaceae, commonly known as allspice, is a bushy green tree native to West Indies and introduced to Indian gardens. The dried unripe berries and leaves of this plant are used as spice which possess the characteristic combined flavour and aroma of cloves, nutmeg, cinnamon and black pepper (1). Fruits of the plant contain an essential oil used as a flavoring agent, as a

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perfume and as a carminative and stimulant. They are also renowned for their anti-bacterial and anti-fungal activities (2).

Ample experimental and epidemiological evidences are there to show that oxidative stress occurs during hyperlipidemia as a result of enhanced generation of free radicals (3, 4). Scavenging of free radicals by plant principles has proved effective in combating many sorts of oxidative dysfunctions (5). In our laboratory, we have established the antioxidant potential of plants such as *Coscinium fenestratum*, *Curculigo orchioides*, *Syzygium aromaticum* etc. (6–8). We know the anti-hyperlipidemic and anti-oxidant effects of many spice species such as turmeric, pepper, cinnamon, cardamom, ginger, garlic, curry leaf etc. (9). The present study is aimed at probing the antioxidant and anti-hyperlipidemic effects of *P. officinalis*.

METHODS

Plant material

Leaves of *Pimenta officinalis* were collected from K.E. College campus, Mannanam, Kottayam. The plant was previously identified and authenticated by the experts in the Post Graduate and Research Department of Botany, St. Thomas College, Pala. The leaves were air dried for a week, chopped and then powdered using an electric grinder. The powder was then extracted in distilled water using a Soxhlet extractor. The extract was concentrated in a rotary evaporator under reduced pressure. The powdered aqueous extract *of Pimenta officinalis* (APO) was kept in airtight polythene containers for further use. The yield of APO was 2.1% w/w.

Experimental animals

18 male albino rats of Sprague-Dawley strain weighing 150–200 g were procured from Small Animals' Breeding Centre of Kerala Agricultural University, Mannuthy, Trichur. They were housed in polypropylene cages maintained in the animal house of School of Biosciences, Mahatma Gandhi University. The animals were maintained at controlled temperature (27 ± 2°C) and natural light - dark cycle. They were fed with Amrut Laboratory pellet diet supplied by Nav Maharashtra Chakan Oil Mills Ltd., Pune. Food and water were provided *ad libitum*. A week's time was allowed for the animals to get acclimatized to the laboratory conditions. Ethical clearance for the handling of animals was obtained from the committee constituted for the purpose.

The animals were divided into 3 groups of 6 rats each as follows. Group I animals served as control, which received lab diet and water *ad libitum*. Group II constituted the hyperlipidemic rats, which received daily, in addition to lab diet and water, high fat diet (HFD) made of a mixture of coconut oil (Kera, manufactured by Kerala Coconut Development Corporation) and vanaspathy (supplied by Hindustan Lever, Mumbai) in the ratio 2:3 w/w. HFD was administered at the dose of 10 g per kg body weight of each animal orally. Group III animals were the spice-administered HFD fed rats, which received daily 75 mg per kg body weight of APO orally in a suspension of water. (A dose dependent preliminary study of HFD rats

using APO with 25, 50, 75 and 100 mg/kg body weight evoked hypolipidemic effect in all the cases, and revealed the 75 mg/kg body weight to be the most effective dose. A pilot study involving 30, 60 and 90 days duration models of hyperlipidemic rats further showed that the 90 days model evoked the maximum hypo-lipidemic effect on administration of APO at the effective dose). Hence the animals were maintained in laboratory conditions for 90 days.

At the end of experimental period, the rats were deprived of food overnight and then sacrificed by cervical dislocation. Blood was collected by an incision made in the jugular veins. Serum was prepared from the collected blood. Tissues like liver and kidney were dissected out, blotted off blood, rinsed in phosphate buffered saline (pH 7.4) and then tissue homogenates were prepared.

Biochemical estimations

Estimation of levels of urea (10), cholesterol (11), triglycerides (12) and phospholipids (13) was done in serum/liver/kidney of different groups. Contents of conjugated dienes (CD) (14) and reduced glutathione (GSH) (15) were assessed in liver and kidneys. The level of thiobarbituric acid reactive substances (TBARS) (16) was measured in the presence of *in vivo* LPO-inducing agents in various groups of animals. Antioxidant enzymes such as superoxide dismutase (SOD) (17), catalase (CAT) (18) and glutathione peroxidase (GPX) (19) were assayed in kidneys and liver of experimental animals.

Statistical analysis :

The results were presented as the mean ± SEM. Student's paired t test was used to analyze statistical significance of the data.

RESULTS

The data pertaining to the values of urea in serum and also those of cholesterol, triglycerides and phospholipids in the serum/liver and kidneys are presented in Table I. All the above parameters registered a significant hike in hyperlipidemic rats (Group II) as compared to control ones

TABLE I : Effect of *Pimenta officinalis* on different biochemical parameters. [Values are mean±SEM of 6 animals in each group].

Parameters		*Group–I*	*Group–II*	*Group–III*
Cholesterol (mg/dL) or (mg/100 g tissue)	In serum	78.7±1.8	172.2±2.6*	91.8±2.1**
	In liver	317.4±2.9	601.6±3.1*	343.2±2.3**
	In kidney	341.2±3.4	416.6±3.6*	354.2±2.8**
Triglycerides (mg/dL) or (mg/100 g tissue)	In serum	8.3±0.2	18.7±0.5*	9.4±0.4**
	In liver	417.5±3.6	670.9±4.5*	501.6±3.9**
	In kidney	77.5±1.6	110.9±2.5*	80.6±1.9**
Phospholipids (mg/dL) or (mg/100 g tissue)	In serum	171.1±3.6	424.6±3.2*	218.2±3.5**
	In liver	2178±8.6	3419±9.2*	2486±9.5**
	In kidney	2014±9.6	3019±8.9*	2287±7.4**

*P<0.01 as compared to Group–I.
**P<0.01 as compared to Group–II.

TABLE II: Effect of *Pimenta officinalis* on antioxidant status of liver and kidneys. [Values are mean±SEM of 6 animals in each group].

Parameters		*Group–I*	*Group–II*	*Group–III*
TBARS (µ mol/100 g tissue)	In liver	0.7±0.03	1.2±0.04*	0.8±0.04***
	In kidney	1.2±0.04	1.6±0.04*	1.2±0.03***
CD (µ mol/100 g tissue)	In liver	50.3±1.3	68.7±1.9*	53.4±1.5***
	In kidney	47.5±1.6	70.9±1.5*	50.6±1.9***
GSH (µ mol/100 g tissue)	In liver	471.1±2.6	384.6±3.2*	458.2±2.5***
	In kidney	373.6±2.6	301.6±1.9	365.7±2.4***
SOD (units/mg protein)	In liver	6.2±0.19	2.9±0.19*	5.8±0.21***
	In kidney	5.9±0.17	2.2±0.18*	5.6±0.19***
CAT (µ mol of H_2O_2/min/mg protein)	In liver	206.8±2.97	127.3±2.57*	196.8±2.14***
	In kidney	66.1±1.5	35.7±1.7*	61.9±1.9***
GPX (units/mg protein)	In liver	157.4±1.9	99.7±1.8*	151.4±2.6**
	In kidney	118.3±2.0	52.7±1.9*	110.8±1.9***

*P<0.01 as compared to Group–I.
**P<0.01 as compared to Group–II.

(Group I). These values barring that of the triglycerides, showed a tendency to return towards normalcy in HFD + APO – administered rats (Group III). Triglycerides in the liver of Group III animals, however, did not come back to normal though it showed a tendency to decline when compared to that of HFD-rats.

Table II depicts the antioxidant status of liver and kidney in different experimental groups. In HFD-fed rats, there was a significant increase in the production of lipid peroxides as manifested by elevated levels of TBARS and CD, and a distinct diminution in GSH content. All these values were found attaining near normal values in HFD + APO-fed rats. Activities of antioxidant enzymes, such as SOD, CAT and GPX in liver and kidneys of different experimental groups recorded a decline in Group II rats, as compared to Group I. Activities of these enzymes were significantly enhanced in APO-administered Group III animals.

DISCUSSION

This study reveals that consumption of HFD causes a condition called hyperlipidemia, manifested by enhanced presence of cholesterol, phospholipids and triglycerides in the body tissues. The urea content in the tissues of affected animals too increases. Administration of APO had a beneficial effect in reversing the hyperlipidemic condition. In the present study, APO administered at the effective dose of 75 mg/kg body weight evoked significant hypolipidemic effect in HFD rats.

Hyperlipidemia evokes oxidative damages in various tissues, which in turn, deregulates the cellular functions leading to various pathological conditions (4). Elevated levels of TBARS and CD observed in HFD fed rats indicate excessive formation of free radicals and activation of lipid peroxidation system in liver and kidneys due to induced hyperlipidemia. TBARS are produced as byproducts of lipid peroxidation that occurs in hydrophobic core of bio-membranes. The tendency of these parameters to retrieve towards normalcy in different APO-administered rats reveals the potential of the spice in combating oxidative dysfunctions

GSH is a major non-protein thiol in living organisms, which plays a key role in coordinating the body's antioxidant defense processes, thus emphasizing its role in maintaining the structural and functional integrity of cells. Decline in GSH contents in the liver and kidneys of hyperlipidemic rats, and its subsequent return towards near normalcy in APO-coadminsitered group unearth the antioxidant effect of *Pimenta officinalis*. Two factors mainly attribute towards the antioxidant effect of a drug, viz., (1) prevention of GSH depletion (20) and (2) destruction of free radicals (21). *P. officinalis* elicits both these effects and hence can be deemed as an antioxidant.

SOD, CAT and GPX constitute a mutually supportive team of defense against ROS (22). The present study revealed a marked decline in the levels of these antioxidant enzymes in HFD-fed rats, especially due to the oxidative stress associated with hyperlipidemia. The activities of all these enzymes showed a significant enhancement in hyperlipidemic rats co-administered with APO, thus proving the antioxidant potential of the spice.

It can be concluded that the oxidative stress caused due to HFD is nullified by APO. The antioxidant effect manifested by the plant may possibly be due to its ability to activate antioxidant enzymes or to replenish GSH. The present study is in perfect conformity with the antihyperlipidemic activity of plant species such as *Syzygium aromaticum* (8) *Piper officinarum* (23) *Azadirachta indica* (24) and *Cassia tora* (25). Thus *Pimenta officinalis* seems to be a promising plant in respect of its hypolipidemic and antioxidant potential, and it necessitates further studies to isolate the antioxidant and antihyperlipidemic principle of this plant.

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