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Research article

Free radical scavenging activity of methanolic leaf extract of *Pimenta dioica* on streptozotocin-induced diabetic rats

K. Yogalakshmi*[1], J. Vaidehi[1]

[1]Department of Zoology, Annamalai University, Annamalai nager-608 002, Tamilnadu, India.

Abstract

The present study was designed to investigate the free radical scavenging activity of *Pimenta diocia* on streptozotocin (STZ)-induced diabetic rats. The methanolic leaf extract of *Pimenta diocia* at the doses of 75 and 150 mg/ kg of body weight was administered orally once in a day to the diabetic induced group for 45 days. Glibenclamide (0.6 mg/kg of body weight) was used as reference drug. The antioxidant properties were assessed by estimating the liver and kidney catalase (CAT), thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and reduced glutathione (GSH). Antioxidant levels were significantly restored towards normal levels in *P.dioica* treated rats when compared with the STZ control. The results of the study indicate that the *Pimenta dioica* leaf methanolic extract exhibit promising antioxidant activity towards diabetic rats.

Key words: Free radical scavenging activity, *Pimenta dioica*, glibenclamide, catalase.

***Corresponding Author: K. Yogalakshmi,** Department of Zoology, Annamalai University, Annamalai nager-608 002, Tamilnadu, India.

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in the production of insulin by the pancreas or by the ineffectiveness of the produced insulin. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body systems and in particular the blood and nervous systems. It is one of the alarming worldwide health problems at present leading to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications [1]. It is expected that about 366 million people are likely to be diabetic by the year 2030 [2]. Hyperglycemia is known to produce reactive oxygen species (ROS) which plays a central role in the complications of diabetes [3]. Diabetes is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical, hydrogen peroxide and hydroxyl radicals or reduction of antioxidant defense

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system. Implications of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free radical generation but also due to non enzymatic protein glycosylation, auto oxidation of glucose, impaired antioxidant enzymes and formation of peroxides. Lipid peroxidation is a key marker of oxidative stress that results in extensive membrane damage and dysfunction [4].

Treatment of diabetes with sulphonylureas and biguanides is associated with adverse side effects. However, complementary medicine has grown in popularity in recent years owing to its minimal side effects and appropriate action. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine are used commonly in India. Many indigenous Indian medicinal plants have been found to be useful in the successful management of diabetes and some of them have been tested for their active ingredients. The World Health Organization (WHO) has also recommended the evaluation of the plant's effectiveness and conditions against chronic ailment in place of chemically synthesized drugs. Despite the development of new drugs and their validation by scientific criteria, research still continues in scientific community around the world to evaluate antidiabetic activities of raw plant materials or isolated natural products without adverse effects.

Pimenta dioica (L.) Merril (Family: Myrtaceae) is commonly known as Allspice in culinary. It takes its name from the aroma of dried berries, which smells like the combination of spices, especially cinnamon, cloves, ginger and nutmeg. Allspice owes its characteristic odour due to the presence of essential oil in the pericarp of the seeds. The plant Allspice is mentioned in the Wealth of India [5]. The natives of Kerala and Mangalore use Allspice leaves as medicine for pain, arthritis, fever and stress. The drug has derived the name "Allspice" since its aroma resembles the aroma of spices such as clove, nutmeg and cinnamon [6]. In India, the leaves of *Pimenta* are used to flavor rice which gives it a typical aroma. Allspice is considered as a very important spice in the meat industry which utilizes the powder of the berries for the tenderizing of meat [7, 8].

In all the previously mentioned pathological conditions, oxidative stress is one of the causes, which trigged the momentum to explore the *P. dioica* leaf extract for in vitro antioxidant activity. Antioxidant and hepatoprotective activity in CCl_4 (Carbon Tetra Chloride) induced liver toxicity of all spice leaves had been reported earlier [9]. In the present study *Pimenta dioica* leaves were subjected to evaluate the antioxidant activities against diabetes.

2. Materials and Methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma–Chemical Co. Bangalore. All other chemicals and reagents used for this study were of analytical grade.

Plant material

Pimenta diocia was collected from Kumuli, Kerala State, India.

Preparation of extract

The *Pimenta dioca* leaves were dried at room temperature and then were powdered using dry grinder and passed through sieve. Hundred grams of *Pimenta dioica* were packed in a soxhlet apparatus and extracted with methanol. The methanolic extracts were concentrated on a rotary evaporator.

Experimental animals

Male Wistar albino rats (150-200 g) were procured from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamilnadu, India, and were housed in polycarbonate cages in an animal room with 12 hours day – night cycle. The animals were allowed free access to tap water and standard laboratory rat food. The animal treatment and protocol employed were approved by the TAEC, Annamalai University (Registration Number -1084 /2014/CPCSEA)

Induction of experimental diabetes

Diabetes was induced in the rats by intraperitoneal (I.P.) injection of streptozotocin (STZ) at a dose of 55 mg/kg b.w dissolved in 0.1 M cold citrate buffer (pH = 4.5) [10]. The rats were allowed to drink 5% glucose solution overnight to overcome the drug- induced hypoglycemia. The blood glucose values above 250 mg/dl on the third day after streptozotocin injection were considered as diabetic rats. Then the treatment was started on the fifth day after streptozotocin injection and it was considered as the first day of treatment.

Experimental design

All animals were randomly divided into five groups with six animals in each group

1. Normal untreated rats
2. Diabetic control rats (STZ) (55 mg/kg bw).
3. Diabetic rats treated with methanolic extract of *Pimenta diocia* leaves (75 mg/kg of body weight)
4. Diabetic rats treated with methanolic extract of *Pimenta diocia* leaves (150 mg/kg of body weight)
5. Diabetic rats treated with standard drug, glibenclamide (0.6 µg/kg of body weight).

Estimation of antioxidant parameters

Tissues (liver and kidney) were dissected out and washed immediately with ice cold saline to remove any blood. The antioxidant enzymes such as superoxide dismutase (SOD) [11], reduced glutathione (GSH) [12] catalase (CAT) [13], thiobarbituric acid reactive substances (TBARS) [14] and glutathione peroxidase (GPx) [15] activity were estimated in the liver and kidney.

Statistical analysis

All antioxidant data are expressed as mean ± S.E. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple tests using SPSS (version 18) of computer software. In all cases, P-value of less than 0.05 was considered to be significant.

3. Results

The antioxidant enzymes such as TBARS, SOD, CAT, GSH, and GPx were analyzed in the liver and kidney of normal and STZ induced diabetic rats and treated with the methanolic leaf extracts of *Pimenta dioica* and the standard drug glibenclamide (Table 1). An increased level of TBARS was observed and the levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were significantly ($p<0.05$) reduced in STZ induced diabetic rats. These adverse changes were reversed to near normal values in the methanolic leaf extract of *P. dioica* treated rats on par with the results obtained during the administration of the standard drug, glibenclamide.

4. Discussion

Antioxidants are substances or nutrients which can prevent or slow down the oxidative damage to the body. When the body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage [16].

Table 1. Effect of *P. dioica* on the liver and kidney antioxidant enzymes in STZ – induced diabetic rats

Organs	Group	Treatment	TBARS	SOD	CAT	GSH	GPx
Liver	I	Normal control	3.90±0.10	112.90±2.28	32.55±0.90	241.28±3.40	34.50±0.81
	II	Diabetic control	8.41±0.16	81.01±1.48	21.60±0.31	220.35±1.32	22.10±0.39
	III	Diabetic + *P. dioica* extract (75 mg/kg b.w)	4.60±0.11	96.39±2.19	26.20±0.51	235.89±3.10	29.45±0.89
	IV	Diabetic + *P. dioica* extract (150 mg/kg b.w)	4.54±0.15	102.48±3.12	28.21±0.51	237.41±0.51	32.04±0.44
	V	Diabetic + glibenclamide (0.6 mg/kg b.w)	4.15±0.09	109.69±3.64	31.47±0.51	240.33±2.31	33.01±0.55
Kidney	I	Normal control	5.13±0.10	146.69±2.01	28.81±0.55	334.98±5.71	32.60±0.89
	II	Diabetic control	7.35±0.15	115.59±2.05	20.01±0.84	302.18±3.13	23.80±0.68
	III	Diabetic + *P. dioica* extract (75 mg/kg b.w)	5.93±0.10	131.12±3.41	27.60±0.50	326.80±4.98	27.02±0.59
	IV	Diabetic + *P. dioica* extract (150 mg/kg b.w)	5.39±0.20	133.10±3.65	28.16±0.55	329.41±6.41	30.12±1.02
	V	Diabetic + glibenclamide (0.6 mg/kg b.w)	5.27±0.15	144.81±2.90	28.61±0.51	331.65±3.41	31.42±1.15

Values are expressed as mean±S.E (n=6) and are significantly different at $p<0.005$ when compared with control groups.

Chronic hyperglycemia in diabetes leads to auto-oxidation of glucose, non-enzymatic protein glycosylation, impaired glutathione metabolism, alteration in

antioxidant enzymes and formation of lipid peroxides. The above events accelerate the production of free radicals and weaken the antioxidant defense system. Hence, attention has been given to naturally occurring antioxidants that counteract the deleterious effects of reactive antioxidants. The increase in oxygen free radicals in diabetes could be primarily due to an increase in the blood glucose levels, which upon auto-oxidation generate free radicals. The increased susceptibility of the tissues of the diabetic animals may be due to the activation of the lipid peroxidation system. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism and increased generation of reactive oxygen species [17]. The antioxidant activity of *P.dioica* in liver and kidney was studied in diabetic rats. After the induction of diabetes by STZ, significantly ($P<0.005$) decreased levels of SOD, CAT, GPx, reduced GSH and increased level of TBARS in liver and kidney were observed compared to normal control rats. These altered above antioxidant levels were reversed significantly ($P<0.005$) to near normal levels after the administration of *P. dioica* 75 and 150 mg/kg dose and glibenclamide 0.6µg/kg dose compared to diabetic control rats. It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation in the tissues [18]. These adverse change were reversed to near normal values in the methanolic extract of *P. dioica* leaf treated rats. Recent studies have clearly demonstrated the importance of medicinal plants in the treatment of experimental diabetes, where oxidative stress induced apoptosis or β -cell death occur [19, 20]. Oral administration of *Asparagus racemosus* (EEAR) showed significant hypoglycemic effects against STZ-induced diabetes in rats. The extract significantly lowered the levels of blood glucose and TBARS and significantly increased the levels of GSH, SOD and CAT [21, 22]. From the present study it could be concluded that the methanolic leaf extract of *P. dioica* possess potent antioxidant properties in STZ induced diabetic rats.

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