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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY OF *TILIACORA ACUMINATA* SEED EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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Abstract

Administration of ethanol extract of *Tiliacora acuminata* seed extract (200mg/kg and 400mg/kg body weight) to alloxan induced diabetic rats for 7 days reduced the elevated level of lipid peroxidation (LPO). The treatment also resulted in significant increase in reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in serum, liver and kidney. The results confirm the antioxidant activity of Tiliacora acuminata seed and suggest that because of its antioxidant effects its administration may be useful in controlling the diabetic complications in experimental diabetic rats.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hypoglycemia, due to absolute or relative deficiency or diminished effectiveness of circulating insulin. The most common form of diabetes is diabetes mellitus, a chronic progressive, systemic condition of impaired carbohydrate metabolism. The prevalence of diabetes for all age – groups worldwide was estimated to be 2.5% in 2000 and 4.4% in 2030 (Wild et al., 2004). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. Its major manifestations include disorder metabolism and inappropriate hyperglycemia. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and sharp reduction of antioxidant defenses. Hence compounds with both hypoglycemic and antioxidative properties would be useful for antidiabetic agents. Many plant extracts and plant products have been shown to have significant antioxidant activity (Nishanthini and Mohan, 2013).

Tiliacora acuminata is a large woody climber; branches cinereous, striate. Leaves are long, ovate, acuminate, cordate, truncate or rounded at the base, flowers yellow in elongate, lax, axillary, racemose panicles belonging to the family Menispermaceae. This plant has been used as an ingredient in many of the ayurvedic preparations and regard as an antidote for snake bite (Selvaraj et al., 2008; Sri and Reddi, 2011). The present study has been aimed to investigate the effect of ethanol extract of seed of *Tiliacora acuminata* on lipid peroxides and enzymatic antioxidants in serum, liver and kidney of alloxan induced diabetic rats.

MATERIALS AND METHODS

The seed of *Tiliacora acuminata* was collected from Ulakaruvi, Kanyakumari District, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Tiliacora acuminata* seed were shade dried at room temperature and the dried seed were powdered in a Wiley mill. Hundred grams of powdered *Tiliacora acuminata* seed was packed in a Soxhlet apparatus and extracted with ethanol. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures (Brinda et al., 1981; Lala, 1993). The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2º C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water ad libitum.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Induction of Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) (Nagappa et al., 2003). Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Tiliacora acuminata* seed (200mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *Tiliacora acuminata* seed (400mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

The animals were sacrificed at the end of experimental period of 7 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes and serum was stored at -4^{o}C until analyses completed. The liver and kidney tissues were excised, rinsed in ice cold saline, cut into small pieces and homogenized with homogenizer in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 10 min. Supernatant was used for enzyme assays for the estimation of non enzymatic and enzymatic antioxidants such as lipid peroxidation (LPO)[Ohkawa et al., 1979]; superoxide dismutase (SOD) (Kakkar et al., 1984), catalase (CAT)(Sinha, 1972), glutathione peroxidase (GPx)(Rotruck et al., 1973) and reduced glutathione (GSH) (Sedlak and Lindsay, 1968).

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of *Tiliacora acuminata* seed revealed the presence of alkaloid, catechin, coumanin, flavonoid, steroid, saponin, phenol, tannin, glycoside, terpenoid and xanthoprotein. Acute toxicity study revealed that the administration of ethanol extract of *T. acuminata* seed upto a dose level of 2000mg/kg bodyweight did not produce significant changes in behavior, breathing, cutaneuos effects, sensory, nervous system responses and gastrointestinal effects in rats. From the results it was observed that ethanol extract of *T. acuminata* seed at the dose of 2000mg/kg were nontoxic. The results (Table 1, 2 &3) showed increased lipid peroxidation (LPO) in serum, liver and kidney of alloxan induced diabetic rats. Lipid peroxidation is one of the characteristic features of chronic diabetes and lipid peroxidation mediated tissue damage has been observed in diabetic conditions (Felliet- Coudray et al., 1999). Hyperglycemia generates reactive oxygen species (ROS), which in turn cause lipid peroxidation and membrane damage (Hunt et al., 1988). Increased concentration of lipid peroxides in the liver are reported to decrease cytochrome P_{450} and cytochrome b_5 activities, which may affect the drugs metabolizing activity in chronic diabetes (Ragini et al., 2011). In the present study, an increase in the levels of LPO was found and these levels were significantly ($p<0.01$) reduced after the supplementation of the ethanol extract of *T. acuminata* seed (400mg/kg body weight) and glibenclamide (Table1, 2 & 3). This indicate that plant extract inhibit oxidative damage due to the antiperoxidative effect of ingredients present in ethanol extract of *T. acuminata* seed. This should be correlated with previous study reported that *Cassia auriculata* flower, *Syzigium cumini*,

Tinospora cordifolia, Scoparia dulcis, Nigella sativa and *Polycarpea corymbosa* (Pari and Latha, 2002; Prince and Menon, 1998; Prince et al., 1999; Latha and Pari, 2003; Nishanthini and Mohan, 2013).

The level of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were significantly reduced in serum, liver and kidney of alloxan induced diabetic rats. The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it into hydrogen peroxide and molecular oxygen (Robinson et al., 1998), while CAT brings about the reduction of hydrogen peroxides and protects tissue from the highly reactive hydroxyl radicals (Brioukhanov et al., 2004). In the present investigation both these enzymes registered low levels of activity in diabetic controls indicating diabetes-induced stress. Such a decline in these enzyme activities has also been reported earlier (Nishanthini and Mohan, 2013). When *T. acuminata* seed extracts administrated to the diabetic rats improved both SOD and CAT activities substantially, reflecting the antioxidant potency of plant extracts.

GSH is a major non-protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphate (GSSG) and the reduction product of the hydroperoxide. In the present study, decline in the activities of these enzymes in alloxan induced rats and attainment of normally in *T. acuminata* seed extract treated rats indicate that oxidative stress elicited by alloxan was significantly reduced by this extract.

The present study reveals that the *T. acuminata* seed extract had antioxidant activity. The bioactive components, responsible for the observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in the seed extracts. In the present study, phytochemical screening reported that the presence of phenolics and flavonoids in extracts which might be the constituents responsible for the antioxidant activities. Further identification and isolation of these compounds may be fruitful.

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Table 1: Effect of *Tiliacora acuminata* seed extract on serum LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	**GPX (u/mg protein)**	**GSH (u/mg protein)**	**SOD (u/mg protein)**	**CAT (u/mg protein)**
I	1.81±0.05	668.16±31.18	34.18±1.23	439.16±28.39	81.16±2.13
II	3.16±0.08**	308.28±20.22**	21.13±1.08**	294.16±20.84**	59.13±1.84**
III	1.73±0.05^{a}	561.84±16.82^{aa}	28.16±1.85^{a}	397.16±20.12^{a}	78.12±1.13^{aa}
IV	1.56±0.06^{aa}	581.16±16.13^{aa}	30.76±1.34^{aa}	402.15±19.85^{aa}	87.84±1.12^{aa}
V	1.73±0.06^{aa}	653.18±20.18^{aa}	35.88±1.92^{aa}	411.29±20.16^{aa}	82.86±1.35^{aa}

Each Value is SEM ± 5 individual observations * $p < 0.05$; ** $p<0.01$ Compared normal control vs -Diabetic rats : a -$p < 0.05$; aa - $p<0.01$ Compared -Diabetic rats vs drug treated

Table 2: Effect of *Tiliacora acuminata* seed extract on Liver LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	0.103±0.011	7.93±0.12	54.16±1.82	5.84±0.75	88.27±2.16
II	0.184±0.024**	3.95±0.27**	19.82±0.93**	3.08±0.84**	58.13±2.15**
III	0.142±0.018ns [a]	5.04±0.08*	39.19±1.05*[a]	4.02±0.27ns	72.16±3.95[a]
IV	0.122±0.027[aa]	6.92±0.15[a]	42.88±1.84[aa]	4.84±0.31[a]	78.24±4.11[aa]
V	0.109±0.022[aa]	8.04±0.19[aa]	57.84±1.92[aa]	5.67±0.16[aa]	85.84±3.81[aa]

Each Value is SEM ± 5 individual observations * p < 0.05 ; ** p<0.01 Compared normal control vs -Diabetic rats : a -p < 0.05 ; aa - p<0.01 Compared -Diabetic rats vs drug treated

Table 3 Effect of *Tiliacora acuminata* seed extract on Kidney LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	0.081±0.001	5.98±0.26	32.14±1.31	18.27±0.92	45.62±1.65
II	1.912±0.007**	2.36±0.18**	14.88±1.05**	8.91±0.84**	18.27±1.16**
III	0.098±0.015[a]	4.06±0.11[a]	21.16±1.54ns	17.81±0.84[a]	32.83±1.08[a]
IV	0.092±0.016[aa]	5.04±0.27[aa]	30.13±1.36[a]	18.89±0.36[aa]	33.16±1.22[a]
V	0.083±0.011[aa]	5.84±0.17[aa]	33.84±1.27[aa]	19.08±0.16[aa]	39.15±1.13[aa]

Each Value is SEM ± 5 individual observations * p < 0.05 ; ** p<0.01 Compared normal control vs -Diabetic rats : a -p < 0.05 ; aa - p<0.01 Compared -Diabetic rats vs drug treated

REFERENCES

Wild, S., Gojka, R., Anders, G., Richard, S. and Hilary K. (2004): Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care., 27 : 1047-1053.

Nishanthini, A. and Mohan.V.R. (2013): Antioxidant activity of *Polycarpaea corymbosa* L. whole plant in alloxan induced diabetic rats. World J. Pharm .Pharmaceut. Sci., 2: 343-351.

Selvaraj, S.J., Alphonse, I. and Britto, S.J. (2008): A new lactone from aerial parts of *Tiliacora acuminata*. Indian J. Chem., 47B: 942-944.

Sri, B.S. and Reddi, T.W.W.S. (2011): Traditional phyto-antidotes used for snakebite by Bagata tribe of Eastern Ghats of Visakhapatnam district, Andhra Pradesh. Int. Multidisci. Res. J., 1: 42-45.

Brinda, P., Sasikala, P. and Purushothaman, K.K. (1981): Pharmacognostic studies on *Merugan kizhangu*. Bull. Med. Ethnobot. Res., 3: 84-96.

Lala, P.K. (1993): Lab manuals of Pharmacognosy. CSI Publishers and Distributers. Kolkata.

OECD. (2002): (Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris.

Nagappa, A.N., Thakurdesai, P.A., Venkat Rao, N. and Sing, J. (2003): Antidiabetic activity of *Terminalia catappa* Linn. fruits. J Ethnopharmacol., 88: 45-50.

Ohkawa, H., Ohishi, N. and Yaki, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem., 95: 351-358.

Kakkar, P., Das, B. and Viswanathan, P.N. (1984): A modified spectrophotometric assay of Superoxide dismutase. Int Biochem Biophysics., 21: 130-132.

Sinha, A.K. (1972) : Colorimetric assay of catalase. Anal Biochem., 47: 389-394.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973): Selenium: biochemical role as a component of glutathione peroxidase. Science., 179: 588-590.

Sedlak, J. and Lindsay, R.H. (1968): Estimation of total protein bound and non-protein sulfhydryl groups in tissue with Ellmans reagent. Anal Biochem., 25: 192- 205.

Feillet-Coudray, C., Rock, E. and Coudray, C. (1999): Lipid peroxidation and antioxidant status in experimental diabetes. Clin Chim Acta., 284:31-43.

Hunt, J., Dean, R.T. and Wolff, S.P. (1988): Hydroxyl radical production and autoxidative glycosylation: Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes and ageing. Biochem J., 256: 205-212.

Ragini, V., Prasad K.V.S.R.G. and Bharathi, K. (2011): Antidiabetic and antioxidant activity of *Shorea tumbuggaia* Rox. Int. J. Innova.. Pharmaceut. Res., 2: 113-121.

Pari, L. and Latha, M. (2002): Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med. J.,43: 617-621.

Prince, P.S.M. and Menon, V.P. (1998): Hypoglycemic activity of *Syzigium cumini* seeds; effect on lipid peroxidation in alloxan diabetic rats. J. Ethnopharmacology., 61:1-7.

Prince, P.S.M., Menon, V.P. and Gunasekaran, G. (1999): Hypolipidemic action on *Tinospora cordifolia* root extract in alloxan diabetic rats. J. Ethnopharmacology., 14: 4-16.

Latha, M. and Pari, L. (2003): Modulatory effect of Scoparia dulucis in oxidative stress induced lipid peroxidation in streptozotocin diabetic rats. J. Med. Food., 6: 379-386.

Nishanthini, A. and Mohan,V.R. (2013): Antioxidant activity of *Melastomma malabathricum* L. leaf in alloxan induced diabetic rats. Pharmascience Monitor., 4: 3489-3498.

Robinson, B.H. (1998): The role of manganese super oxide dismutase in health and disease. J Inherit. Met. Dis., 21:598-603.

Brioukhanov, A.L. and Netrusov, A.I. (2004): Catalase and superoxide dismutase: distribution, properties, and physiological role in cells of strict anaerobes. Biochemistry., 69:949-962.