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Research Paper

Antioxidant and Antiperoxidative Effects of *Ipomoea staphylina* in Streptozotocin Induced Diabetic Rats

P. Shobana Devi[1] and M. Shabana Begum*[2]

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ABSTRACT

Ipomoea staphylina Linn. Is traditional practice in the treatment of stomach disorders, pain, purgation, rheumatism, and also in inflammation. In this study, ethanol extract of *Ipomoea staphylina* was subjected to analyze its antidiabetic activity in STZ provoked diabetes animals. Diabetes rats were remedied with *Ipomoea staphylina* (200 milligrams/kg) ethanol extract for 45 days using a noteworthy decline in the levels of blood glucose in diabetic animals. The antioxidant activity of ethanol extract has shown improved levels of Thiobarbituric acid reactive substances (TBARS), hydroperoxide, lowered, glutathione peroxidase (GPx), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) within the pancreas, liver, and kidney of diabetic rats. Furthermore, the results are similar to groups treated with glibenclamide, a standard drug. The results of this study recommend that *Ipomoea staphylina* may be an excellent healing property for diabetes mellitus patients.

***Key words*:** Antioxidants, Ethnomedicine, *Ipomoea staphylina,* Streptozotocin, Diabetes

Diabetes Mellitus (DM) is made up of a variety of hyperglycemic problems or lowered blood insulin secretions [1]. DM is a serious health condition that affects millions of people worldwide. In 2025, 300 million people will are afflicted by DM [2]. Research conducted in India reported that metropolitan communities are improving rapidly in getting DM for ten years [3]. The International Diabetes Federation (IDF) reviewed that there will be about 40.9 million diabetic patients in India, which from the season 2025, will grow furthermore to 69.9 million [4]. Enhanced free radicals advancement and oxidative stress are viewed with an essential role in DM pathogenesis and past due difficulties [5]. A free radical is an individual molecule with more than one unpaired electrons. Free radicals lead to antioxidant shield ingestion, which can cause cell function interference and oxidative problems for the membranes and increase lipid peroxidation intolerances. Improved productions of lipid peroxidation and reactive oxygen species (ROS) have been detected to be concerned in the pathogenesis of numerous noted and volatile etiological ailments along with the harmful measures of several compounds [6]. Antioxidants showed a substantial role in safeguarding the body against reactive oxygen damage [7].

Recently the study of new types of antioxidants from numerous herb resources continues to be intensively investigated [8]. DM administration without having quality results is still a problem in the health care process. People are increasingly demanding to use all-natural antidiabetic drugs since insulin and oral hypoglycemic drugs have unfavorable adverse reactions [9]. Plants and their active compounds provide worthwhile causes of healing products for the treating of DM [10].

Ipomoea staphylina (IS) is uncovered in woodland and trash. It is a prolonged-durable timbered shrub with pink flowers. It might be seen in the therapeutic of respiration infections [11]. It is utilized to deal with digestive issues, have the anthelmintic capability, and bronchial discomfort. The assessment describes its inhibitory action against inflammation, lipoxygenase, glucosidase, and α-amylase [12]. Chemical compounds comprise of Sitosteryl-3-O-β-D-glucoside, and bioactive chiro-deoxyinositol continues to be determined from IS leaves.

***M. Shabana Begum**
shobirose2013@gmail.com

[1-2]Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram - 637 408, Namakkal, Tamil Nadu

MATERIALS AND METHODS

Fresh IS leaves were collected and taxonomically authenticated by the botanist Dr. S. John Britto at The Rapinat Herbarium, where the specimen was deposited with the voucher number RVP 001.

Preparation of IS leaf extracts

The fresh IS Leaves were cleaned and rinsed with distilled $H_2O$and mildly smeared with filter paper and dried in the dark. The Mechanical grinder was used for crushing the dried leaves and placed in a 4°C clean container for further use. 20 g of IS powder was extracted with 200ml solvent ethanol and filtered at room temperature every 3 hours with the help of Whatman No.1 filtering material. Combined extracts were then evaporated at 40°C to dryness. The desiccated extracts were preserved at 4°C until further usage.

Preparation of Ipomoea staphylina plant extract

Plants have already been rinsed with freshwater, shade dried out, and soil at room temperatures. The powder was

extracted with n-ethanol. Then 2.5 kg of the air-dried powder was added with ethanol and double distilled water in 10 Lt Aspirator by soaking (24 h × 3 days) at room temperature. These extracts were evaporated by using rotary evaporator to obtain respective dry extracts. The extracts were dissolved in Tween 80 for pharmacological studies. Extracts have maintained to be used in a 4°C freezer.

Animals used in research

Male Wister rats of 180-200 g body weight were acquired and maintained at the Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram, India Fed on healthy pellet diet (Amrut, Pune, India) and water *ad libitum*. The report of the study was accepted by Muthayammal College of Arts and Science's Institutional Ethical Committee (1416/PO / a/11/CPCSEA & 17.06.2013).

Induction of diabetes mellitus

DM was experimentally caused by an intraperitoneal administration of Streptozotocin (STZ) (55 mg/kg BW) suspended in 0.1M cold citrate buffer (pH 4.5) in 12-hour fasted rats. Subsequent 6h of STZ administration, the rats were treated with 10% blood sugar solution for the next 24h to avoid hypoglycemia. No mortality or any other adverse reactions have been noted. Rats with modest diabetic issues (i.e., glucose levels >250 mg/dl) exhibiting glycosuria and hyperglycemia were chosen for experimental work after having a week of development and aggravation of DM.

Experimental design

Group I: Control rats.

Group II: Diabetic induced rats.

Group III: Diabetic rats administered orally with *Ipomoea staphylina* ethanol extract (ISEE) of leaves (200 mg/kg body weight/rat) for 45 days

Group IV: Diabetic rats administered orally with glibenclamide (600 µg/kg body weight/rat) [13].

The rats were fasted and sacrificed with cervical decapitation overnight, following the last treatment (45 days). The blood was hoarded, and after centrifugation, the plasma was collected. Then, the glucose estimation was done. The tissues of the liver, kidney, and pancreas were directly removed from the experimental animals and kept in ice-cold containers. We are then homogenized with an appropriate buffer solution, centrifuged, and stored the supernatant. Biochemical estimates have been made inhomogeneous on the very same day of sacrifice.

Biochemical estimations

Fasting glucose levels were assessed using the Kit method [14]. Thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HP) have been calculated employing [15] and [16] methods and also calculated lowered glutathione in the tissues (GSH) [17]. The function of antioxidant enzymes such as SOD [18], CAT [19], and GPx [20] have also been analyzed. Ascorbic acid has become measured by [21] method. [22] methods were adopted to evaluate vitamin E antioxidant.

Statistical analysis

All conclusions happen to be demonstrated as Mean ± SD in each group for six animals. All the compiled data were statistically examined utilizing SPSS10 software. Theory Hypothesis test approaches included one-way variance analysis (ANOVA) followed by the least significant difference (LSD) test. The significance level at alpha=0.05 was considered to statistical significance.

RESULTS AND DISCUSSION

The ethanol extract of *Ipomoea staphylina* can boost glucose tolerance considerably ($P<0.05$). Within an hour of drug administration, blood glucose levels decreased substantially. Together with the ISEE, blood sugar was reduced to normal. The administration of *Ipomoea staphylina* in STZ-stimulated diabetic rats has demonstrated an essential lowering of blood glucose and increased quantities of blood insulin (Table 1). The concentration of lipid hydroperoxides, Thiobarbituric acid reactive substances (TBARS), has shown in (Table 2-3). Tissues Thiobarbituric acid reactive substances (TBARS) and hydroperoxides have been considerably increased during DM in comparison to the related normal group. *Ipomoea staphylina* has reduced lipid peroxidation in diabetes rats substantially. (Tables 4-5) displayed the functions of Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx) in control and experimental rat groups within the liver and kidney. GPx, CAT, and SOD activities in cells, for example, kidney and liver system, in diabetes control rats were significantly lower compared with normal rats. When *Ipomoea staphylina* was given orally to diabetic person rats, the antioxidant levels were substantially higher when competed with Group I rats. GSH, Vitamin C, and Vitamin E in Group II organs, liver, kidney, and pancreas have been significantly reduced. But, the diabetic animals in Group III and IV showed increased antioxidant levels compared to Group I (Tables 6-8).

Table 1 Effect of *Ipomoea staphylina* on plasma glucose, insulin and body weight in normal and experimental diabetic rats

Groups	Glucose (mg dl^{-1})	Insulin (µU ml^{-1})	Body weight (g)
Control	80.32±1.9	17.2±1.95	156.4±2.9
STZ Control	345.83±16.9a***	7.3±0.7a*	116.4±11.8 a*
STZ + ISEE of leaves	91.7±2.9 b***	15.1±0.8 b*	147.3±17.1 b*
STZ + Glibenclamide	121.4±12.5b***	14.7±1.3b*	139.4±8.7 b*

Values are mean ± SD, n = 6

Values are statistically significant at $^{*}p<0.05$, $^{***}0.001$

[a]Significantly different from control

[b]Significantly different from STZ control

The present study was executed over to discover the antidiabetic in vivo activity of ISEE. The discoveries of the anti-diabetes process, ISEE extract have given the technological basis for the efficacy of this plant for DM treatment. The ethanol extract revealed a significant reduction in glucose range in both glucose and STZ induced diabetic animals. The extract has created the most significant antidiabetic activity, and it is equitable to glibenclamide's

hypoglycemic activity in diabetic rats. It is, therefore, evident that the ethanol extract has enriched using energetic principles. The plant has lowered glucose levels to normalcy in animals stocked with glucose. The medication can also work by potentiating the pancreatic secretion or by improving the uptake of sugar.

Table 2 Effect of *Ipomoea staphylina* on plasma TBARS and lipid hydroperoxides in normal and diabetic rats

Groups	TBARS	Lipid hydroperoxides
	(nmol/ml)	
Normal control	2.39 ± 0.17	0.79 ± 0.01
Diabetic control	3.74 ± 0.34[a*]	1.59 ± 0.01[a*]
Diabetic + ISEE of leaves (200 mg)	2.70 ± 0.22[b*]	0.90 ± 0.01[b*]
Diabetic + glibenclamide)	2.31 ± 0.17[b*]	0.71 ± 0.01[b*]

Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from Diabetic control [*]P < 0.05

Table 3 Effect of *Ipomoea staphylina* on lipid hydroperoxides in the liver, kidney, and pancreas of normal and diabetic rats

Groups	Liver	Kidney	Pancreas
	(nmol/g wet tissue)		
Normal control	45.94 ± 3.30	63.1 ± 4.50	0.24 ± 0.01
Diabetic control	127.01 ± 9.20[a*]	94.01 ± 7.29[a*]	0.38 ± 0.01[a*]
Diabetic + ISEE of leaves (200 mg)	63.4 ± 4.80[b*]	69.9 ± 5.31[b*]	0.23 ± 0.01[b*]
Diabetic + glibenclamide)	42.9 ± 3.30[b*]	62.96 ± 4.80[b*]	0.19 ± 0.01[b*]

Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from Diabetic control [*]P < 0.05

Table 4 Effect of *Ipomoea staphylina*on the activities of superoxide dismutase, catalase and glutathione peroxidase in the liver of normal and diabetic rats

Groups	SOD ([*]Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/min/ mg protein)	GPx (µg of GSH consumed/min/ mg protein)
Normal control	11.9 ± 0.75	67.3 ± 5.5	10.6 ± 0.79
Diabetic control	5.36 ± 0.49[a*]	46.2 ± 4.29 [a*]	5.4 ± 0.50 [a*]
Diabetic + ISEE of leaves (200 mg)	9.31 ± 0.79[b*]	57.9 ± 5.20 [b*]	8.4 ± 0.67 [b*]
Diabetic + glibenclamide)	11.9 ± 0.85 [b*]	64.14 ± 5.03 [b*]	10.12 ± 0.61 [b*]

[*]SOD units: one unit is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in 1 min
Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from diabetic control [*]P < 0.05

Table 5 Effect of *Ipomoea staphylina* on the activities of superoxide dismutase catalase and glutathione peroxidase in the kidney of normal and diabetic rats

Groups	SOD ([*]Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/min/ mg protein)	GPx (µg of GSH consumed/min/ mg protein)
Normal control	10.9 ± 0.70	46.5 ± 1.9	5.5 ± 0.39
Diabetic control	6.21 ± 0.50[a*]	32.5 ± 1.9 [a*]	3.81 ± 0.29 [a*]
Diabetic + ISEE of leaves (200 mg)	9.43 ± 0.72[b*]	40.3 ± 3.21 [b*]	4.93 ± 0.30 [b*]
Diabetic + glibenclamide)	10.5 ± 0.6 [b*]	46.7 ± 2.01 [b*]	5.5 ± 0.41 [b*]

[*]SOD units: one unit is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in 1 min
Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from Diabetic control [*]P < 0.05

Table 6 Effect of *Ipomoea staphylina* on plasma glutathione, vitamin C and vitamin E in normal and diabetic rats

Groups	Glutathione	Vitamin C	Vitamin E
	(mmol/L)		
Normal control	0.69 ± 0.04	0.14 ± 0.01	0.07 ± 0.002
Diabetic control	0.34 ± 0.02[a*]	0.05 ± 0.01[a*]	0.02 ± 0.001[a*]
Diabetic + ISEE of leaves (200 mg)	0.5 ± 0.02[b*]	0.09 ± 0.01 [b*]	0.03 ± 0.002 [b*]
Diabetic + glibenclamide)	0.65 ± 0.03 [b*]	0.15 ± 0.01 [b*]	0.06 ± 0.002 [b*]

Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from Diabetic control [*]P < 0.05

Table 7 Effect of *Ipomoea staphylina* on glutathione in the liver, kidney, and pancreas of normal and diabetic rats

Groups	Liver	Kidney	Pancreas
	(mg/100 g tissue)		
Normal control	32.19 ± 2.39	23.85 ± 1.77	18.4 ± 1.261
Diabetic control	17.9 ± 1.59[a*]	12.7 ± 0.89 [a*]	12.5 ± 0.43 [a*]
Diabetic + ISEE of leaves (200 mg)	26.2 ± 1.9[b*]	18.9 ± 1.79 [b*]	17.9 ± 1.3 [b*]
Diabetic + glibenclamide)	35.4 ± 2.43 [b*]	23.7 ± 2.1 [b*]	18.6 ± 1.1 [b*]

Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from Diabetic control *P < 0.05

Table 8 Effect of *Ipomoea staphylina* on vitamin C and vitamin E in the liver and kidney of normal and diabetic rats

Groups	Vitamin C (µmol/mg tissue)		Vitamin E (µmol/mg tissue)	
	Liver	Kidney	Liver	Kidney
Normal control	1.49 ± 0.10	1.1 ± 0.05	0.79 ± 0.04	0.52 ± 0.05
Diabetic control	0.87 ± 0.05[a*]	0.77 ± 0.06 [a*]	0.49 ± 0.06 [a*]	0.37 ± 0.02 [a*]
Diabetic + ISEE of leaves (200 mg)	1.13 ± 0.08[b*]	1.0 ± 0.05 [b*]	0.65 ± 0.03 [b*]	0.49 ± 0.02 [b*]
Diabetic + glibenclamide)	1.41 ± 0.10 [b*]	1.20 ± 0.05[b*]	0.71 ± 0.05[b*]	0.50 ± 0.02[b*]

Values are mean ± SD, n = 6
[a]Significantly different from control
[b]Significantly different from Diabetic control *P < 0.05

During all sorts of diabetic problems, due to organized devastation of pancreatic insulin-secreting β-tissues by STZ, the extra quantity of ROS was produced. Better ROS levels lead to injury to the muscle and good lipid peroxidation [23]. Moreover, islet cellular material is far more at risk of free of charge extreme attacks because of the relatively lower process of antioxidant digestive enzymes like SOD, CAT, GST and GPx [24]. Because of this, following STZ infusion, most islet tissue is affected by damage or impairment. Lacking the insulin level of resistance reimbursement on account of elevated blood insulin release contributes to increased glucose levels. Long-term hyperglycemia, which injuries the antioxidant shield system, is attributed to more excellent free radicals noticed in diabetes rats [25]. Our present study proved a tremendous surge in diabetic rats of TBARS tissues. The improved TBARS subjects of diabetic induced rats show that injuries caused by peroxidative may bring about diabetic issues. Within the *Ipomoea staphylina* and glibenclamide dealt with group levels of TBARS, and hydroperoxides from the liver organ and kidney have been considerably below within the diabetic control group. The effect mentioned shows that *Ipomoea staphylina* can propagandize antioxidant activity and safeguard the cells from lipid peroxidation.

All types of DM are allocated to improved development of free-radicals or fragile antioxidant defense alternatives [24]. During DM, the ROS exhaust process might be collected as enzymatic (SOD, CAT, GST, and GPx) and non-enzymatic (GSH, ascorbic acid, and tocopherol). Lowered antioxidant tissues in the kidney and liver of diabetic rats verify earlier published review [24]. Bloodstream insulin insufficiency from your diabetic issues delivered on impairment in using sugar, creating enhanced oxygen-free radicals generation [1]. Research has noted decreases in STZ-stimulated tissue SOD action when evaluated in Group I [26]. SOD is a vital protective enzyme that enhances the dismutation of O_2•– to generate H_2O_2 and molecular oxygen, decreasing the intense free radicals activities. ROS could incapacitate and lowered tissue SOD function [27]. The noticed decrease in SOD activity could be a consequence of H_2O_2 inactivation or enzyme glycation [28]. In this particular review, it was found out that *Ipomoea staphylina* helped bring on a tremendous rise in diabetic rat liver and kidney SOD function. It indicates *Ipomoea staphylina* will be able to minimize reactive oxygen-free-radicals and boost hepatic antioxidizing enzyme activity.

Catalase (CAT) is a heme protein that catalyzes hydrogen peroxide, lessening, and shields the tissues against extremely reactive OH- radicals. The decline in the CAT process could affect enzyme glycation deactivation. CAT decreases on the H_2O_2 created by the dismutation impulse and ceases the production of OH-, thus guarding the cell components of peroxisomes through oxidative damage [29]. The minimized CAT functions in rats resolved with STZ action in H_2O_2 deposition, which creates harmful effects [27]. In this study, it was noticed that *Ipomoea staphylina* triggered a considerable boost in diabetic rats. This method is primarily on account of the antioxidant nature of *Ipomoea staphylin* and could embroil a mechanism associated with free radicals scavenging activity.

GSH, a non-enzymatic antioxidant, has proven to supply defense in opposition to ROS through efficiently preventing free radicals along with other ROS directly and indirectly by enzymatic reactions [30]. Research indicates that hepatic GSH levels in STZ-stimulated diabetes rats are considerably reduced when compared with normal rats [31]. It is well known that GSH is implicated in the basic safety of standard cell structure and function by preserving redox homeostasis, squeezing free-radicals, and engaging in detoxing side effects. Winterbourn reported that it is a potent scavenger of free radicals in addition to co-substrate for peroxide recuperation by glutathione peroxides [32]. It was proposed that the reduction in hepatic GSH would be the reaction to lowered functionality or increased oxidation of GSH due to oxidative stress in all forms of DM [33]. Increased oxidative stress, arising from a tremendous boost in lipid peroxidation aldehyde products, will probably lessen the GSH materials in the tissues. Within this review, the elevation of GSH levels inside the liver and kidney was discovered in diabetes rats cured with *Ipomoea staphylina* and glibenclamide. It is suggested that *Ipomoea staphylina* and glibenclamide may either improve GSH biosynthesis or lessen oxidative stress resulting in significantly less GSH deterioration or have both outcomes.

Glutathione Peroxidase (GPx) is a significant enzyme for peroxide, removing the leading to intracellular hydrogen

peroxide builds up as a consequence of extended depressive disorders within the measures of this enzyme. GST abilities as peroxidase and lowers harmful peroxides through the process, resulting in a reduction of peroxide-caused damage [34]. GPx and GST combined activities with glutathione within the breakdown of H_2O_2 or other natural and organic hydroperoxides to non-harmful effects at the expense of decreased glutathione [35]. The minimized procedure of GPx may be described as a consequence of significant-stimulated inactivation and glycation in the enzyme. Glutathione can be a substrate of GPx enzyme and, in effect, increased amounts of glutathione increase the action of GPx, and, due to this, GPx process is now mediated to scavenge free radicals in diabetes rats. The minimized motion of GPx within the liver and kidney has become identified during all forms of DM and can even final result in several harmful effects attributable to the deposition of hazardous chemical contaminants. In this situation, other findings have claimed a reduction within GPx activity within the liver and kidney of diabetes rats [36]. With this research, the administration of *Ipomoea staphylina* and glibenclamide boosted the measures of GPx throughout diabetic rat tissues.

Antioxidant intake by diet plays a significant role in supporting an oxidative system. Vitamin C, vitamin E, and other micronutrients protect humans, including aging and DM, against several diseases [37]. A critical antioxidant vitamin C can scavenge singlet oxygen, superoxide, and hydroxyl radicals directly. Vitamin E is the best lipid-soluble antioxidant in the living environment. The counteracts LPO and retrieves decreased ascorbic acid and GSH [38]. In the liver and kidney of diabetic rats, both vitamin C and vitamin E have decreased significantly. *Ipomoea staphylina* was used to increase the levels of vitamin C and vitamin E.

CONCLUSIONS

From the results of this study, it was concluded that *Ipomoea staphylina* has a free radical scavenging activity that can influence its defensive property on free radicals and enhance its action on the protection of cellular antioxidants. This operation helps protect against oxidative damage in diabetes mellitus caused by STZ.

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