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Anti-Diabetic Effect of Diospyros Malabarica against Alloxan Induced Diabetes in Rats

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A B S T R A C T

The Present study was under taken to evaluation of anti-diabetic potential of *Diospyros malabarica(DM)* leaves in low dose Alloxan and high fat diet induced diabetes in rats at concentration at doses of (200 and 400 mg/kg/ p.o) showed significant hypoglycemic activity on normal rats. The extract also excreted significant anti-hyperglycemic effect in Alloxan induced hyperglycemia and resulted in increase in plasma protein content decrease in alkaline phosphate, cholesterol and triglyceride levels when compared with those in the diabetic control group. However, there were no significant changes in body and kidney weights of the *Diospyros malabarica* extract treated animals, compared to those of the untreated diabetic rats is control. However, the DM extract showed a potential antioxidant activity by increasing catalase activity and reducing lipid peroxidation in liver, (Histopathological studies for liver and kidney). The Results demonstrate anti-diabetic activity of the defatted methanol extract of DM leaves.

Keywords: Anti-Diabetic activity, Diospyros malabarica, glibenclamide, Wistar rats, hyperglycemic activity, high fat diet, Alloxan.

A R T I C L E I N F O

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1. Introduction

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is a silent killer that kills 1 person every 8 seconds in the world and 4 million persons a year [1]. The Hyperglycemia is one of the

common manifestations of diabetes [2]. Chronic hyperglycemia causes damage to the eyes, kidneys, nerves, heart and blood vessels. The term Diabetes, coined by Aretaeus of Cappadocia, is derived from the Greek word, diabainein that literally means passing through, or siphon, a reference to one of diabetes major symptoms- excessive urine production [3]. Diabetes is classified into diabetes mellitus and diabetes insipidus. Diabetes insipid us is characterized by the persistent excretion of excessive quantities of dilute urine and by thirst because of deficiency of production of ADH, a hormone secreted by posterior pituitary gland. Diabetes mellitus is a metabolic disorder characterized by resistance to the action of insulin, insufficient insulin secretion, or both. The clinical manifestation of diabetes mellitus is hyperglycemia i.e., high glucose blood sugar [4].

Herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects.[5] *Diospyros malabarica* (Family: Ebenaceae), commonly known as Bandadamara in Tirumala hills, is a succulent, prostrate herb, with fleshy leaves . It is found growing abundantly all over India and is eaten as a leafy vegetable [6]. It contains more omega-3-fatty acids than any other leafy vegetable plant. Besides vitamins (mainly vitamin C, vitamin B), carotenoids and dietary minerals have been reported. Some alkaloid igments large amounts of Norepinephrine, a neurohormone that has vasopressor and hypertensive activities have been reported in this plant. Other constituents include dopamine, coumarins, flavonoids, saponins and urea [7]. Analgesic the pharmalogical studies have shown Tumour inhibiting and wound healing activity. *Diospyros malabarica* has been claimed to possess anti -diabetic activity in traditional system of medicine but no systematic, scientific work has been done to prove this activity [8].

2. Materials and Methods

Collection and authentication

The leaves of Diospyros Malabarica were collected in the month of January 2014 from forest area of Tirumala Hills, Thirupathi, and Chittor district. Andhra Pradesh. It was shade dried away from sunlight and stored suitably. The plant material was taxonomically identified by the Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateshwara University, Thirupathi, Andhrapradesh, India.

Extraction

Diospyros Malabrica was used in the form of crude 50% of Hydro alcoholic extract and this extract was prepared according to the traditional system of medicine. The shade dried and coarsely powdered leaves (1kg) were extracted with 50% Hydro alcoholic compounds in the cold for 72 hours. The extract was filtered and distilled on water bath, a reddish brown syrupy mass was obtained and it was finally dried at low temperature under reduced pressure in a rotary evaporator. A crude residue (75g) was obtained giving a yield of 7.5%. The antidiabetic effects were evaluated by oral administration of the extract to the Alloxan induced diabetic rats.[9]

Preliminary phytochemical screening

The hydro alcoholic extract of Diospyros malabarica. Was tested for phytoconstituents such as carbohydrates, Alkaloids, Tannins, Triterpenoids, Amino acids phenolic compounds, glycosides, saponins using standard phyto-chemical methods. [10]

Animals:

Experimental animals of male Wistar albino rats weighing 150-200g were obtained from Raghavendra enterprises. The animals were housed in stainless steel cages at a controlled room temperature of 24C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the experimental animals were divided randomly in to 5 groups (n=6). The experimental protocol was approved by the Institutional Animal Ethical Committee.[11]

Chemicals

Glibenclamide and Alloxan were obtained from Sigma-Aldrich, Bangalore. Petroleum ether and Ethanol, chloroform and all other reagents used were of analytical grade. Sod, Catalase, GPX were obtained from thermal diagnostics Ltd., and Span diagnostics Ltd.

Instruments

UV-Visible spectrophotometer (Analytical systems, Model no: AUV 2060), electronic balance (Shimadzu, Model no: DS-852 J), homogenizer (Ever shine, Model no: 607) and Cooling centrifuge (Remi, Model no: C-24 BL).

Acute toxicity study

Acute toxicity studies were conducted by following OECD 423 guidelines for safe dose administration to animals the results of cute toxicity study revealed that LD50 values of hydro alcoholic extract of Diospyros malabarica were high and apparently showed the safety of extracts. The treatment of rats with hydro alcoholic extract of Diospyros malabarica did not change the autonomic or behavior response in rats [12]. The zero % mortality for hydro alcoholic extract of Diospyros malabarica was found at the doses of 2000mg/kg. Hence 1/10th of the dose was considered as therapeutic dose for the evaluation of antidiabetic activity of Evaluation of Anti-Diabetic Activity Of Diospyros malabarica Leaves In Low Dose Alloxan and High Fat Diet Induced Diabetes In Wistar Rats [13].

Alloxan Induced diabetes

Diabetes was induced by a single i.p dose of 100-120 mg/kg of Alloxan (2, 4, 5,6 tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) (S.D Fine – Chem. Ltd., Mumbai, India), in sterile saline. Induces permanent diabetes. In rats used in the dose of 150 mg/kg [14].

Experimental procedure

Adult Wistar rats weighing 150- 200g were used for the study. The animals were divided in to 5 groups of 6 each. Group-I served as normal healthy control. Group-II (Diseased control). Group-III (Standard) diabetic rats given Glibenclamide (150mg/kg i.p). Group-IV (Test-1) diabetic rats given Diospyros malabarica extract (HAEM) (200 mg/kg body weight). Group-V control rats given Diospyros malabarica extract (HAEM) (400 mg/kg body weight). The crude extract was administered for a period of 30 days.[15]

Collection of blood samples

The blood samples were collected from the retro-orbital venous plexus of rats without any coagulant for the

separation of serum. After collecting the blood in micro centrifuge tubes they were kept for 1 hr at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min and stored until analyzed for various biochemical parameters.[16]

Estimation of parameters

Serum glucose was estimated by GOD/POD method of in vivo parameter of GSH (Glutathione), CAT levels and Histopathlogical study was assayed.

3. Results and Discussion

For the present study, the rats were divided into five groups and treatment was given for a period of 28days. Diabetes mellitus was induced by using High fat diet and low dose of Alloxan (100mg/kg, i.p) at last two consecutive days. After 48hrs, of induction the biochemical parameters were assessed and the results obtained are discussed below. The preliminary phytochemical tests performed on HAEDM were found to be Tannins, phytosterols, saponins and Alkaloids, phenols, proteins, steroids.

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals [16]. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants such as Super oxide dismutase (SOD), Catalase (CAT), Reducing Glutathione (GSH) are terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions [17].

Heart was excised at the end of experimental and prepared homogenate assess in-vivo parameters. Alloxan and high fat diet treated (control) group rats showed significant ($p<0.0001$) decreasing of SOD levels when compared with normal animals. Rat treated with standard Glibenclamide showed significant ($p<0.001$) increase of antioxidant levels SOD and significantly when compared with the control group and Rats were pre-treated with HAEDM showed significant ($p<0.001$) increase of SOD levels when compared with the disease control group was shown in table 1 & Fig. 1.

Heart homogenate was prepared and assess the Catalase levels in homogenate. Alloxan and high fat diet treated (control) group rats showed significant ($p<0.001$) decreasing of CAT levels when compared with normal animals. Rat treated with standard Glibenclamide showed significant ($p<0.001$) increase of antioxidant levels CAT and significantly when compared with the control group and Rats were pre-treated with HAEDM showed significant ($p<0.001$) increase of CAT levels when compared with the disease control group was shown in Table no.2

Pancreas homogenate was prepared and assess the Glutathione levels in homogenate. Alloxan and high fat diet treated (control) group rats showed significant ($p<0.001$) decreasing of GSH levels when compared with normal animals. Rat treated with standard Glibenclamide showed significant ($p<0.001$) increase of antioxidant levels GSH and significantly when compared with the control group and Rats were pre-treated with HAEDM showed significant ($p<0.001$) increase of GSH levels when compared with the disease control group was show in Table 3 & Fig 3.

Table 1: Effect of HAEDM on SOD levels

S.No	Groups	SOD (U/mg protein)
1	Normal	19.87 ± 0.47
2	Disease Control	6.66 ± 0.66$^{\#\#\#}$
3	Standard	16.83 ± 0.47***
4	Haess (200mg/kg)	14.00 ± 0.57**
5	Haess (400mg/kg)	15.67 ± 0.49***

Values were expressed as mean ± S.E.M of 6 observations, ### Indicates $p<0.001$ when compared to respective normal group,*** Indicates $p<0.001$ when compared to respective disease control group,**Indicates $p<0.01$ when compared to respective disease control group

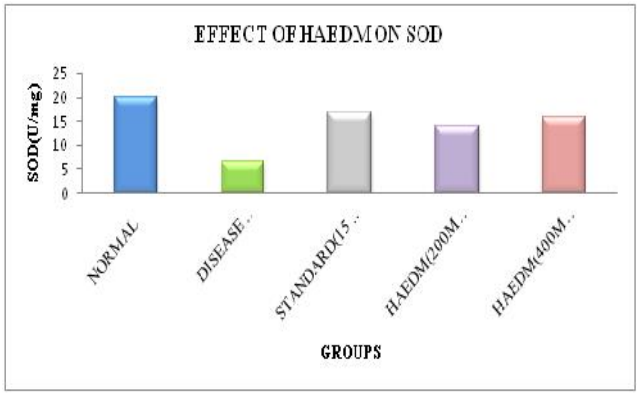


Figure 1: Effect of HAEDM on SOD levels

Values were expressed as mean ± S.E.M of 6 observations, ### Indicates $p<0.001$ when compared to respective normal group, *** Indicates $p<0.001$ when compared to respective disease control group, **Indicates $p<0.01$ when compared to respective disease control group

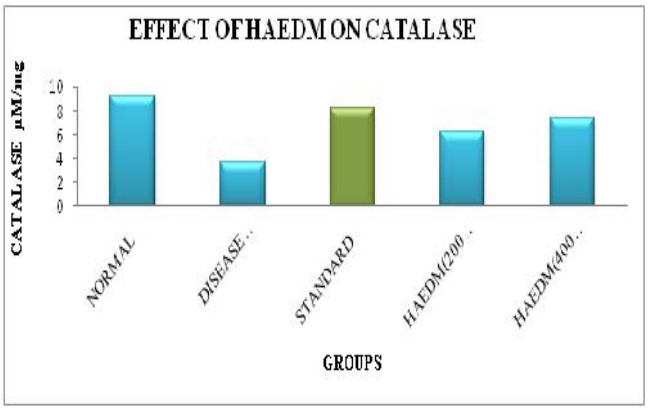


Figure 2: Effect of HAEDM on CATALASE levels

Values were expressed as mean ± S.E.M of 6 observations,### Indicates $p<0.001$ when compared to respective normal group,*** Indicates $p<0.001$ when

compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group

Table 2: Effect of HAEDM on CATALASE levels

S.No	Groups	Catalase (µM H_2O_2 consumed/mg protein)
1	Normal	9.16 ± 0.47
2	Disease Control	3.66 ± 0.33###
3	Standard	8.16 ± 0.30***
4	HAEDM (200mg/kg)	6.16 ± 0.30**
5	HAEDM (400mg/kg)	7.33 ± 0.33***

Values were expressed as mean ± S.E.M of 6 observations ### Indicates p<0.001 when compared to respective normal group,***Indicates p<0.001 when compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group.

Table 3: Effect of HAEDM on GSH levels

S.No	Groups	GSH (µM of GSH/mg protein)
1	Normal	9.01 ± 0.21
2	Disease Control	4.01 ± 0.29###
3	Standard	7.96 ± 0.15***
4	HAEDM (200mg/kg)	6.25 ± 0.07**
5	HAEDM (400mg/kg)	7.01 ± 0.20***

Values were expressed as mean ± S.E.M of 6 observations,### Indicates p<0.001 when compared to respective normal group,*** Indicates p<0.001 when compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group

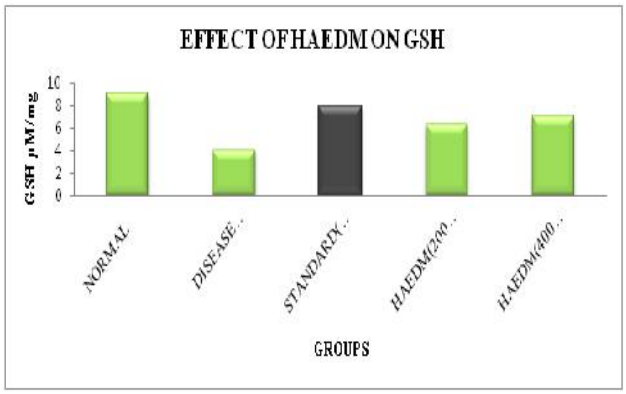


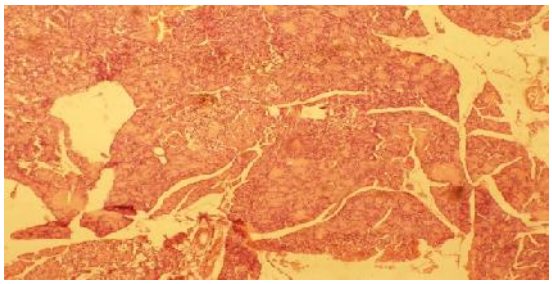
Figure 3: Effect of HAEDM on GSH levels

Values were expressed as mean ± S.E.M of 6 observations,### Indicates p<0.001 when compared to respective normal group,*** Indicates p<0.001 when compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group

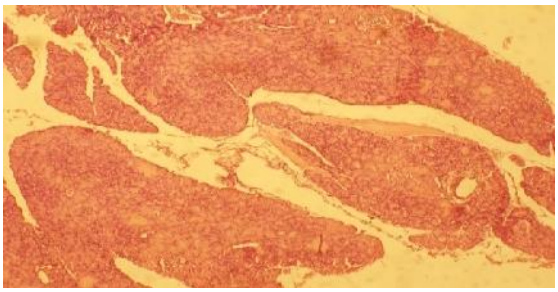
Histopathological Studies

Histopathological studies, pancreas obtained from the excised heart was immediately fixed in 10% buffered neutral formalin solution[18]. The Histological examination of the pancreas shows the necrosis of the islet tissues with the alveolar cells moderately destroyed; there was also moderate congestion of the blood vessels (Figure 1) in the diabetic non-treated group. In the extract-treated group, the architecture of the pancreas appeared intact. The interlobular, intraocular and the alveolar granules were seen (Figure 2). There was slight necrosis of the pancreas around the islet tissues in the glibenclamide-treated group (Figure 3). Microscopically examination of pancreatic section of diabetic non-treated group (Figure 4) showed various degrees of pathological changes such as centrilobular fatty degeneration, cloudy swelling, and vacuolar change of the hepatocytes as well as necrosis of hepatic cells [19]. Microscopically examination of liver section of diabetic extract treated control group (Figure 5) showed normal arrangement of hepatocytes with clear broad of central vein at portal layer [20].

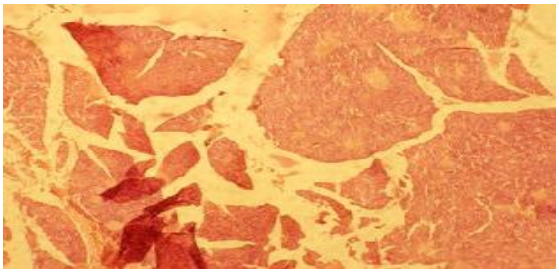
Histopathology of Pancreas



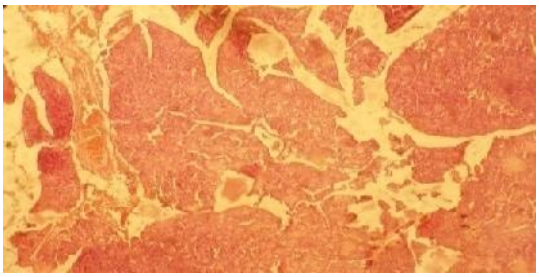
Control (Group-1)



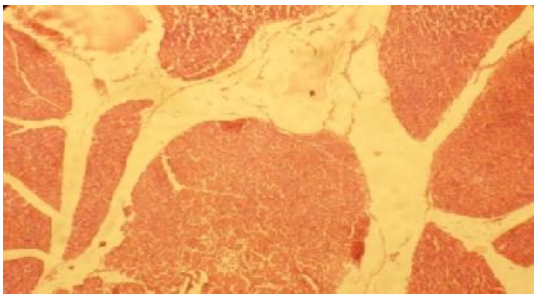
Diabetic control (Group-2)



Standard (Group-3)



Low Dose (HAEDM, 200mg/kg) (Group-4)



High Dose (HAEDM 400mg/kg) (Group-5)

Statistical Analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Bonferroni's test using computer based fitting program (Prism graph pad 6.0). Statistical significance was set accordingly [19].

4. Conclusion

While the antidiabetic effect of the extract may derive from its hypoglycemic effect, the mechanisms of the hypoglycemic effect are yet to be elucidated [21]. The hypoglycemic effect in normal and diabetic rats suggests an insulin-like effect probably mediated via peripheral glucose consumption [22]. Also, postprandial hyperglycemia is related to postprandial hyper insulinemia and its suppression by the extract suggests an insulin-like effect. Phytochemical analysis of the extract revealed the presence of flavonoids, alkaloids, glycosides and tannins, which are typical plant constituents [23]. Thus, there are chances that any of these constituents may possess anti-diabetic properties [24]. The histological studies also indicated that 150 mg/kg of glibenclamide produced some histological changes whereas the 200 and 400 mg/kg doses of the plant extracts did not produce any histological change [25]. This showed that this plant is not only effective as medicinal agent but also has high safety margin.

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