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EVALUATION OF *MOMORDICA DIOICA* SEED EXTRACT FORALPHA AMYLASE INHIBITORY ACTIVITY AND ANTIOXIDANT POTENTIAL IN RAT MODEL

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ABSTRACT

The effect of ethylacetate extract of *Momordica dioica* seeds by *in-vitro* alpha amylase assay and *in vivo* anti-oxidant potential in streptozotocin induced diabetic rats was studied. Type II diabetes was induced in Wistar rats by a single intraperitoneal injection of streptozotocin (STZ) at the dose of 30 mg/kg body weight. *In vitro* α-amylase activity was performed by using dinitrosalicylic acid. Experiment for *in vivo* antioxidant effect was carried out after a continuous treatment with ethylacetate extract for a period of 15 days at the dose of 100 and 200 mg/kg body weight. Serum glucose levels were estimated by GOD-POD method, and ethylacetate extract of *Momordica dioica* seeds at the dose of 200 mg/kg body weight showed significant reduction in serum glucose levels at 2hrs, 4 hrs, 6 hrs and 8 hrs respectively in STZ-induced diabetic rats ($^{a}p<0.001$). In *in vitro* α-amylase assay, there was a significant inhibition with IC_{50} of 58±0.091 µg/ml in comparable to the standard Acarbose. Treatment for a period of 15 days showed significant elevation of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). ($^{a}p<0.001$). The results revealed that the *Momordica dioica* seeds proved to possess potent α-amylase, anti-diabetic and antioxidant activities.

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INTRODUCTION

Momordica dioica Roxb. Ex. Wild is a perennial, dioceous climbing creeper belonging to the family Cucurbitaceae. Its commonly called as Parora, kakora originated in the Indo –Malayan region [1, 2]. Fruits of plant are green and generally used as vegetable. It possesses many medicinal properties like diuretic, alexiteric, stomachic, laxative, hepato protective, and has anti-venum property. It is also used to cure asthma, leprosy, excessive salivation, prevent the inflammation caused by lizard, snake bite, elephantiasis, fever, mental disorders, digestive disorders and troubles of heart and to treat discharge from mucous membrane [3, 4]. Fresh fruit juice is prescribed for hypertension. Tender fruits are rubbed on skin for pimples and acne. Seeds are roasted and taken for eczema and other skin problems. Leaves of the plant are anti-helminthic, aphrodisiac. They are also used to cure tridosha, fever and alter pitta, jaundice, asthma, bronchitis, piles, hepatic damages, mental digestive disorders, bleeding piles bowel affection and urinary complaints. Roots of the *Momordica dioica* are full of medicinal values. Juice of root is stimulant; astringent, antiseptic .The mucilaginous tubers are ant-ihelmintic, spermicidal, anti-fertility abortificant, used in case of bleeding piles, similar bowel afflictions and urinary complaints [5]. It has been reported that *M.dioica* fruit extract showed anti-ulcer potential of hydro-alcoholic extract in pylorus ligation induced ulcer model in wistar rats [6]. It has been evaluated that the *M.dioica* fruit aqueous extract exhibited antioxidant activity in alloxan insulted diabetic rats [7].

Also the fruits of the *M.dioica* possessed in vitro antioxidant and nephro protective activity of ethylacetate and ethanol in albino wistar rats. However, still more scientific evaluation is necessary to be a potent anti-hyperglycemic agent in diabetes.

Streptozotocin is a nitrosurea compound produced by Streptomyces achromogenes, which specially induced DNA strand breakage in β-cells causing diabetes mellitus. It causes destruction of pancreatic β-cells by probably through generation of oxygen free radicals [8]. There were decreased levels of antioxidant enzymes, superoxide dismutase and catalase in streptozotocin induced diabetic rats. Hence, our aim is to study the potentiality of anti-diabetic agent that overcomes the oxidative stress in diabetes. Metformin (50 mg/kg p.o) was used as standard oral hypoglycemic agent.

MATERIALS AND METHODS:

Collection of Plant material and extraction:

Fresh fruits of *M. dioica* (5 kg) were collected locally, during July-August, 2011 and seeds were removed mechanically and dried under shade. They were identified and authenticated by SriVenkateshwara University, Botany department, Tirupati. The seeds were powdered in electric grinder. The powder was subjected to ethylacetate extraction, in Soxhlet apparatus and was run about 10 cycles. After filtration through Whatman filter paper, the filtrates were dried in desiccator.

Chemicals:

Streptozotocin was purchased from Sisco Research laboratories pvt ltd.Acarbose was purchased from Glucobay, Bayer Pharma, India. Metformin was a gift sample from Ranbaxy pvt ltd, Mouhali, Punjab, India. Malanoldehyde, 1, 1, 3, 3-tetra ethoxypropane,thiobarbituric acid were purchased from NR chemicals, India. Hydrochloric acid, hydrogen peroxide, sucrose, Kcl, E.D.T.A., phosphate buffer were procured from Rankem, India. Alpha amylase, bovine serum albumin, Starch, Dinitro salicylic acid was procured from Sigma-Aldrich, St. Louis, USA. Nitrobluetetrazoleum was purchased from SRL, India. Methanol was procured from SDFCL, India. Trichloroacetic acid was purchased from Merck, India. All the other chemicals used were of analytical grade.

Preliminary Phytochemical Screening: [9]

Ethylacetate extracts of *M.dioica* (EEMD) was screened for the presence of various phytoconstituents like alkaloids, flavanoids, steroids, tannins, glycosides, triterpenoids and saponins.

Alpha-amylase inhibition assay of EEMD by the DNS procedure [10].

The α-amylase inhibitory activity for EEMD was determined based on the spectrophotometric assay using Acarbose as a reference compound. The EEMD was allowed to react with α-amylase and 2mM of phosphate buffer (pH- 6.9) to give concentrations of 10, 40 and 80 µg/ml. Incubate this reaction mixture for 20 minutes, and add 0.1ml 1% starch solution. The same was performed for the controls where the enzyme was replaced by buffer. After incubation for 5 minutes, 0.5 ml of dinitro salicylic acid reagent was added to both control and test. They were kept in boiling water bath for 5 minutes. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Animals

Animal Protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experimentation on Animals) through its reference no: IAEC/SVCP/2011/006, Dated: 26/7/11. Male Wistar rats, weighing (180-250 gms) were obtained from NIN (National Institute of Nutrition, Hyderabad. The animals were acclimatized to the experimental room at a temperature of 23 ± 2^0 C, controlled humidity conditions (50-55%) and 12 hr light and 12 hr dark cycles.

They were fed with standard food pellets (Hindustan Lever, Hyderabad) and water *ad libitum*.

Acute toxicity studies:

Ethylacetate extracts of *M.dioica* seeds were studied for acute oral toxicity as per OECD (Organization for Economic Cooperation and development, 2001) guidelines No. 423[11]. The extract did not produce any signs of toxicity when given in doses up to 2000 mg/kg by an oral route. Hence, for further studies 100 & 200 mg/kg dose of the extract were selected.

Induction of Experimental Diabetes: [12]

The animals were fasted for 12 h prior to the induction of diabetes. Diabetes was induced by a single intraperitoneal injection (i.p) of a freshly prepared Streptozotocin (STZ) solution (Dose: 30-60mg/kg)in acetate buffer 0.1 M, pH 4.5 to rats. Control rats were received only the buffer. Diabetes was identified by polydipsia, polyuria and by measuring non-fasting blood glucose levels 48 h after injection of STZ. Rats with blood glucose levels of above 250 mg/dl were considered to be diabetic and used for the studies. EEMD (100 and 200 mg/kg in 0.5% CMC) and Metformin (50 mg/kg in Tween -80) were administered to diabetic rats for a period of 15 days and blood sugar level was measured.Blood glucose levels were estimated on 11th day at 0 hr, 1 hr, 2hr, 4 hrs, 6 hrs and 8 hrs by Glucose-oxidase method and absorbance was measured at 505 nm by UV-Spectrophotometer (ELICO-Sl-159) [13]. On 16th day animals were sacrificed and the liver was removed and homogenized for *in vivo* antioxidant activity.

***In vivo* antioxidant activities:**

Lipid Peroxidation is based on the reaction of Malondialdehyde (MDA) one of the products of lipid peroxidation with TBA (Thiobarbituric acid) to form TBARS (Thiobarbituric acid reactive substances), which has a pink color with absorption maxima at 540 nm [14]. Catalase (CAT) and superoxide dismutase (SOD) activity was determined using standard methods [15, 16].

Statistical analysis:

Results are expressed as mean±standard error of the mean. Data was analyzed by one-way Anova followed by Dunnett's tests. All analysis was done using Graph pad prism software 6.0. The p value $^{a}p<0.0001$ was considered to be extremely significant.

RESULTS

Preliminary Phytochemical analysis:

Preliminary Phytochemical analysis showed the presence of alkaloids, flavanoids, steroids, tannins, glycosides, triterpenoids and saponins in ethylacetate extract of *M.dioica* seeds.

Alpha-amylase inhibition assay of EEMD:

The results revealed that EEMD showed a significant inhibition of α-amylase enzyme (Table 1). EEMD showed 6.6±0.006, 26.6±0.006 and 46.3±0.008 of α-amylase enzyme inhibition at concentrations 10, 40 & 80 µg/ml with IC_{50} value of58µg/ml. Acarbose was used as reference compound at concentrations 10, 40 & 80 µg/m,l showed 6±0.005, 27±0.003 and 60.1±0.006 of α-amylase enzyme inhibition and IC_{50} value was 35 µg/ml.

Table 1 Alpha-amylase inhibition assay of ethyacetate extract of *M. dioica*:

Sample	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml
Acarbose	10	6±0.005	
	40	27.0±0.003	35±0.029
	80	60.1±0.006	
EEMD	10	6.6±0.006	
	40	26.6±0.006	58±0.091^{a}
	80	46.3±0.008	

$^{a}p<0.0001$ considered significant, Vs standard. The data was expressed in mean±S.E.M. n=3 in each group.

Effect of EEMD on serum glucose levels:

EEMD (200mg/kgb.w) and Metformin (50 mg/kgb.w) showed significant ($^{a}p<0.001$) fall in blood glucose levels at 2 hrs, 4 hrs, 6 hrs and 8 hrs respectively when compared with diabetic control group (Table 2).

Table 2 Effect of ethylacetate extract of *M.dioica* (EEMD) on serum glucose level in diabetic rats.

Treatment and Dose	Serum glucose levels (mg/dl)					
	0 hr	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
Normal	94.4±3.8	86.5±3.21	91.3±2.03	82.9±0.85	85±0.36	82.6±0.95
Diabetic control (DC)	268.5±5.5^{a}	272.2±3.95^{a}	280.9±3.26^{a}	281±4.78^{a}	275.8±1.14^{a}	271.3±1.14^{a}
DC+EEMD (100 mg/kg)	295.1±7.3	275±6.5	277.5±10	270±3.65	271.1±0.87	269±0.85
DC+EEMD (200 mg/kg)	298.1±9.31	245.8±7.19	207.5±1.88^{a}	182.5±2.66^{a}	173±0.57^{a}	171.5±1.17^{a}
DC+Metformin (100 mg/kg)	280±7.3	262±3.45	152.3±4.08^{a}	138.3±1.82^{a}	137±0.51^{a}	129.1±0.98^{a}

$^{a}p<0.0001$ considered significant, Vs standard. The data was expressed in mean±S.E.M. n=6 in each group.

***In vivo* antioxidant activity:**

Table 3 shows the effect of administration of EEMD on MDA, CAT and SOD in liver tissue of different groups of rats. There was a significant ([a]p<0.0001) elevation in tissue MDA of 46.33±1.67 nmoles/g in diabetic rats as compared to normal rats. Treatment with EEMD at a dose of 200 mg/kg for 14 days resulted in significant decrease ([a]p<0.0001) in liver tissue MDA with 27.75±2.52 nmoles/g. Catalase & Superoxide dismutase in diabetic control rats was significantly ([a]p<0.0001) depleted in liver tissue when compared to normal rats. EEMD treatment at a dose of 200 mg/kg significantly ([a]p<0.0001) restored CAT & SOD levels with 9±1.06 and 29.6±1.22 U/mg of protein as compared to diabetic control rats.

Table 3 Effect of ethylacetate extract of M.dioica (EEMD) on MDA, CAT and SOD levels in liver homogenates of STZ-induced diabetic rats.

Treatment	MDA (nmoles/g of tissue)	CAT (U/mg of protein)	SOD (U/mg of protein)
Normal control	14.34±1.37	20.25±1.83	56.5±0.85
Diabetic control (DC)	56.33±1.67[a]	5.167±1.16[a]	17.67±0.88[a]
DC+EEMD(100 mg/kg)	41.33±1.67[b]	6.58±1.33	22.5±0.56
DC+EEMD (200mg/kg)	25.17±1.14[a]	12±0.54[a]	35.5±0.76[a]
DC+ Metformin (50mg/kg)	18±1.48[a]	11.67±0.954[a]	48±0.98[a]

[a]p<0.0001 considered significant, Vs standard. The data was expressed in mean±S.E.M. n=6 in each group.

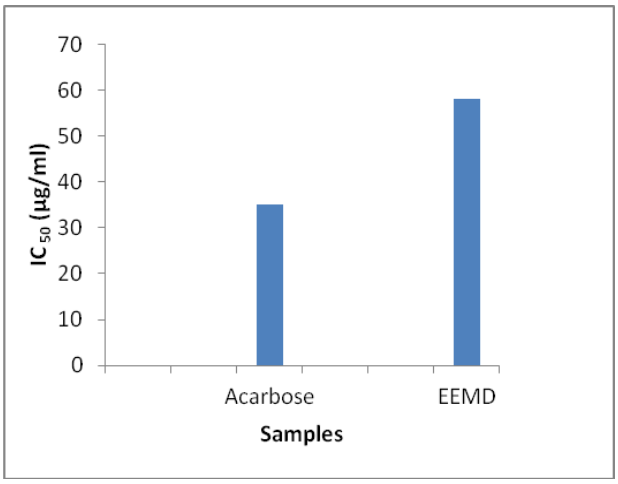


Fig. 1. Effect of EEMD on alpha amylase activity.

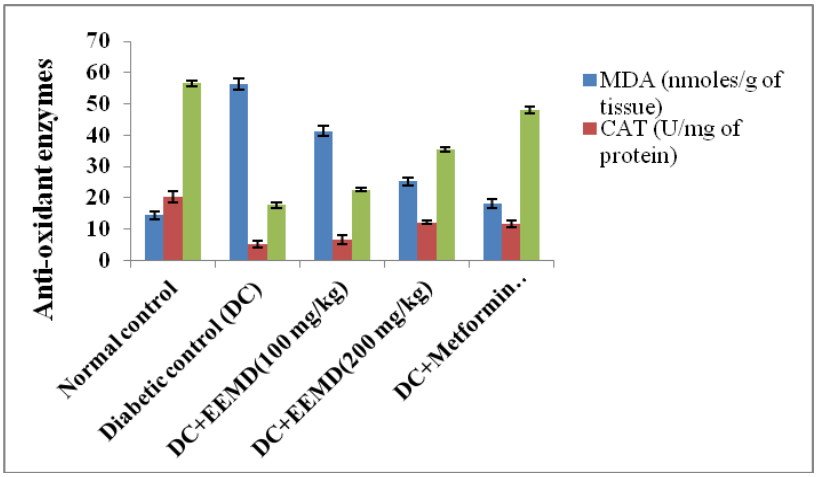


Fig. 2. Effect of ethylacetate extract of *M.dioica* (EEMD) on MDA, CAT and SOD levels in liver homogenates of STZ-induced diabetic rats.

DISCUSSION

In the present study the effects of EEMD on oxidative stress in STZ-induced diabetic rats was examined. The mechanisms behind free radicals and the possible role of oxidative stress in the pathogenesis of diabetes and diabetic complications have been extensively studied for years in patients and animal models [17]. Diabetics and experimental models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which causes depletion of antioxidative defense system and promotes de novo generation of free radicals [18]. Streptozotocin (STZ) is most commonly used to induce diabetes in rats. This causes the death of pancreatic β-cell by alkylation of DNA resulting in reduced synthesis and release of insulin. This results in fragmentation of DNA by means of production of reactive oxygen species. [19] STZ selectively destroys the pancreatic cells that secrete insulin, which causes less active pancreatic cells and produces diabetes mellitus [20]. The toxic effect of STZ is not restricted to abolishment of pancreatic β-cells but also cause oxidative stress [21]. The STZ induced diabetic rats showed significant increase in blood glucose levels. STZ induction is associated with formation and accumulation of free radicals which leads to a number of deleterious effects [22] .Continuous exposure of the system to free radicals results in decreased activities of SOD and catalase.

One of the therapeutic approaches for decreasing post-prandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes for example alpha-amylase in the digestive organs. We therefore investigated the inhibitory effects of ethylacetate extract from *M.dioica* seed on alpha-amylase. Many plant extracts and natural products suppress the glucose production from carbohydrate in the gut or glucose absorption from the intestine. Inhibition of alpha amylase enzyme in the digestive tract of human is being considered to be effective in controlling diabetes by decreasing the absorption of glucose from starch [23]. The results showed that ethylacetate extract of *M.dioica* showed more percentage inhibition on alpha amylase activity, which may be due to the presence of potential α-amylase inhibitors (alkaloids, flavanoids, terpenoids or glycosides). The results presented in Table 2 indicated that *Momordica dioica* seed extract produced anti-hyperglycemic activity in a dose dependent manner. Further at a dose of 200 mg/kg the extract produced significant anti-hyperglycemic activity when compared with the reference compound metformin. Here the glucose lowering activity of *Momordica dioica* may be attributed to both pancreatic (enhancement of insulin secretion) and extra pancreatic (peripheral utilization of glucose) mechanisms.

The present data indicates that STZ-induced diabetes disrupts the actions of antioxidant enzymes. The decreased activities of these enzymes may be due to the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH) that reduced the activity of these enzymes [24,25]. In the present study, EEMD potentiated the *in vivo* antioxidant activities. Elevation of LPO (lipid peroxidase) is attributed to the enhanced production of the reactive oxygen species. In the present study, we observed a MDA formation, the index of lipid peroxidation, was significantly increased in the liver of STZ-treated animals. EEMD supplementation potentially reduced MDA level, suggesting that EEMD might have antioxidant principles to produce such a response. CAT & SOD protects the cellular system against the toxic effects of lipid peroxidation. A marked depletion in both the enzymes of a liver was observed in diabetic control rats shown in Table 3.Furthermore, EEMD treatment showed a significant restoration in CAT & SOD of diabetic rats.

CONCLUSION

The findings of the present study showed positive effect of *M.dioica* on rats with STZ-induced disturbances in the glucose level and the antioxidant status. Thus, EEMD is beneficial in the control of diabetes and oxidative stress by activation of enzymatic antioxidants. The study indicated that *M.dioica* may have active principle(s) which are responsible for the potent anti-diabetic activity. However, more efforts are still required for the isolation, characterization and biological evaluation of the active principle (s) of the *M.dioica* seed extract. Further studies can be carried out for more exploration on mechanism of drug action.

CONFLICT OF INTERESTS

There is no conflict of interests from authors.

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REFERENCES

1. Rashid M M. Bangladeshi shabjee.1st ed. Bangla academy. Dhaka, Bangladesh. 1976; 494.
2. Singh A K. Cytogenetics and evaluation in the cucurbitaceous In ; Bats D M, Robinson R W, and Jaffy C, (eds) Biology and utilization of cucurbitaceae .Comstock Publishing Associates, Cornell Univ. Press ,Ithaca , New York and London.1990; 10.
3. Kirtikar K R, Basu B D. Indian Medicinal Plants. International Book Distributors, Dehradun. 1999; 11-29.
4. Kirtikar K R, Basu B D.Indian Medicinal Plants. LalitMohan Basu, Allahabad, India. 1981;11.
5. Satyavati GV, Gupta A K, Tandon N.In Medicinal Plants of India, 1987; ICMR New Delhi. 267.
6. 6) Vijayakumar M, BavaniEswaran M, Oha S K, Rao C H V, Rawat A K S.Antiulcer activity of Hydroalcoholic extract of *Momordica dioica* roxb. Fruit, Indian Journal of Pharmaceutical sciences. 2011:73-79.
7. Jain A, Singhai A K. Effect of *Momordica dioica*. Roxb on gentamicin model of acute renal failure. Nat Prod Res. 2010:137-139.
8. Shanmugasundaram E R V, Baskaran K. Use of *Gymnema sylvestre* leaf extract in the control of blood glucose in insulin dependent diabetes mellitus. J Ethnopharmacol. 1990; 281-290.
9. Khandelwal K R. Practical Pharmacognosy. Techniques and Experiments.NiraliPrakashan, Pune, 19th edition. 2008.

10. Bernfeld P,Amylase, α and β in methods in Enzymology, S. P Colowick and N O Kaplan, Edited by Academic press, New York, USA. 1955; 149.
11. OECD guidelines for testing of chemicals. Acute Oral Toxicity-Acute Toxic Class Method
12. 2001; 423.
13. Vogel H G, Vogel W H. Chapter K: Anti-diabetic activity, Drug Discovery and Evaluation, Pharmacological Assays. New York edited by Springer Publication. 2002; 535-540.
14. Trinder P. Enzymatic determination of blood glucose. Ann ClinBiochem. 1969; 24.
15. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by Thiobarbituric acid reaction,Annal Biochem. 1979; 351-355.
16. Aebi H.Catalase. In Bergmeyer Hu (ed) Methods in enzymatic analysis. Academic press: New York. 1983; 276.
17. Kono Y. Generation of superoxide radical during auto oxidation of hydroxylamine and an assay for superoxide dismutase, Arch Biochem Biophys. 1978; 186-188.
18. Baynes J W. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991; 405-415.
19. Hammers H D, Martin S, Federslin K, Geisen K, Briwnle M. Amino guanidine treatment inhibit the development of experimental diabetic retinopathy, Proc Natl Acad Sci, U S A 88.1991; 115-55.
20. Mahimainathan L, Das E, Venkatesan B, Choudhury G G. Mesengial cell hypertrophy by high glucose is mediated by down regulation of the tumour suppressor PTEN. Diabetes. 2006; 21-25.
21. Gilman AG, Rall T W, Nies A S, Tayer P S. Eds., Goodman and Gilman's the Pharmacological Basis of Therapeutics, edited by Pergoman Press. 1990. New York, NY, USA.
22. Lei Y C, Hwang J S, Chan C C, Lee C T, Cheng T J. Enhanced oxidative stress & endothelial dysfunction in Streptozotocin diabetic rats exposed to particles, Environ. Res. 2005: 35-40.
23. Santhakumari P, Prakasam A, Pugalendi K V. Modulation of oxidative stress parameters by treatment with *Piper betle* leaf in streptozotocin induced diabetic rats, Indian J Pharmacol. 2003 ; 373-380.
24. Hara Y, Honda M. The inhibition of α-amylase by tea poly phenols. Agric Biol Chem.1990; 1939-45.
25. Kaleem M, Sheema S, Bano B. Protective effects of *Piper nigrum* and *Vinca rosea* in alloxan induced diabetic rats, Indian Journal of Physiology and Pharmacology. 2005; 49-65.
26. Vincent A M, Russel J W, Low P, Feldman E L. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocrine Review. 2004: 6-12.



