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EVALUATION OF ANTIOXIDANT PARAMETERS OF *MUKIA MADERASPATANA*

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ABSTRACT: The present study was an attempt to investigate the effect of extracts and fractions of *Mukia maderaspatana* on glycemia, lipid profile, lipoprotein level and antioxidant profile in STZ induced diabetic rats for 21 days. Diabetes was induced using streptozotocin (50 mg/kg i.p) and after the induction of diabetes the animals were given with MMEE (100 mg/kg, 200 mg/kg), MMCF (100 mg/kg) and MMBF (100 mg/kg) orally for 21 days. Blood glucose levels were determined by using GOD-POD method with diagnostic kits. The lipid and lipoprotein level was estimated by using the respective kits. The administration of the extracts orally for 21 days showed that there was an amelioration of the lipid and lipoprotein levels significantly. After 21 days the parameters like HDL, LDL, VLDL, TC, TG, Albumin, Creatinine, total protein and glucose were estimated. The treatment with the extracts and fractions of *Mukia maderaspatana* improved the lipid level and lipoprotein level to a normal condition which may be attributed to its potent antidiabetic activity. The levels of urea and creatinine were significantly decreased after the treatment of STZ diabetic rats with MMEE, MMCF, and MMBF. The treatment of diabetic rat with *Mukia maderaspatana* caused a noticeable elevation in serum total protein and albumin levels as compared with normal levels. The treatment with MMEE, MMCF, and MMBF ameliorated the changes induced by STZ.

INTRODUCTION: Diabetes is a condition primarily defined by the level of hyperglycemia giving rise to a risk of microvascular damage (retinopathy, nephropathy, and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes-related microvascular complications, increased risk of macrovascular complications (ischemic heart disease, stroke, and peripheral vascular disease) and diminished quality of life.

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Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to severe damage to many of the body's systems, especially the nerves and blood vessels.

Over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received a scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended

that traditional medicinal herbs be further investigated. Herbal medications are the most commonly used alternative therapy for blood sugar control; however, their safety and efficacy need to be further evaluated by well-designed, controlled clinical studies.

In the Indian system of medicine, *Mukia maderaspatana* was used as a bitter, sweet, refrigerant, carminative, sudorific, expectorant, anodyne and tonic. It is also used in vitiated conditions of pitta, burning sensation, dipsia, flatulence, colic, constipation, ulcers, cough, asthma, neuralgia, nostalgia, odontalgia and vertigo [36]. But the pharmacological and scientific evidence for its antidiabetic effect is yet to be proved. So based on the above fact, it can be evaluated for antidiabetic and antioxidant property in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS:

Chemicals Used: STZ, diagnostic kits, MMEE (100 mg/kg and 200 mg/kg), MMCF (100 mg/kg) and MMBF (100 mg/kg). The doses were selected based on the acute toxicity studies under OECD guidelines 423. The acute toxicity study was carried out for 14 days, and there were no signs of toxicity.

Extraction and Fractionation of the Plant: The plant was collected from the forests of polavaram in WG district of Andhra Pradesh and was authenticated by the botanist. The entire plant was dried and made into a coarse powder and then extracted using soxhlet apparatus with ethanol as solvent, and the menstruum was dried by evaporating ethanol, and the solid was taken and was then fractionated using chloroform and n-butanol.

Animals Used: Healthy, adult Wistar rats of both sexes (180-220g), were obtained from the central animal house. The animals were kept in a well-ventilated room, and the animals were exposed to 12 hrs day and night cycle with a temperature between 20 ± 3 ºC. The animals were housed in large spacious, hygienic polypropylene cages during the experimental period. The animals were fed with water and rat feed *ad libitum*, supplied by this institution. All the experiments were performed after obtaining prior approval from IAEC with number 1581/PO/a/11/CPCSEA.

Induction of Diabetes: Non-Insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 50 mg/kg streptozotocin. The elevated glucose levels confirmed hyperglycemia in plasma, determined at 72 h. The rats with permanent NIDDM (250-350 mg/dL) were used for the study.

EXPERIMENTAL MODELS:

Oral Glucose Tolerance Test (OGTT): [47] The oral glucose tolerance test was performed in overnight fasted (18 h) normal animals. Rats divided in to six group (n-6) were administered with 10 mg/kg Glibenclamide, 100 mg/kg, 200 mg/kg ethanolic extract, 100 mg/kg chloroform fraction, and 100 mg/kg n-butanol fraction, respectively. Glucose (2 g/kg) was fed 30 min. after the administration of extracts. Blood was withdrawn from the retro-orbital sinus under ether inhalation (to minimize the distress) at 0, 30, 60, 90, and 120 min. of extract administration. The fasting blood glucose levels were estimated by glucose oxidase-peroxidase method.

STZ Induced Diabetic Model: The Wistar rats weighing 180-220 gm of either sex were used for the experimental study. The animals were divided into seven groups of 6 animals each.

Grouping of the Animals:

Group I	Untreated Control
Group II	Diabetic control
Group III	Positive control (Glibenclamide 10 mg/kg b.w i.p)
Group IV	MMEE 100 mg/kg, p.o
Group V	MMEE 200 mg/kg, p.o
Group VI	MMCF 100 mg/kg, p.o
Group VII	MMBF 100 mg/kg, p.o

The test drug was administered for 21 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100 mg/kg each of two successive fractions made in aqueous and given by orally. The blood was collected by sinuous orbital under light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 min. Body weight, urine sugar, glucose was analyzed every week, and fluid intake was analyzed every day and lipid and lipoprotein profile from serum and tissue homogenate (TC, TG,) were analyzed after 21 days [29, 30]. Total protein, albumin, creatinine, urea were also analyzed by serum.

On the day of termination of the study, the animals were sacrificed; liver and kidney were excised and stored in 10% buffered neutral formalin for histopathological studies.

RESULTS AND DISCUSSION: The present study was an attempt to investigate the effect of extracts and fractions of *Mukia maderaspatana* on glycemia, lipid profile, lipoprotein level and antioxidant profile in STZ induced diabetic rats. The phytochemical screening showed the presence of alkaloids, tannins, terpenes, phenols, flavonoids which are responsible for the antidiabetic activity and also for free radical scavenging activity.

The goal of blood glucose tests is to find out whether there is the availability of large amounts of glucose in the blood. The combination of increased hepatic glucose production and reduced metabolism in peripheral tissues leads to elevated plasma glucose levels. [41] The treatment with MMEE, MMCF, and MMBF in STZ induced diabetic rats significantly decreased the elevated serum glucose levels from first week onwards.

To check the safety profile of the extract/fractions, it was subjected to acute toxicity study which confirmed the absence of any toxicity or mortality at a higher dose of 2000 mg/kg. Thus, the extract can be classified into the safe drug category according to the "Global Harmonized Classification System" quoted in the OECD guidelines 1996. Based on the acute toxicity studies four dose levels were selected for the evaluation of various pharmacological properties *i.e.*, MMEE (100 mg/kg, 200 mg/kg), MMCF (100 mg/kg) and MMBF (100 mg/kg) [54]. The antioxidant enzymes such as superoxide dismutase, catalase, and glutathione reductase and malonaldehyde act as protective enzymes in the defense system. Increased levels of MDA, SOD and decreased levels of GSH and CAT in the diabetic state may be due to inactivation caused by reactive oxygen species. In the present study, the levels of both GSH and CAT were significantly increased and MDA, SOD was significantly decreased after 21 days treatment of MMEE, MMCF AND MMBF as shown in **Table 1** and **Table 2** [36].

TABLE 1: EFFECT OF MMEE, MMCF, AND MMBF ON *IN-VITRO* ANTIOXIDANT PARAMETER

S. no.	IC_{50} Value ± S.E.M. (µg/mL)					
	Extract/ Compound	Hydrogen peroxide	Nitric oxide	Lipid-peroxidation	Deoxyribose	Superoxide
1	MMEE	330.42 ± 0.220	801.00 ± 0.520	48.660 ± 0.166	45.88 ± 0.145	298.54 ± 0.273
2	MMCF	387.28 ± 0.360	>1000	64.106 ± 0.578	72.01 ± 0.830	501.66 ± 0.270
3	MMBF	285.46 ± 0.414	765.62 ± 0.311	47.230 ± 1.514	41.28 ± 0.394	247.84 ± 0.542
	Standard					
	Rutin	36.23 ± 0.015	68.300 ± 0.152	-	-	1000.20 ± 0.200
	αTocopherol	-	-	91.380 ± 0.198	-	-
	Ascorbic acid	-	-	-	11.24 ± 0.003	-

Means (-) not done; Average of three determinations

TABLE 2: EFFECT OF MMEE, MMCF, AND MMBF ON *IN-VIVO* ANTIOXIDANT PARAMETER FROM PANCREAS HOMOGENATE

S. no.	Treatment	GSH (µg/mg of protein)	SOD (unit/min/gm tissue)	CAT (µmol of H_2O_2/min/gm tissue)	MDA (µg/mg of protein)
1	Untreated control	19.30 ± 0.720	0.657 ± 0.158	3.686 ± 0.104	3.816 ± 0.043
2	Diabetic control	10.54 ± 0.383 ##	0.866 ± 0.271#	1.474 ± 0.076###	8.692 ± 0.216###
3	Diabetic + Glibenclaminde (10 mg/kg)	15.05 ± 1.350 **	0.500 ± 0.111*	3.785 ± 0.088***	4.15 ± 0.18***
4	Diabetic + MMEE (100 mg/kg)	14.88 ± 0.114 **	0.137 ± 0.038*	2.502 ± 0.104***	4.50 ± 0.092***
5	Diabetic + MMEE (200 mg/kg)	17.192 ± 0.283 **	0.173 ± 0.037*	3.118 ± 0.045***	3.87 ± 0.05***
6	Diabetic + MMCF (100 mg/kg)	14.29 ± 0.319**	0.250 ± 0.087*	2.515 ± 0.051***	4.69 ± 0.05***
7	Diabetic + MMBF (100 mg/kg)	13.58 ± 0.1037**	0.12 ± 0.033*	2.58 ± 0.082***	6.53 ± 0.07***

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01,*P<0.05 as compared to diabetic control.
P<0.001, ##P<0.01, #P<0.05 as compared to untreated control.

Alpha-amylase catalyzes the hydrolysis of alpha-1,4-glucosidic linkages of starch, glycogen and various oligosaccharides and alpha-glucosidase further breaks down the disaccharides into simpler

sugars readily available for intestinal absorption. The inhibition of these enzymes is effective in controlling diabetes mellitus by diminishing the absorption of glucose decomposed from starch. The treatment with MMEE, MMCF, and MMBF significantly decreased the enzyme alpha amylase ($P<0.01$) as shown in **Table 3** [37].

TABLE 3: EFFECT OF ALPHA-AMYLASE ACTIVITY FROM SERUM

S. no.	Treatment	Alpha-amylase (mg/dL)
1.	Untreated control	247.00 ± 12.89
2.	Diabetic control	119.39 ± 2.59 ###
3.	Diabetic + Glibenclaminde (10 mg/kg)	229.83 ± 6.50 ***
4.	Diabetic + MMEE (100 mg/kg)	290.50 ± 15.24 ***
5.	Diabetic +MMEE (200 mg/kg)	283.67 ± 16.21 ***
6.	Diabetic + MMCF (100 mg/kg)	312.01 ± 6.71 **
7.	Diabetic + MMBF (100 mg/kg)	312.33 ± 10.38 **

Value are expressed in mean ± SEM (n=6).
P<0.001, as compared to untreated control.
** P<0.01, *** P<0.001 as compared to diabetic control.

Histopathology reports of liver and kidney gave additional support to the study. Liver sections of normal animals showed the normal architecture with well brought out the central vein, well-preserved cytoplasm and prominent nucleolus whereas the diabetic group section showed the presence of feathery degeneration, micro, and macrocellular fatty changes and inflammatory cells around portal tract. The other groups showed good protection from STZ induced changes in the liver. The sections of normal rat kidney showed the normal nephro-morphology whereas the diabetic section showed fatty degeneration. The other groups showed the less pathological changes of the kidney [31].

CONCLUSION: From the results, it can be stated that the extracts and fractions of *Mukia maderaspatana* decreased the levels of serum glucose, ameliorated lipids and lipoproteins. It also improved glucose uptake with free radical scavenging properties.

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