INVIVO ANTIOXIDANT ACTIVITY AND ANTI HYPERGLYCEMIC RELEVANT ENZYME INHIBITION PROPERTIES OF PETROLEUM ETHER EXTRACT OF TRADITIONALY PROCESSED OCIMUMCANUM LEAVES

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ABSTRACT

Objective: To investigate the antihyperglycemic and antioxidant properties of the *petroleum ether extract of* Ocimum canum leaves *in streptozotocin-induced diabetic rats.*

Place and Duration of Study: Pinacle Biomedical Research institute (PBRI),Bhopal april 2013-december 2013.

Methods: *Hyperglycemia was induced in rats by streptozotocin (STZ, 65 mg/kg body weight). Three days after STZ induction, diabetic rats received Ocimum extrac tat 100 and 200 mg/kg body weight daily for 28days. Glibenclamide (*600 µgm/kg*) served as reference. Blood glucose levels were measured on every 7th day during 28 days. Serum biochemical parameters* low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), atherogenic index and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)*were estimated.* antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) , serum thiobarbituric (TBAR) were measured in the diabetic rats . *Therefore,* Ocimum canum *demonstrated remarkable antidiabetic activity in STZ induced diabetic rats. The potential antidiabetic action is plausibly due to its modulation of endogenous antioxidant status.*

Results: Aadministration of the extracts for 28 days caused a significant ($P < 0.01$) reduction in blood glucose levels in diabetic mice. . The extracts also improved other altered biochemical parameters associated with diabetes. Furthermore, the extracts have favorable effects on the histopathological changes of the pancreas, in STZ induced diabetic mice. The extracts also showed significant ($P < 0.05$) antioxidant activity.

Conclusions: Ocimum canum possesses antihyperglycemic and antioxidant properties as well improves lipid profile.

Key words: Anti diabetic, ALP, herbal drug, plant extract,antioxidant

INTRODUCTION

Herbal medicines are the oldest remedies known to mankind. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. In many journals, national and international, we find an increasing number of research publications based on herbal drugs. Many analysis-based studies regarding pharmacological research in India have been conducted in the past [1]. Diabetes is a metabolic disorder characterized by hyperglycemia resulting deficiency of insulin secretion by pancreas, ineffectiveness of produced insulin, or both [2]. It is the most important non-infective epidemic to hit the globe in the present millennium. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicated that about 366 million people are likely to be

diabetic by the year 2030[3]. Hyperglycemia can be handled initially with oral synthetic agents and insulin therapy. However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries [4]. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment [5]. In the natural system of medicine many plants have been claimed to be useful for the treatment of diabetes mellitus. The dependence of large rural population on medicinal plants for treatment of diabetes is because of its availability and affordability [6]. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Although a large number of medicinal plants have been tested for their antidiabetic effects, but it remains to be investigated in several other Indian medicinal plants. The excessive oxidative stress is observed in the diabetes [7]. So, the present study was conducted to evaluate antidiabetic, antihyperlipidaemic and antioxidant activities of Ocimum Canum leaves in streptozotocin induced diabetic mice. Ocimum is the most ancient tree of India, generally known as a “sacred plant”. The leaves of Ocimum Canum are used in the treatment of diabetes but there was no significant proof for its antidiabetic efficacy. Also the plant contains many flavonoids and sterols/triterpenoids as its main constituents, which are known bioactive principles for antidiabetic potential [8-9]. Flavonoids are also known to regenerate the damaged -cells in diabetic mice [10-11]. From here it was thought worthwhile to find out the efficacy of the plant.

METHODOLOGY:

2.1Plant material

Leaves of *Ocimum canum were* collected in the month of November 2011 from its natural habitat from nearby Dasapalla forest division, Nayagarh district of Odisha,india. The plant was authenticated by Dr. C. H.V.RAO from National Botanical Research Institute (NBRI)Lucknow. and voucher specimens(NBRI/GU/11-556) .The leaves were cleaned and dried under the shade to avoid degradation of volatile oil.

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2.2.Preparation of plant extracts

The plant material was powdered to coarse powder and extracted with petroleum ether (60-80℃) in Soxhlet apparatus at a temperature not exceeding 60℃.. The extracts were concentrated under reduced pressure in rotary evaporator to yield a crude semi-solid mass. It was then dried and used.

2.3. Preliminary phytochemical screening

A portion of residue from each extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols and triterpenoids in the leaves extracts.

2.4. Animals

Experiments were performed using Swiss albino male mice (25-35 g). Animals were maintained under standard environmental conditions i.e ambient temperature of (22-23)℃, relative humidity 30-70%, an artificial dark and light cychloroform of 12 h each, fed with a standard pellet mice diet from ((golden feed, New Delhi) and water regularly)

2.5. Acute Toxicity Studies-

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 (5). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight 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 1000 mg/kg body weight. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No. - 1283/c/09/CPCSEA) protocols prior to conducting the experiments having Protocol approval reference number is PBRI/12/IAEC/PN-340

2.6-Experimental Design:
In the investigation, a total of 40 rats (32 diabetic surviving rats and 8 normal rats) were taken and divided into four groups of 8 rats each.
Group I: Normal, untreated rats
Group II: Diabetic control rats
Group III: Diabetic rats given petroleum ether extract of *Ocimum Canum* leaf (100 mg/kg of body weight)
Group IV: Diabetic rats given ethanol extract of *Ocimum Canum* leaf (200 mg/kg of body weight)
Group V: Diabetic rats given standard drug glibenclamide (600µg/kg of body weight)

2.7. Biochemical estimation
Collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total cholesterol, serum triglycerides and total protein (14).
Estimation of liver and kidney biochemical parameters
Lipid peroxidation, i.e. thiobarbituric acid reactive substances (TBARS) was estimated and expressed as mM/100 g of tissue (15). Reduced glutathione (GSH) was determined and expressed as mg/100 g of tissue (16). Catalase (CAT) activity was assayed and expressed as µ moles of H2O2 decomposed/min/mg of tissue (17).

2.8. Antioxidant assay
On the 10th day following study, the animals were deprived of food overnight and
Sacrificed by cervical dislocation. The livers were dissected out, washed in ice-cold saline, and patted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris- HCl buffer and processed for estimation of lipid peroxidation by the method of Fraga *et al*. (18).
A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of glutathione by the method of Ellman *et al*. (19). The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4 ℃. The su pernatant thus obtained was used for the estimation of SOD by the method described by Kakkar *et al*.[20} and CAT activity was measured by the method of Maehly *et al*.(21).
2.9. Histopathology
For histopathological study, animals from all groups were anaesthized with mild ether anaesthesia and dissected. pancreas are excised out of the animal's body and put immediately into 10% formalin solution in a stoppered container. These samples were then sent to diagnostic lab fixation (using Bouin's solution), dehydration, embedding (in paraffin), sectioning (with standard microtome) and staining (Haematoxylin or eosin). The slides so prepared were than examined by pathologist and the pictures were clicked with the help of a binocular microscope fixed with a camera.

2.10. Statistical analysis
The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests to determine level of significance. A value of $P<0.01$ was considered significant results are expressed as mean 嶂 SEM
3. Results

Preliminary phytochemical analysis of Ocimum Canum leaves showed the presence of flavonoids, tannins, saponins, sterols and triterpenoids which are known bioactive principles [22]. Flovonoids are also known to regenerate the damaged 毬-cells in diabetic mice [23]. The overall study showed the LD50 of oral toxicity of all extracts to be above 2 000 mg/kg b.w. in mice. So, the extracts are safe for long term administration. The effects of Glibenclamide and petroleumether extracts on blood glucose levels in normal and diabetic mice after treatment of 28 days are shown in Table 1, in which all extracts showed significant reduction ($P<0.01$). It was observed that standard drug glibenclamide lowered the blood glucose levels significantly bringing it back to normal which is an indication of the presence of some β-cells, as glibenclamide is known to stimulate insulin secretion from β-cells.(24). Standard drug and different extracts

showed dose-related reductions in the serum concentrations ofALP,AST,ALT,PRO,CHO,HDL, LDL, and VLDL (Table 2). Percentage anti radical activity of all extracts is summarized in Table 3. All the extracts exhibited good but varying levels of antioxidant activity.

TABLE 1:
Effect of Petrolium ether extract of *Ocimum canum* leaf on blood glucose, after prolonged treatment (mean±SEM)

Group	0 day	7 day	21 day	28 day
Normal	94.5±4.5	97.33±4.46	98.66±3.56	100.33±2.15
Diabetic control STZ 45 mg/kg	329.16±25.50	324.33±24.04	314±22.63	311±22.34
STZ+ OC(Pet ether) low dose 100 mg/kg	275.83±16.16	248.66±15.41	187±7.69	163.33±4.20
STZ+ OC (Pet ether) high dose 200 mg/kg	272.5±16.40	242.33±15.13	181.83±7.29	157.66±4.16
Std group Glibenclamide 600 µgm/kg	291±14.10	236.16±9.82	165.33±2.64	135±2.72

TABLE 2:
Effect of petroleum ether extract of Ocimum canum leaf on the serum lipid profile of normal, diabetic induced and drug treated rats.

	Parameters							
Group	ALT	AST	ALP	PRO	CHO	HDL	LDL	VLDL
Normal	36.76±0.88	57.80±0.59	56.94±0.78	15.86±0.41	80.88±0.27	18.32±0.13	24.49±.7	14.1±.74
Diabetic control	63.63±0.49	90.81±0.81	85.14±0.65	6.43±0.23	115.95±1.35	29.00±0.14	87.22±0.71	37.13±1.5
STZ+ OC(Water) low dose 100 mg/kg	57.68±0.46	74.57±0.53	72.94±0.69	11.71±0.24	87.08±0.65	21.51±0.43	53.09±1.3	30.26±1.1
STZ+ OC(Water) high dose 200 mg/kg	47.57±0.33	65.19±0.52	65.47±0.35	14.95±0.39	82.26±0.29	18.88±0.21	40.31±1.4	24.8±0.75
Std group	43.86±0.53	60.59±0.45	60.63±0.33	15.44±0.30	80.79±0.23	18.59±0.17	36.13±1.4	20±0.94

Each value is SEM of 6 animals, Comparisons were made between normal control to diabetic control: * $p < 0.05$ and comparisons were made between diabetic control to drug treated groups: a $p<0.05$ level

Graph-2

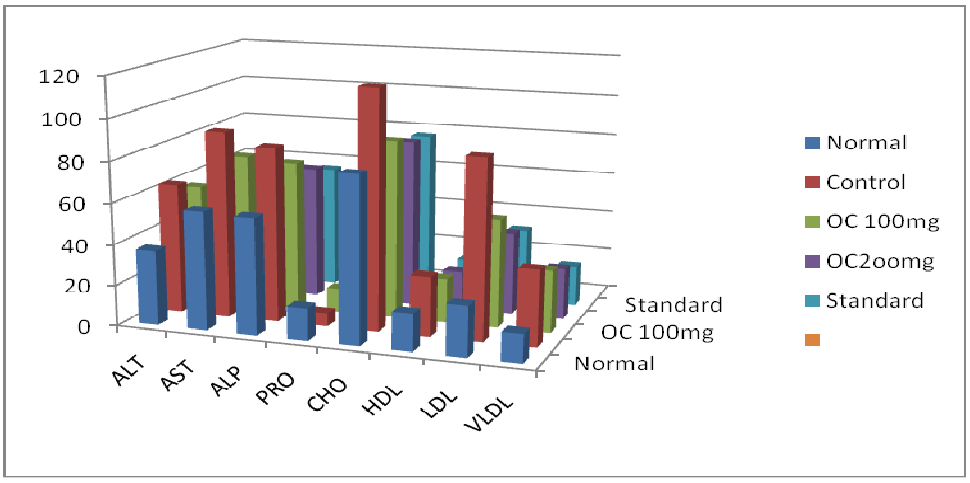
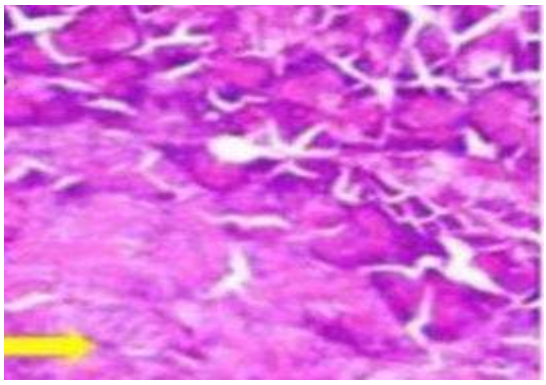
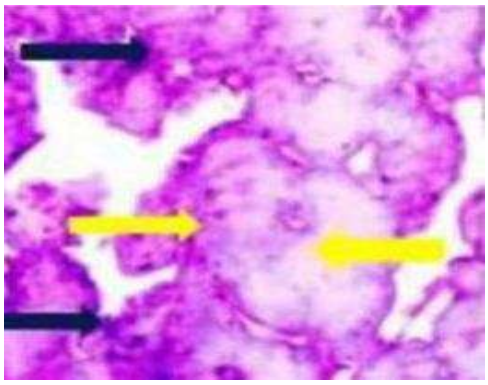


Fig-1-Histopathological changes in Pancreases

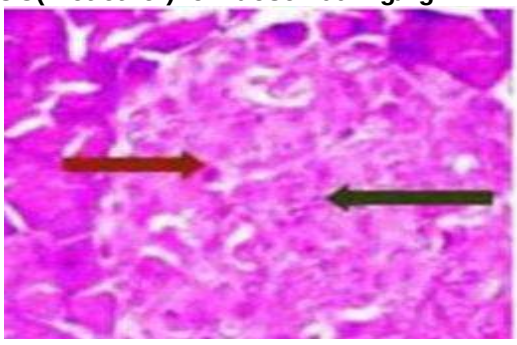


OC (Pet ether) high dose 200 mg/kg

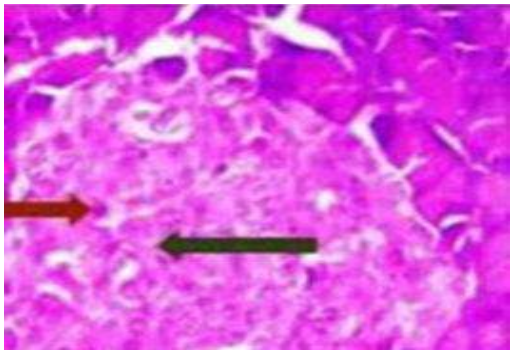


OC(Pet ether) low dose 100 mg/kg

DIABETIC CONTROL



NORMAL



Std group Glibenclamide 600 µgm/kg

TABLE 3:
Effect of petroleum ether extract of *Ocimum canum* leaf on the CAT, SOD, GSH, AND TBAR activity of normal, diabetic induced and drug treated rats

Parameters

Group	SOD	GSH	CAT	TBAR
Normal	**3.54±0.09**	**17.40±0.05**	**19.09±0.01**	**1.93±0.006**
Diabetic control	**1.39±0.08**	**6.63±0.15**	**6.71±0.10**	**2.87±0.004**
STZ+ OC(Water) low dose 100 mg/kg	**1.83±0.05**	**10.86±0.08**	**11.77±0.34**	**2.74±0.012**
STZ+ OC(Water) high dose 200 mg/kg	**2.72±0.06**	**14.60±0.11**	**14.82±0.08**	**2.35±0.008**
Std group	**2.97±0.02**	**16.46±0.08**	**18.15±0.09**	**2.18±0.008**

Antioxidant parameter in different groups of treated rats. Group I: Normal, Group II: Diabetic control rats Group III: STZ+ OC(PE) low dose 100 mg/kg Group IV: STZ+ OC(PE) high dose 200 mg/kg Group V: Std group Glibenclamide 600 µgm/kg

4. DISCUSSION

STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood glucose in animals[25] .The present study indicate that Petroleum ether extract of Ocimum Canum leaves showed antidiabetic properties against STZ induced diabetic model and also it proved to have antioxidant activity. A significant reduction ($P< 0.01$) was observed in petroleum ether (200 mg/kg) . The presence of flavonoids contents might be the possible mechanism for antidiabetic activity of this plant..

The reduction in the level of serum cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), atherogenic index and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) confirms the possibility that major functions of the extract are on the protection of vital tissues (kidney and liver) including the pancreas, thereby reducing the causation of diabetes in experimental animals. The portal tracts showed portal triad with portal vein, hepatic artery and bile duct, where as the diabetic mice liver tissue section showed marked structural alterations in the liver as a result of absence of insulin. The results indicated a primary and secondary effect of diabetic state on the kidney of mice.

Petroleum ether extracts (100 and 200 mg/kg b.w.) treated diabetic kidney, the damaged capillary loops with increase in the thickness of the wall, glomeruli and tubules without proteinuria and haemorrhage. The primary effect, the diabetes factor was associated with hyperglycemia and was responsible for dialation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes[26,27].

Dieresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus[28]. The ultra structure of diabetic pancreas showed considerable reduction in the islet langerhans and depleted islets. aqueous extracts (100 and 200 mg b.w.) treated pancreas the cells seem to have gathered together and small preserved islets similar to the normal. The liver damage is partially reversed by both extracts.

The present study also indicates that Ocimum Canum can partially inhibit renal toxicity as observed from serum creatinine. All the above observations suggest that Ocimum Canum can be a promising significant antidiabetic and antioxidant properties evidenced by physical, biochemical and Histopathological parameter. Further study need to be done to elucidate the mechanism of action involved in antidiabetic and antioxidant activities.

As a conclusion, it could be speculated that the observed antihyperglycemic activity of Ocimum Canum leaves might be related to the presence of flavonoids, sterols and triterpenoids and saponins as active constituents. The present investigation has also opened an avenue for further research especially with reference to the development of potent formulation for diabetic mellitus from ocimum canum plant.

CONCLUSION:

It can be concluded from the data that *Ocimum Canum* extract supplementation is beneficial in controlling the blood glucose level, improves the lipid metabolism and prevents diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats. This could be useful for prevention or early treatment of diabetic disorders

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ETHICAL APPROVAL

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No. - 1283/c/09/CPCSEA) and the Protocol approval reference number is PBRI/12/IAEC/PN-340.

COMPETING INTERESTS

Authors have declared that no competing interests exist

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author D.K.D designed and guided the study. Author A.K.D performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed the analyses of the study. Author J.M managed the literature searches. All authors read and approved the final manuscript.

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