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ANTIDIABETIC ACTIVITY AND MODULATION OF ANTIOXIDANT STATUS BY OCIMUM CANUM IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

The aquous extract of *Ocimum Canum*. (Family: Lamiaceae) leaf was investigated for its antidiabetic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of streptozotocin (45mg/kg, I.P). The aquous extractof *Ocimum canum* at a dose of 100mg/kg and 200mg/kg of body weight was administered to diabetes induced rats for a period of 28 days. The effect of aquous extractof *Ocimum canum* leaf extract on blood glucose, plasma insulin, glycosylated haemoglobin, serum lipid profile 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) of all groups were analyzed.Antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) , serum thiobarbituric (TBAR) were measured in the diabetic rats. The aquous extractof *Ocimum canum* leaf elicited significant reductions of blood glucose ($p<0.01$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes.. From the above results it is concluded that aquous extractof *Ocimum canum* possesses significant antidiabetic, antihyperlipidaemic and antioxidant effects in streptozotocin induced diabetic rats.

Keywords: Ocimum Canum, Antidiabetic, Antihyperlipidaemic, Antioxidant, streptozotocin

Introduction

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action. It is one of the oldest diseases affecting millions of people all over the world. According to recent estimates the prevalence of diabetes mellitus is 4% worldwide and that indicates 143 million persons are affected which will increase to 300 million by the year 2025 . Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes (Sumana et al 2001). Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals (Srivastava et al 1993). In the present investigation, *Ocimum Canum*. leaves were tested for their antidiabetic efficacy. *Ocimum canum* (Family: Lamiaceae) is widely used in Indian traditional medicines have been used in successful management of various disease conditions like bronchial asthma, chronic fever, cold,cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite etc and in treatment of gastric, hepatic,cardiovascular & immunological disordersthe present study was designed to investigate the antidiabetic efficacy of aquous extract of *Ocimum canum*leaf in streptozotocin induced diabetic rats.

MATERIALS AND METHODS:

Plant 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 from National Botanical Research Institute (NBRI)Lucknow. The leaves were cleaned and dried under the shade to avoid degradation of volatile oil.

Preparation of plant extract for Phytochemical Screening and Antidiabetic Studies: The *Ocimum canum* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Ocimum canum* leaves was packed in a Soxhlet apparatus and extracted with water. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures(Anonymous et al., 1990). The concentrated aquous extract were used for antidiabetic studies.The phytochemicals like Alkaloids, Carbohydrates,,tannins, flavonoids, terpinoids are found.(Behera etal.,2012)

Animals: Normal healthy male Wistar albino rats 200 ± 25 gm were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) , light and dark (12:12 h).

Rats were feed standard pellet diet ((golden feed, New Delhi and water regularly) .

Antidiabetic potential assessment: After overnight fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced in rats by I.P. injection of STZ dissolved in

0.1M cold sodium citrate buffer (pH 4.5) at a dose of 45 mg/kg body weight) The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia.

Control rats were injected with citrate buffer alone. After a 72 hrs for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats. Fasting blood glucose estimation and measurement ware done on day 0, 7, 14, 21 and 28 of the study.

Acute Toxicity Study: 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 (. OECD,2002). The animals were kept fasting for over night 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).

Induction of Experimental Diabetes: Rats were induced diabetes by the administration of simple intraperitioneal dose of streptozotocin (45 mg/kg). Two days after streptozotocin injection, rats screened for diabetes having glycosuria and hypoglycaemia. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design: In the investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each. Group I: Normal, untreated rats Group II: Diabetic control rats Group III: Diabetic rats given aquous extractof *Ocimum canum* leaf (100 mg/kg of body weight) Group IV: Diabetic rats given aquous extractof *Ocimum canum* leaf (200 mg/kg of body weight) Group V: Diabetic rats given standard drug glibenclamide (600µg/kg of body weight)

Biochemical Analysis: The animals were sacrificed at the end of experimental period of 28 days by decapitation. Blood was collected, sera separated by centrifugation . Serum glucose was measured by the O-toluidine method (Sasaki et al., 1972). Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit (Anderson et al 1993).

Glycosylated haemoglobin (HbA1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan (Karunanayake et al.,1985). Glycosylated haemoglobin, serum lipid profile 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) was measured by the method of King and Armstrong (King et al.,1934). Catalase (CAT) (Bergmayer et al.,1983), superoxide dismutase (SOD) (Madesh et al., 1998), lipid peroxidation (LPO) (Rehman et al 1984), reduced glutathione (GSH) (Pagila et al.,1967), serum thiobarbituric (TBAR) (Goldberg et al., 1983) were analyzed in the normal, diabetic induced and drug treated rats.

Statistical Analysis: The data were analyzed using student's t-test statistical methods. For the statistical tests p values of less than 0.01 and 0.05 was taken as significant.

RESULTS AND DISCUSSION

The phytochemical screening of aquous extract of Ocimum canum leaf revealed the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids. Acute toxicity study revealed the non-toxic nature of the aquous extract of Ocimum canum leaf. The streptozotocin induced diabetic rats elicited significant rise in blood glucose from 94.5±4.5**to 329.16±25.50mg/dl ($p<0.01$).On the contrary, diabetic rats treated with aquous extract of Ocimum canum exhibited decrease blood glucose significantly at a dose of 100 mg/kg and200 mg/kg body weight (Table 1).

TABLE 1: Effect of aquous 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(Water) high dose 200 mg/kg	290.33±27.64	271.83±26.04	199±17.10	160±5.73
STZ+ OC(Water) low dose 100 mg/kg	294±28.32	277.5±27.04	205.16±16.92	168.5±5.77
Std group Glibenclamide 600 µgm/kg	291±14.10	236.16±9.82	165.33±2.64	135±2.72

Fig. 1: *Blood glucose levels in different groups of treated rats*

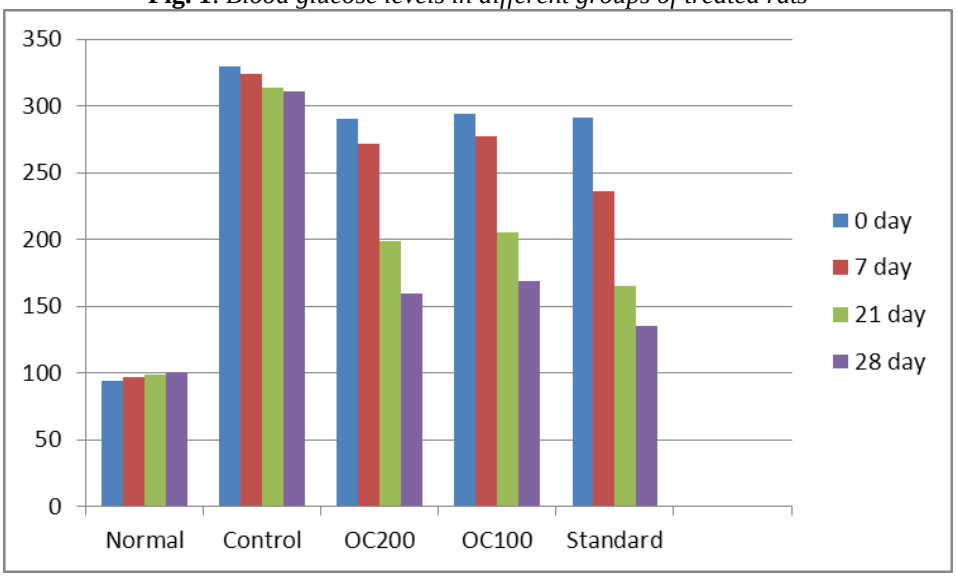


Fig. 1: *Blood glucose levels in different groups of treated rats. Group I: Normal, Group II: Diabetic control rats Group III: STZ+ OC(Water) high dose 200 mg/kg Group IV: STZ+ OC(Water) low dose 100 mg/kg Group V: Std group Glibenclamide 600 µgm/kg*

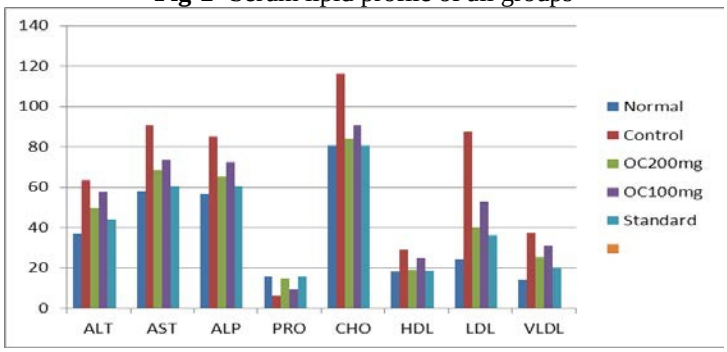
The hypoglycemic aquous extract of *ocimum canum* leaf was found to be decrease the blood glucose level. Streptozotocin induced diabetic rats showed significant decreased ($p<0.01$) blood glucose level compared with normal rats.. The levels of serum lipid profile ALT,AST, ALP, PRO, CHO, HDL,LDL,VLDL of control and streptozotocin induced diabetic rats were presented in **Table 2**.

TABLE 2: Effect of aquous 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±.7 4
Diabetic control	63.63± 0.49	90.81± 0.81	85.14± 0.65	6.43±0. 23	115.9 5±1.3 5	29.00± 0.14	87.22± 0.71	37.13± 1.5
STZ+ OC(Water) high dose 200 mg/kg	49.80± 0.34	68.25± 0.30	65.33± 0.45	14.83± 0.64	83.87 ±0.36	19.14± 0.24	40.41± 1.3	25.37±. 55
STZ+ OC(Water) low dose 100 mg/kg	57.46± 0.49	73.63± 0.49	72.42± 0.35	9.45±0. 32	90.69 ±1.35	24.93± 0.81	53.13± 0.11	30.8±.6 5
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±0.9 4

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

Fig-2- Serum lipid profile of all groups



A significant reduction in serum lipid profile ALT, AST, ALP, PRO, CHO, HDL, LDL, VLDL observed.. **Table 2** summarized the effect of streptozotocin on the activity of the hepatic marker enzymes in serum. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi et al., 2009). In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as atherogenic index and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of all groups were examined.. Elevated level of this enzyme in diabetes may be due to extensive damage to liver in the experimental animals by streptozotocin.

Treatment with aquous extract of *Ocimum canum* in streptozotocin induced diabetic rats produces a significant ($p<0.05$) decline in ALP level. The levels of serum lipid profiles,LDL-C, VLDL-C, and HDL-C in control and experimental animals were investigated . Streptozotocin induced rats showed significantly increased serum lipid profiles except CHO and PRO when compared with normal rats. The glibenclamide and aquous extractof *Ocimum canum* leaf treated rats showed a significant decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL-C level decreased in streptozotocin induced diabetic rats when compared to normal rats. On administration of aquous extract of *Ocimum canum* leaf and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents risk factor for coronary heart diseases (Mironova et al,. 2000). The significant reduction of serum lipid levels in diabetic rats after *Ocimum canum* treatment may be directly attributed to decreased glucose levele .

Effect of Ocimum canum on in- vivo antioxidant parameters

The superoxide dismutase activity was found to be reduced in erythrocytes ofanimals treated with streptozotocin . GSH is a major non-

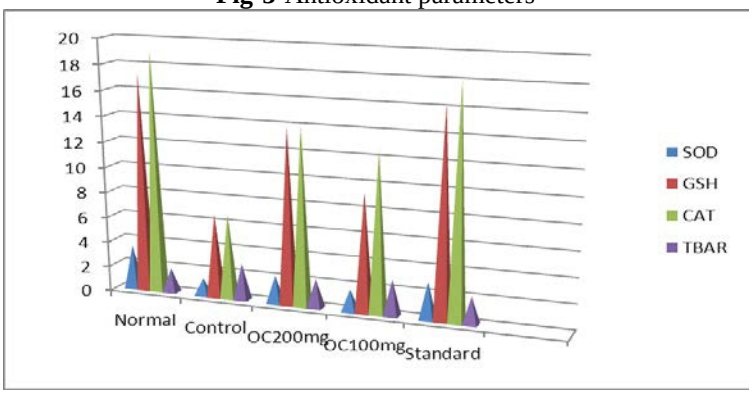
protein thiol in living organisms, which plays a central role in co-ordinating the body's antioxidant defense processes. (Latha et al., 2003) Perturbation of GSH status of a biological system can lead toserious consequences. SOD, CAT and TBAR constitute a mutually supportive team of defense against reactive oxygen species (ROS) The results (**Table 3**) showed decreased oxidant enzymes of streptozotocin induced diabetic rats.. These indicate that, plant extract inhibit oxidative damage due to the antiperoxidative effect of ingrediants present in aquous extractof *O.canum*.

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

	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) high dose 200 mg/kg	2.23±0.04	13.9±0.07	14.03±1.13	2.27±0.055
STZ+ OC(Water) low dose 100 mg/kg	1.73±0.07	9.39±0.11	12.61±0.32	2.87±0.004
Std group	2.97±0.02	16.46±0.08	18.15±0.09	2.18±0.008

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; aa p<0.01 level

Fig-3-Antioxidant parameters

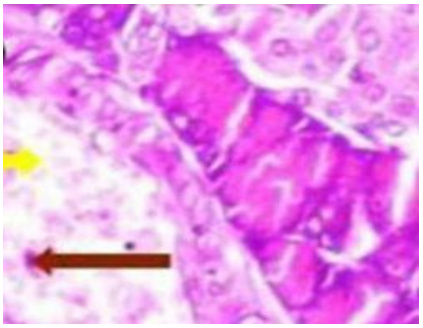


The levels of superoxide dismutase (SOD), catalase (CAT) reduced glutathione (GSH) and serum thiobarbituric (TBAR) were significantly (p<0.05) reduced in streptozotocin induced rats. These adverse changes were reversed to near normal values in aquous extract of *O.Canum* leaf treated. It is well known that CAT, SOD , GSH and TBAR play an important role as protective enzymes against free radical formation in tissues (Oberlyet al.,1974). The present study indicates the reduction in the activity of SOD, CAT, GSH and TBAR in streptozotocin induced rats. These results reveal

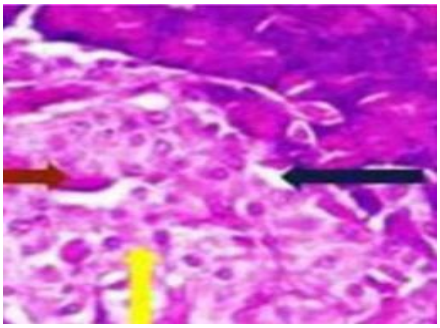
the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

Histopathology

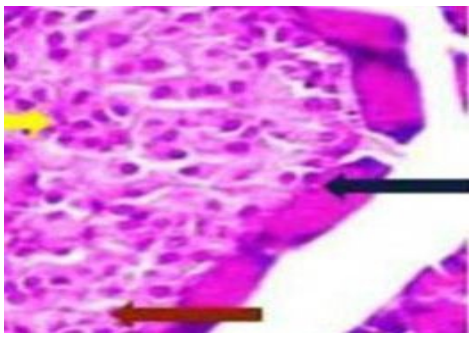
For histopathological study, animals from all groups were anaesthized with mild ether anaesthesia and dissected. pancreas are excised out of the animal's body and put immediately into 10% formalin solution in a stoppered container. These samples were then sent to diagnostic lab fixation (using Bouin's 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.



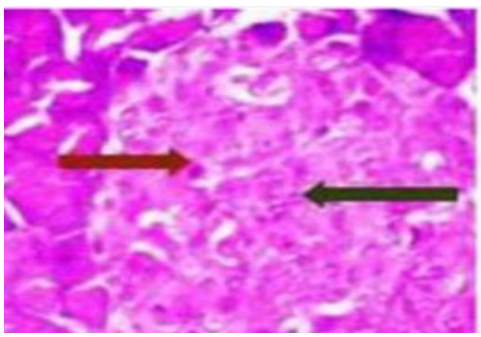
STZ+ OC(Water) high dose 200 mg/kg



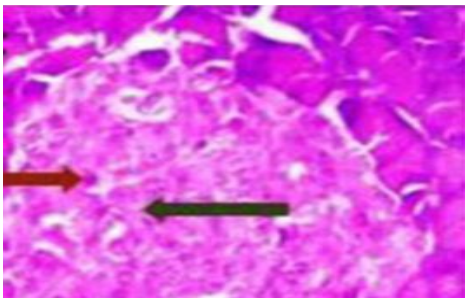
STZ+ OC(Water) low dose 100 mg/kg



DIABETIC CONTROL



NORMAL



Std group Glibenclamide 600 µgm/kg

CONCLUSION

In conclusion, the present study has shown that the aquous extract of the leaves of *Ocimum canum* has antidiabetic and antihyperlipidaemic effects. Since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, tannins, carbohydrate and phenols . Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles(Oliver et al., 1986 and Rhemann et al. 1989). Flavonoids are known to regenerate the damaged beta cells in the streptozotocin diabetic rats (Chakravarthy et al., 1980). Phenolics are found to be effective antihyperglycemic agents (Manickam et al., 1997). In the present study, the phytochemical analysis of aquous extractof *Ocimum canum* leaf clearly points out the presence of above said active phytochemicals. It denotes that, the antidiabetic effect of aquous extractof Ocimumcanum leaf may be due to the presence of more than one antihyperglycemic principle and their synergistic effects.

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References:

Anderson L, Dinesen B, Jorgonsen PN, Poulsen F and Roder ME(1993): Enzyme immune assay for intact human insulin in serum or plasma. Clinical Chemistry.; 39: 578-582.

Anonymous(1990): Phytochemical investigation of certain medicinal plants used in Ayurveda. Central Council for Research in Ayurveda and Siddha, New Delhi.

Behera etal(2012) Journal of Drug Delivery & Therapeutics; 2012, 2(4), 122-128

Bergmayer HU(1983): UV method of catalase assay. In Methods of Enzymatic Analysis, Weiheim Deer field Beach, Florida, Bansal. ; 3: 273.

Chakravarthy BK. Gupta S, Gambir SS and Gode KD(1980): Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. Indian Journal of Pharmacology. ; 12:123-127.

Goldberg DM, Spooner RJ(1983): Glutathione Reductase, In: Methods in Enzymatic Analysis, VCH Weinhem, Germany. ; 258-265.

Karunanayake EH and Chandrasekharan NV(1985): An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establishment of reference values for Sri lanka. Journal of the National Science Council of Sri Lanka. ; 13:235-258.

King EJ. Armstrong AR(1934): Determination of serum and bile phosphatase activity. Cannadian Medical Association Journal. ; 31: 56-63.
Latha M, Pari L(2003): Modulatory effect of *Scoparia dulcis* in oxidative stress induced lipid peroxidation in streptozotocin diabetic rats. Journal of Medical Food. ; 6:379-386.
Madesh M and Balasubramanian KA(1998): Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics.; 35: 184-188
Manickam M, Ramanathan M, Farboodinary Jahromi MA, Chansouria JPN and Ray AB(1997): Antihyper glycemic activity of phenolic from *Pterocarpus marsupium*. Journal of Natural Product; 60: 609-610.
Mironova MA, Klein RL and Virella GT, Lopes-Virella MF(2000): Anti-modified LDL antibodies, LDL-Containing immune complexes and susceptibility of LDL to invitro oxidation in patients with type2 diabetes. Diabetes; 49:1033-1049.
OECD,(2002) (Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; acute oral Toxicity- Acute Toxic Class method. OECD. Paris
Oberly WR and Buettner RG(1974): Role of superoxide dismutase in cancer. Cancer Research. ; 35: 1141-1149.
Oliver-Bever B(1986): Medicinal plants in tropical West Africa, Cambridge University press, London. ; 245-267.
Pagila DE, Valentine WN (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical medicine. ; 70: 158-169.
Preethi KC, Kuttan R(2009): Hepato and reno protective action of *Calendula officinalis* L. flower extract. Indian Journal of Experimental Biology. ; 47:163-168.
Rehman S(1984): Lead- induced regional lipid peroxidation in brain. Toxicology Letter. ; 21: 333-337
Rhemann AU and Zaman K(1989): Medicinal plants with hypoglycemic activity. Journal of Ethnopharmacology; 26:1-55.
Sumana G and Suryawarshi SA (2001): Effect of *Vinca rosea* extracts in treatment of streptozotocin diabetes in male albino rats. Indian Journal of Experimental Biology.; 39: 748-758.
Srivastava Y, Bhat HV, Vermal Y and Venkaiah K(1993): Antidiabetic and adaptogenic properties of *Momordica charantia* extract; an experimental and Clinical evaluation. Phytotherapy Research. ; 7: 285-289.
Sasaki T, Masty S and Sonae A (1972): Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinshbo Kagaku.; 1: 346-353.