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Effect of Ocimum sanctum and *Allium sativum* on Lipid Peroxidation and Antioxidant Enzymes in Alloxan Induced Diabetic Rats

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ABSTRACT

In diabetes, persistence hyperglycaemia has been reported as a cause of increased production of free radicals. Hyperglycemia induces oxidative stress and becomes the main factor for predisposing the complications in diabetes. The study is being aimed to find out the status of lipid peroxidation by measuring the levels of peroxidation end products namely,malondialdehyde (MDA) and4-hydroxynonenal (4HNE)nitrate and nitrite as well as antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) which might be helpful in risk assessment of various complications of diabetes mellitus. Treatment of the diabetic animals with *Ocimum sanctum* (Holy basil/ Tulsi) and *Allium sativum* (garlic) extract for two weeks showed protective effects evidenced by reversal of most of the parameters studied including plasma glucose level as compared to control rats.

KEY WORDS: OXIDATIVE STRESS, ANTIOXIDENTS, LIPID PEROXIDATION, OCIMUM SANCTUM AND ALLIUM SATIVUM.

INTRODUCTION

Diabetes mellitus is a serious health problem affecting millions of individuals worldwide. By the year 2025, the World Health Organization (WHO) predicts that over 300 million people worldwide will have diabetes mellitus. The reported prevalence of diabetes in adults between the ages of 20 and 79 is as follows: India8.31%, Bangladesh 9.85%, Nepal 3.03%, Sri Lanka 7.77%, and Pakistan 6.72%. Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or/ and when the body cannot effectively use the insulin it produces. Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose level that result from defects in insulin secretion, or action, or both (Lakhtakia et al., 2013, Gyawali et al., 2015, Animaw et al., 2017, Alotaibi et al., 2018).

Islets of Langerhans are organelles present within the pancreas and are mainly responsible for the production of insulin, glucagon, somatostatin and pancreatic polypeptide upon stimulation (Xavier et al., 2018). There is evidence that β cell dysfunction results from prolonged exposure to high glucose, elevated free fatty acids level or a combination of both (Jezek et al., 2018). β cells are particularly sensitive to ROS due to inadequate expression of free radical quenching enzymes (Tan et al., 2018). The capability of oxidative stress to damage mitochondria and ultimately decrease in insulin secretion is therefore, obvious (Park et al., 2019).

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Oxidative stress, a potentially harmful imbalance between the levels of pro-oxidants and antioxidants (Pizzino et al., 2017). It can cause cellular injury and tissue damage by promoting several reactions e.g., lipid perxidation, DNA damage, protein glycationetc (Liguori et al., 2018). Lipid peroxides may increase the participation of advanced glycation end-products in the development of chronic vascular complications (Chen et al., 2018). The chemical modification of proteins and lipids by ROS is speculated to contribute to the pathogenesis of diabetic complications. ROS also causes base modifications and strand breaks in DNA also (Sengupta et al., 2018).

Under normal conditions, free radicals are formed in minute quantities and are rapidlyscavenged by natural cellular defense mechanisms comprisingof enzymes like superoxide dismutase (SOD), glutathioneperoxidase (GPx), glutathione reductase (GR) and catalase (CAT) (Kurutas et al., 2016). Increased production of ROS which disturbs the antioxidant defence system of the cell by increasing the functional levels of superoxide anion ($O2^{-}$.), hydroxyl radical (OH.) and hydrogen peroxide (H_2O_2) (Lu et al., 2018). There are reports that in diabetics the disturbed equilibrium between prooxidants and antioxidants alters the metabolic status of body (Sarangarajan et al., 2017).

Plants have always been the source of drugs since ancient times. Many of the currently available drugs have been derived directly or indirectaly from plants (Calixto et al., 2019). Many herbal medicines have been recommended for the treatment of diabetes (Moradi et al., 2018). The effect of *Ocimum sanctum* on the glucose, serum lipid peroxidase and antioxidant enzymes are documented (Governa et al., 2018). Antidiabetic drugs can act in different ways such as stimulation of beta cell of pancreatic islets to release insulin to resist the hormones which rise blood glucose, increase the number and sensitivity of insulin receptor, increase glycogen content, enhance the use of organ glucose in the tissue, free radicals scavenging, resist lipid peroxidation, correct the metabolic disorder of lipid and protein and promote microcirculation in the body (Lankatillake et al., 2019). The aim of the present study is to investigate the potential of *Ocimum sanctum* (holy basil or Tulsi) and *Allium sativum* (garlic) in controlling the levels of glucose, nitrate/ nitriteand antioxidant enzymes in alloxan induced diabetic rats as compared to control and diabetic rats (Jayant et al., 2016).

MATERIAL AND METHODS

Chemicals used in the present study were of highest purity/ analytical grade. Sodium chloride, potassium chloride, sodium carbonate, sodium bicarbonate, disodium hydrogen phosphate, sodium dihydrogen phosphate, triton X-100, sodium hydroxide, ethylenediaminetetraacetic acid, reduced glutathione, sodium azide, ethanol, and magnesium chloride were purchased from HIMEDIA Chemicals, India. Glutathione, Folin-Ciocalteu's reagent, glutathione reductase, nicotinamide adenine dinucleotide phosphate(reduced) were purchased from sigma Aldrich chemicals private limited, Mo, USA.

Male albino rats of Wistar strain (weight 120±20g) were used in the proposed study. Animals were obtained from the animal facilities of Defence Research and Development Establishment, Gwalior, India, and were maintained under controlled conditions of temperature (250 ± 2^0C), relative humidity of (50 ± 15%), and normal photoperiod (light-dark cycle of 12 h) in the animal room of our department on standard pellet diet and tap water ad *libitum.* Animals were housed throughout the experiment in polypropylene cages (with each cage housing six animals) containing paddy husk as bedding and allowed to acclimatize to the environment of animal room for seven days before the start of the experiment.

Thirty six rats were randomly divided into six groups of six rats each. Animals were divided into six groups and were given following treatments:

Group 1 : Control (normal blood glucose level).
Group 2 : Treated control group (treated with leaves extract of *Ocimum sanctum*, 2.5 mg/kg body weight) (Kaushal et al., 2019).
Group 3 : Treated control group (treated with extract of pods of *Allium sativum*, 0.25 mg/kg body weight) (Azantsa et al., 2018).
Group 4 : Diabetic (I.V. injection of alloxan 70 mg/kg body weight).
Group 5 : Treated diabetic group (treated with leaves extract of *Ocimum sanctum*, 2.5 mg/kg body weight).
Group 6 : Treated diabetic group (treated with extract of pods of *Allium sativum*, 0.25 mg/kg body weight).

Type I diabetes was induced by giving single intravenous injection of alloxan monohydrate, 70 mg/kg body weight, dissolved in 0.9% solution of sodium chloride (Jayant et al., 2016). The animals were checked for blood glucose level 48 h after alloxan injection, and the rats with blood sugar level above 200 mg/dl were considered as diabetic and were used for the experiment.

Ocimum sanctum (Holy basil or Tulsi) leaves were obtained from botanical garden of Jiwaji University Gwalior, cleaned and aqueous extract was prepared and 2.5 mg/kg body weight was given orally to the rats of group 2 and 5 with the help of cannula, daily, for two weeks. *Allium sativum* (Garlic)pods were purchased from the local herbal market, cleaned, and aqueous extract of *Allium sativum* pods was prepared and 0.25 mg/kg body weight was given orally to the rats of group 3 and 6 with the help of cannula, daily for two weeks.

Rats were humanely killed 24 h after the last treatment by cervical dislocation; different tissues were excised off, washed with 0.9% NaCl and used for different

estimations. Animals were handled, ethically treated and humanly killed as per the rules and instructions of Ethical Committee of Animal Care of Jiwaji University, Gwalior, India, in accordance with the Indian National law on animal care and use. Malondialdehyde (MDA) and 4-hydroxynonanal (4HNE), the two major end products of lipid peroxidation, were estimated by the method of Jacobson (1999) with minor modifications. A 10% tissue homogenate was prepared in TrisHCl buffer (20 mM, pH 7.4). Prior to homogenization, 10 µL 0.5 M

butylatedhydroxytoluene(BHT) in acetonitrile was added per 1 ml of tissue homogenate. After homogenization, the homogenate was centrifuged at 3000 g for 10 minat 4° C and clear supernatant was used for the assay. Briefly 200 µL of supernatant was transferred to 650 µL of 10.3 mM 1-methyl-2-phenylindole in acetonitrite and vortex mixed. To assay MDA + 4HNE, 150 µL of 15.4 M methanesulfonic acid (MSA) was added,vortexed and incubated at 45°C for 40 min . To assay MDA alone, 150 µL of 37% HCl was added instead of MSA, vortexed, incubated at 45°C for 60 min. After incubation, samples were kept on ice, centrifuged at 9500 g for 5 min and absorbance was measured at 586 nm. The levels of MDA and 4HNE are expressed as nmol g^{-1} tissue using extinction coefficient 1.1×105 M-1 cm^{-1}.

Nitrate and nitrite levels in the tissues were estimated as described by Miranda (2001). Tissue homogenate was prepared in 0.9% NaCl and for nitrate estimation, reaction mixture contained 100 µl supernatant, 100 µl of VCl_3 (saturated solution) and 100 µl of Griess reagent (prepared by mixing equal volumes of 2% sulfonamide in 5% HCl and 1% N[1-naphthyl] ethylenediaminedihydrochloride). Absorbance was measured at 540 nm. For nitrite estimation, 100 µl of distilled water was added instead of VCl_3. For standard curve varying concentrations of $NaNO_3$ or $NaNO_2$ ranging from 2 to 20 µmole was taken in 100 µl and added either VCl_3 (for nitrate) or distilled water (for nitrite) and 100 µl Griess reagent. Concentration of nitrate or nitrite was expressed as µmole gm-1 tissue.

Superoxide dismutase (SOD, E.C.No. 1.15.1.1): SOD activity was assayed by estimating the inhibition of auto-oxidation of epinephrine (Misra et al., 1972). The 10% tissue homogenate was prepared in 0.9% NaCl, centrifuged at 15,000 g for 15 min and the corresponding supernatant was used for enzyme assay. Reaction mixture containing 0.5 ml sodium carbonate buffer (0.3 M, pH 10.2), 0.5 ml EDTA (0.6 mM), 0.5 ml homogenate and 1.0 ml water, was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.5 ml epinephrine (1.8 mM) and absorbance change per min was recorded for 5 min at 480 nm. Specific activity is expressed as % inhibition of auto-oxidation of epinephrine by the enzyme min-1 mg-1 protein.

Catalase (CAT, E.C.No. 1.11.1.6): CAT activity was estimated by the method of Aebi (1984). The 10% tissue homogenate was prepared in 1.15% KCl, centrifuged at 5000g for 10 min and the corresponding supernatant was used for CAT estimation. Reaction mixture containing 0.8 ml phosphate buffer (K2HPO4/ NaH_2PO_4, 50 mM, pH 7.0), 0.1 ml homogenate and 0.1 ml triton X-100 (0.02%) was incubated at room temperature for 10 min. Reaction was initiated by addition of 2.0 ml H_2O_2 (0.03 M prepared in potassium phosphate buffer, pH 7.0) and absorbance change per min was recorded for 5 min at 240 nm. Specific activity is expressed as µmole H_2O2 decomposed min^{-1} mg^{-1} protein.

Glutathione peroxidase (GPx, E.C.No. 1.11.1.9): GPx activity was estimated as described by Paglia (1967). The 10% homogenate was prepared in 1.15% KCl, centrifuged at 5000 g for 10 min and the corresponding supernatant was used for GPx estimation. Reaction mixture containing 0.3 ml sodium phosphate buffer (0.1 M, pH 7.4), 0.1 ml GSH (0.15 M), 0.05 ml sodium azide (2.25 M), 0.05 ml homogenate, 0.1 ml NADPH (0.84 mM) and 0.05 ml glutathione reducatse (2 U/ml) was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.05 ml H2O2 (0.001 mM) and absorbance change per min was recorded for 5 min at 340 nm. Specific activity is expressed as nmole NADPH oxidized min-1 mg-1 protein.

Protein estimation: Protein in the tissue samples was precipitated by 10% TCA and the precipitate dissolved in 0.1 N NaOH for estimation. Protein was estimated by the method of Lowry (1951) using bovine serum albumin as standard.

Statistical analyses: Results are expressed as mean ± S.E. of six different sets of observation taken on different days. All the statistical analyses were performed using one-way analyses of variance (ANOVA) with post hocDunnett's multiple comparison test applied across treatment groups for each tissue. Significance level was based on $p< 0.05$.

RESULTS AND DISCUSSION

The blood glucose level of all the rats was tested by taking the blood from the tail vein and using electronic glucometer. Administration of alloxan (70 mg/kg, i.v) led to 4-fold elevation of fasting blood glucose levels, which was maintained for a period of 2 weeks(Table 1). It was observed that oral administration of aquous extract of *Ocimum sanctum* and *Allium sativum*, significantly decreased the blood glucose levels in diabetic as compared to the blood glucose level of control rats. Theresults of the present study showed that oral administration ofextract of *Ocimum sanctum* and *Allium sativum* daily for 14 days, to the diabetic rats caused 18.7%, and 15.4% decrease on 7th day and 42.8%, and 40.8% decrease in the blood glucose level on day 14th of the start of treatmet when cmpared with respective untreated diabetic rats. The results clearly showed the hypoglycemic potential of *Ocimum sanctum* and *Allium sativum* extracts.

The results of the present study that showed increased lipid peroxidation (LPO) in the tissues of alloxan induced diabetic rats. The results of the presnt study

clearly showed that alloxan adminstration in rats caused accumulation of malondialdehyde (MDA) and 4-hydroxynonanal (4HNE), the two major end products of lipid peroxidation, in the liver and the brain of rats when comapared with control. MDA and 4HNE levels were increased 28.03% and 55.6% in the liver and 51.11% and 69.8% in the brain, respectively, when compared with the control (Table 2). When the diabetic rats were given *Ocimum sanctum* treatment for two weeks, 14.8% and 27.2% decrease in MDA levels while 28.1% and 34.4% decrease in the 4HNE and *Allium sativum* treatment for two weeks, 11.4% and 23.5% decrease in MDA levels while 24.5% and 30.5% decrease in the 4HNE levels of the liver and the brain, respectively, were observed when compared with control group (Table 2). When the *Ocimum sanctum* and *Allium sativum* treated diabetic rats were compared with control group, percent increase of MDA and 4HNE levels were 9.1%, 10.1% and 13.5%, 15.5% in the liver and 11.8%, 11.3% and 17.7%, 17.9% in the brain, respectively. The results clearly showed antioxidative potential of *Ocimum sanctum* and *Allium sativum*.

Table 1. Effect of oral treatment of extracts of *Ocimum Sanctum* and *Allium sativum* on glucose level in alloxan induced diabetic rats

		Level of glucose in 14 days experimental animals		
S No.	Groups	0 day	7th day	14th day
1.	Control	104.00±1.53	105.67±1.33	106.67±1.33
2.	Control + *Ocimum sanctum*	107.33±2.19#	106.33±2.19#	104.67±1.86#
3.	Control + *Allium sativum*	107.67±2.91#	106.67±2.6#	105.67±2.6#
4.	Diabetic	423.33±7.84***	418.67±12.06***	423.33±7.84***
5.	Diabetic + *Ocimum sanctum*	422.19±12.81***	340.33±18.26***	242.33±5.04***
6.	Diabetic + *Allium sativum*	424.00±7.37***	354.00±18.77**	250.67±10.17***

Glucose concentration is expressed as mg/dl.
Results are expressed as mean ± S.E. of six set of observation.
Significance is based on p>0.05 #, p<0.05 *, p<0.01 **, p<0.001 ***

Table 2. Effect of oral treatment of extracts of *Ocimum Sanctum* and *Allium sativum* for 14 days on the levels of MDA and 4HNE in the tissue of alloxan induced diabetic rats.

		MDA		4HNE	
S No.	Groups	Brain	Liver	Brain	Liver
1.	Control	27.27±0.26	61.42±0.52	16.06±0.3	25.61±0.40
2.	Control + *Ocimum sanctum*	21.82±2.33#	60.00±0.45#	11.67±0.4***	21.97±0.15**
3.	Control + *Allium sativum*	23.18±2.15#	61.06±0.66#	12.58±0.4**	23.33±0.4*
4 .	Diabetic	41.21±0.15***	78.64±0.27***	27.27±0.26***	39.85±0.4***
5.	Diabetic + *Ocimum sanctum*	30.00±0.26***	66.97±0.4***	17.88±0.4***	28.64±0.52***
6.	Diabetic + *Allium sativum*	31.51±0.15***	69.7±0.3***	18.94±0.55***	30.15±0.66***

MDA and 4HNElevels are expressed as n mole/gm.
Results are expressed as mean ± S.E. of six set of observation.
Significance is based on p>0.05 #, p<0.05 *, p<0.01 **, p<0.001 ***

Administration of *Ocimum sanctum* showed antioxidative effect in the control group also by reducing the levels of MDA and 4HNE in the liver and the brain, both. When the control rats were given *Ocimum sanctum and Allium sativum* treatment for two weeks, decrease in MDA and 4HNElevels were observed when compared with untreated control group however the changes were not significant (Table 2).

Table 3. Effect of oral treatment of extracts of *Ocimum sanctum* and *Allium sativum* for 14 days on nitrate and nitrite in the tissues of in alloxan induced diabetic rats.

		Nitrate		Nitrite	
S No.	Groups	Brain	Liver	Brain	Liver
1.	Control	117.27±0.69	143.03±0.80	50.76±0.66	59.7±1.45
2.	Control + *Ocimum Sanctum*	108.03±0.99**	135.18±1.07**	45.45±2.84*	51.51±0.76**
3.	Control + *Allium sativum*	114.24±0.55*	139.21±0.12**	45.45±1.64**	52.64±0.64*
4.	Diabetic	245.91±1.31***	266.7±1.43***	86.81±1.2***	96.06±0.61***
5.	Diabetic + *Ocimum Sanctum*	152.72±0.7***	165±1.39de***	61.51±0.55***	63.48±1.18***
6.	Diabetic + *Allium sativum*	155.54±0.28***	169.09±0.52***	63.03±0.66***	65.45±1.64***

Nitrate and nitrite levels are expressed as µ mole / gm.
Results are expressed as mean ± S.E. of six set of observation.
Significance is based on p>0.05 #, p<0.05 *, p<0.01 **, p<0.001 ***

Levels of nitrate and nitrite were increase in allaxan induced diabetic rat tissues. The results of the present study showed that there was significantly high concentration of nitrate and nitrite in the liver and the brain of diabetic rats. Dabetic rat tissues showed nitrate and nitrite level 109.7%, 71.02% and 86.5%, 60.9% increase in brain and liver tissues when compared with control rat tissues. Diabetic rat tissues given oral administration of *Ocimum sanctum* and *Allium sativum* extracts for 14 days,diabetic treted rats 38%, 36.7% and 29.1%, 27.4% decrease in the brain and 38.1%, 36.6% and 33.9%, 31.9% decrease in the liver of the nitrate and nitrite, respectivelywhen compered with diabetic rat tissues. Treatment of control rats with *Ocimum sanctum* and *Allium sativum* extracts for 14 days, caused about 7.8%, 2.6% and 10.9%, 10.4% decrease in the brain and 5.5%, 2.6% and 13.7%, 11.8% decrease in the liver the level of nitrate and nitrite when compared with control rat tissues (Table3).

The levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly reduced in the tissues of alloxan induced diabetic rats. The hepatic activities of SOD, CAT and GPx were decreased by 50.8%, 64.7%, 46.3% and 47.1%, 70.7%, 52.0% decrease, respectively in brain of diabetic rats compared with control rats (Table 4). Oral administration of *Ocimum sanctum* and *Allium sativum* extract for 14 days showed protective effects against alloxan diabetes induced alterations in the activities of SOD, CAT and GPx. The activities of SOD, CAT and GPx were increased by 51.9%, 81.9%, 72.9% and 47.8%, 78.7%, 68.2% in the liver and 49.3%, 112.3%, 74.4% and 41.9%, 97.1%, 71.2% increased in the brain of diabetic rats given respectively *Ocimum sanctum* and Allium sativum extract for 2 weeks when compared with diabetic rat tissues while the same *Ocimum sanctum* and *Allium sativum* extracts were given to the control rats, marginal changes in the activity of SOD, CAT and GPx were observed in both the tissues which were not significant(Table 4).

Reactive oxygen species (ROS) cause lipid peroxidation and damage protein by chemical modifications through cross-linking and fragmentation (Fabrice et al., 2019). Therefore oxidative stress has been considered to contribute to the pathological processes of diabetic complications. The results of the present study revealed significant elevation in the levels of MDA and 4HNEin the tissues of diabetic rats, an indication of increased oxidative stress (Bigagli et al., 2019). Treatment of diabetic rats with *Ocimum sanctum* and *Allium sativum* extracts reduced not only the blood glucoselevel but also the MDA and 4HNE levels were also decreased in the liver and the brain of rat tissues (Joudaki et al., 2019).

In diabetes hyperglycemia has been reported to cause increased production of ROS through auto-oxidation and non enzymaticglycation (Prakash et al., 2017). Under normal physiological condition there is a critical balance in the generation of oxygen free radicals and its antioxidant defense systems used by organism to deactivate and protect themselves against free radical toxicity (He et al., 2017). The results of the present study showed thatdecrease activity of antioxidant enzymes (SOD, CAT, GPx) in the tissues of diabetic rats and increase in the tissues oxidative stress (Ighodaro et al., 2018). Diabetic rats treated with Ocimum sanctum and Allium sativum extractsincrease the activity of antioxidant enzymes such as SOD, CAT and GPx in liver and brain tissues in diabetic rats (Govindappa et al., 2015).

Diabetes mellitus increase production of nitrate and nitrite in the tissues of diabetic rats. *Ocimum sanctum* and *Allium sativum* extracts decrease activity of nitrate and nitrite in the tissues of diabetic rats.

Table 4: Effect of oral treatment of extracts of *Ocimum Sanctum* and *Allium sativum* for 14 days on the activity of superoxide dismutase, catalase and glutathione peroxidase in the tissues of in alloxan induced diabetic rats.

			SOD		CAT		GPx
S No.	Groups	Brain	Liver	Brain	Liver	Brain	Liver
1.	Control	225.31±2.20	306.32±5.14	20.31±0.35	28.41±0.88	832.74±30.58	1991.96±20.37
2.	Control + *Ocimum sanctum*	226.19±1.62#	309.25±4.82#	20.90±0.53#	28.81±1.18#	838.13±19.97#	2018.47±39.61#
3.	Control + *Allium sativum*	225.24±1.3#	306.72±5.16#	20.47±0.45#	28.57±1.17#	833.81±26.67#	1999.45±25.53#
4.	Diabetic	119.03±17.26**	150.59±10.21***	5.94±0.04***	10.02±0.20***	400.73±28.64***	1070.47±23.76***
5.	Diabetic + *Ocimum sanctum*	177.76±5.48*	228.69±9.88**	12.61±0.92**	18.23±1.62**	698.89±31.42**	1851.89±25.51***
6.	Diabetic + *Allium sativum*	168.86±6.52#	222.61±8.52**	11.71±0.61***	17.91±1.57**	686.12±39.4**	1800±33.74***

SOD, CAT and GPx activation is expressed as μ mole/ min/ mg protein.
Results are expressed as mean ± S.E. of six set of observation.
Significance is based on $p>0.05$ #, $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Ocimum sanctum and *Allium sativum* extracts showed antidiabetic potential (Suanarunsawat et al., 2016).There are about 800 plants which have been reported to show antidiabetic potential (Suryavanshi et al., 2019). A wide collection of plant derived active principles representing numerous bioactive compounds has established their role for possible use in the treatment of diabetes (Suryavanshi et al., 2019). The most common and effective antidiabetic medicinal plants of Indian origin are Babul (*Acacia arabica), bael(Aeglemarmelose), church steeples (Agrimoniaeupatoria), onion (Allium cepa), garlic (Allium sativum), ghritakumarai (Aloe vera), neem (Azadirachtaindica)* etc. All these plants are a rich source of phytochemicals having medicinal value.

Diabetes mellitus decreased the activity of antioxidant enzymes and increased serum glucose, nitrate/nitrite, MDA and 4HNE levels. Oral administration of aqueous extracts of *Ocimum sanctum* and *Allium sativum* increased antioxidant enzymes activity and decrease serum glucose, nitrate/nitrite, MDA and 4HNE in the tissues of diabetic rats. Further human studies are necessary to found the role of these herbal drugs in controlling type I diabetes and its complications (Sharifi-Rad et al., 2016).

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