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Modulatory effect of *Calendula officinalis* on altered antioxidant status and renal parameters in diabetic rats

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Abstract

Calendula officinalis (Family *Compositae*) flowers are recognized as safe substance for food use by Food and Drug Administration. Present study was aimed to determine the modulatory effect of floral extracts of *C. officinalis* administrations on mean blood glucose (MBG), per cent glycosylated hemoglobin (HbA1c), lipid profile [(total cholesterol (TC), triglycerides (TG), low and high density lipoproteins (LDL, HDL)], antioxidant and renal parameters in streptozotocin (STZ) induced diabetic rats. Increased ($P < 0.05$) levels of MBG and HbA1c fraction indicate the induction of diabetes in rats. Enhanced ($P < 0.05$) TC, TG, LDL, total oxidant status (TOS), oxidative stress index (OSI), malondialdehyde (MDA) levels, and renal indices were observed in blood of diabetic rats. However, levels of HDL, protein profile, total antioxidant status (TAS), glutathione (GSH), total thiols (TTH) and activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-s-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH) were significantly reduced in diabetic rats. Repeated administrations of ethanolic floral extract of *C. officinalis* reduced the enhanced levels of MBG, HbA1c and TC while restored OSI, TTH, GSH, CAT, SOD, GST and MDA levels; it also increased activities of G6PDH and GPx in diabetic rats compared to untreated diabetic rats. Hypoglycemic, hypolipidemic, restored antioxidant level, and reduced altered renal functions by the floral extract of *C. officinalis* in diabetic rats. Further, the modulatory effect was better in aqueous as compared to ethanolic floral extract of *C. officinalis.*

Keywords: *Calendula officinalis*, malondialdehyde, hypoglycemic, antioxidant, diabetes

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Introduction

Diabetes mellitus, a most common endocrine disorder is the major cause of ill health worldwide (1). The disorder will continue to grow globally due to an aging population, growth of population size, urbanization, high prevalence of obesity and sedentary life style (2). Defect in insulin secretion from ß-cells of pancreas and/or insulin action on the central and peripheral organs are often primary causes of manifestation of disease (3). Experimental diabetes in animals provides considerable insight into the physiological and biochemical derangement in important vital organs of the body particularly in renal diseases. Studies showed diabetic complications are primarily due to increased non-enzymatic and progressive glycation of proteins with consequently increased formation and accumulation of glucose derived advanced glycation end products (AGEs) (4,5) in tissue leading to oxidative damage. Further persistent decrement in the level of insulin adversely deranges the carbohydrate and lipid metabolism in mammalian tissues leading to secondary complications.

Application of phytochemicals for the treatment of various ailments has been used since dawn of civilization. Herbal drugs are undoubtedly attractive and reliable alternative for management of diseases due to their easy accessibility, efficacy and importantly being natural herbs are considered to be safe (6). Ethno-botanical reports suggest that more than 800 plants to

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possess antidiabetic potential (7). Many traditional plant formulations are being used and claiming to be effective for the management of diabetes throughout world. Botanicals are being rich in natural antioxidants (flavonoids, carotenes, terpenoids etc) not only improve insulin secretion from ß-cells of pancreas but also neutralizes excessive generated free radicals due to deranged cellular metabolism thereby reducing the diabetic complications (8).

Calendula officinalis (Family *Compositae*) is being used in folklore system of medicine due to its high medicinal potential. Dried flower is used as a spice and falls under generally recognized as safe (GRAS) category by the USFDA. Various calendula preparations are approved for their use in traditional medicine by the European Medicines Agency (9). Calendula extract contains a number of phytoconstituents and its chemical composition depends on the extraction method and/or part of plant used for the extraction. Floral extract of *C. officinalis* have high concentration of terpenoids and flavonoids compounds compared to other parts of the plant (10,11). Additionally, floral extract of the plant are rich source of polyunsaturated fatty acids (12), mineral substances (13) and vitamin C (14) which adds to its ethanomedicinal potential. *Calendula* flower is often used in skin care products; it assists the cell rejuvenation and wound healing (15). Studies have reported that plant also posses hepatoprotective (16,17), nephroprotective (17,18) anti-inflammatory (19) potential of the plant extracts. Therefore, present study was aimed to determine the modulatory effect of *C. officinalis* administrations on glycemic index, antioxidant and renal parameters in streptozotocin (STZ) induced diabetic rats.

Materials and method

Collection and preparation of extracts

The flowers of *C. officinalis* were collected from different parts of Jammu (India). Plant sample was taxonomic identified by Taxonomist, Department of Botany, University of Jammu (AU-2875). Sufficient fresh flowers were collected and air-dried in shade (temperature not exceeding 40 °C) for 3-4 weeks. Air dried flowers were pre-crushed and later pulverized into fine powder using electric blender. The aqueous extract was prepared by soaking dry powder in 1:10 ratio in distilled water for 72 h with intermittent shaking. After 72 h of soaking, the contents were filtered through filter paper (0.45 µm) and filtrate was concentrated under reduced pressure using rotatory evaporator (temp 50-55 °C, 10-15 rpm). The ethanolic extract was prepared by using ethyl alcohol in extract container of soxhlet apparatus according to standard method. The dried aqueous and ethanolic floral extracts of *C. officinalis* were stored at 4 °C in air tight containers. Per cent extractability of aqueous and ethanolic floral extracts of *C. officinalis* was 23.62 and 17.61% respectively (11). The extracts were reconstituted in 0.1 % carboxy methyl cellulose (CMC) for oral gavage in wistar rats.

Chemicals and experimental animals

Streptozotocin (2-deoxy-2-({[methyl (nitroso) amino] carbonyl} amino)-β-D-glucopyranose); ABTS (2,2′-azinobis (3-ethylbenzothiazoline 6-sulphonate) were procured from the Sigma-Aldrich, USA and other chemicals used in the study were analytical grade. Adult wistar rats of either sex weighing 180-200gm were procured form Indian Institute of Integrative Medicine, Jammu, INDIA used for the experimental study. Procured rats were acclimatized in the laboratory conditions for a period of more than 2 weeks prior to start of experiment. Animals were provided standard pelleted ration and *ad libitum* drinking water under standard managemental conditions (22 ± 3 °C 50-60% relative humidity and 12 h light-dark cycles). The experimental protocol was approved and monitored by Institutional Animal Ethics Committee (IAEC) and all the experimental animals were kept under constant observation during entire period of study (FVSc/C-11/2456-68).

Experimental design

Diabetes was induced by single intra-peritoneal injection (55 mg/kg) of STZ, freshly dissolved in 0.1 M cold citrate buffer (pH 4.5) (20). Five days after STZ injection, fasting blood glucose of the rats was estimated and the rats having blood glucose above 225 mg/dl were considered as diabetic and included in the experimental trial. Eighteen normal rats divided in Group I, II and III, received 1ml/day CMC, aqueous and ethanolic floral extracts of *C. officinalis*, respectively. Whereas 24 diabetic rats were divided into four groups viz. IV rats were diabetic and receiving no

treatment and served as diabetic control and Group V received glibenclamide (10 mg/kg orally) as a standard antidiabetic drug. Group VI and VII diabetic rats received 300mg/kg BW aqueous and ethanolic floral extract of *C. officinalis*, respectively. All treatments were administered daily for 21 days in between 10.00-11.00 AM. Dose of the plant extract was calculated on the basis of lethal dose and other pharmacological potential (9,15,18).

Collection and processing of samples

3-4 mLblood sample from each rat was collected directly from cardiac puncture in sterilized tube containing heparin after 21 days of repeated administrations. Blood glucose level was determined immediately using glucometer (Contour® TS, Bayer Pharmaceuticals Pvt. Ltd. India). Part of blood sample was used for the determination of reduced glutathione (GSH), hemoglobin (Hb) and glycosylated fraction of hemoglobin (HbA1c). The remaining part of blood samples was centrifuged at 4000 rpm for 10 min; plasma was collected in glass vials for the estimation of plasma proteins, lipid, muscular and renal parameters. Glycosylated hemoglobin (HbA1c) fraction was determined based on ion exchange method (21) based kit (Transasia Bio-Medicals Ltd, India) using UV-visible spectrophotometer (UV-1601, Shimadzu).

Assaying of antioxidant and renal parameters

Total antioxidant status (TAS) was determined spectrophotometrically by using 2,2′-azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS) according to the standard method (22); final TAS values were expressed as mM of ascorbic acid equivalents in the plasma. Similarly, TOS level was measured using a novel automated method developed by Erel (23) and results expressed in terms of μmol H_2O_2 Equiv./L. The percent ratio of TOS to TAS level was used for determination of oxidative stress index (OSI) (24). The activity of G6PDH was assessed based upon the ability of enzyme to catalyze the conversion of Glucose-6-phosphate and $NADP^+$ to 6-phosphogluconolactone and NADPH (reduced nicotinamide adenine dineucleotide phosphate) as per the method described by Deutsch (25). The enzymatic parameters viz. catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were determined as using standard methods (26,27,28,29). Total thiols (TTH) level was determined in plasma as per the standard protocol and the concentration of total thiols (mM) was expressed using reduced glutathione as a standard (30). Similarly, malondialdehyde (MDA) level in plasma (nmole of MDA formed/mg of Hb/h) was determined to estimate the membrane lipid peroxidation (31). The level of reduced blood glutathione (GSH) was determined as per the standard method (32). Activities of aspartate and alanine aminotransferase (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), levels of plasma proteins, albumin, creatinine (CR), blood urea nitrogen (BUN), lipid profile viz. total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) were determined by standard kits (Transasia Bio-Medicals Ltd, India) using Chemistry Analyzer (CHEM-7, ERBA, Mannheim).

Statistical analysis

The values are presented in mean ± standard error. Biochemical and antioxidant parameters were analyzed for analysis by analysis of variance in completely randomized design using the Duncan Multiple Range Test at 5 % level of significance (SPSS 16.0, IBM, USA).

Results

Alterations in MBG, HbA1c, plasma proteins, lipids, antioxidant and renal parameters of blood were assessed in diabetic rats and modulatory effect following repeated administrations of aqueous and ethanolic floral extracts of *C. officinalis*.

Effects on mean blood glucose and glycosylated Hb (HbA1c)

Single oral dose administration of STZ significantly ($P < 0.05$) increased the MBG level as compared to normal rats. Glibenclamide treatment in diabetic rats significantly ($P < 0.05$) reduced the MBG level and these values are significantly ($P < 0.05$) higher from the control group (Table 1). Repeated administrations with either aqueous or ethanolic floral extract to diabetic rats significantly ($P < 0.05$) reduced MBG as compared to diabetic rats but levels were still significantly ($P < 0.05$) higher than the normal control. Further aqueous extract treated group exhibited significantly ($P < 0.05$) lower

Table 1: Effect of aqueous and ethanolic floral extracts of *C. officinalis* administrations on mean blood glucose (MBG) hemoglobin (Hb) and glycosylated Hb (HbA1c) in plasma of diabetic rats

Groups	MBG	Hb	HbA1c
Normal control	$86.83^{a} \pm 5.18$	$12.89^{bc} \pm 0.29$	$5.19^{a} \pm 0.16$
Aqueous extract	$99.67^{a} \pm 7.12$	$11.37^{bc} \pm 0.31$	$5.58^{a} \pm 0.21$
Ethanolic extract	$90.50^{a} \pm 5.02$	$11.48^{bc} \pm 0.38$	$5.30^{a} \pm 0.15$
Diabetic control	$205.33^{d} \pm 7.15$	$8.45^{a} \pm 0.45$	$8.75^{d} \pm 0.21$
Diabetic + Glibenclamide	$124.67^{b} \pm 5.08$	$11.63^{c} \pm 0.48$	$6.33^{b} \pm 0.15$
Diabetic + Aqueous extract	$126.00^{b} \pm 11.75$	$11.66^{b} \pm 0.27$	$6.17^{b} \pm 0.36$
Diabetic + Ethanolic extract	$177.50^{c} \pm 7.70$	$9.95^{b} \pm 0.28$	$7.91^{c} \pm 0.23$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a, b, c & d) in a column are statistically different from one another at 5 % level of significance
Values of Hb (hemoglobin) are expressed in gm/dl
Values of HbA1c and MBG (mean blood glucose) are expressed in Per cent glycosylated Hb and mg/dl respectively

MBG as compared to ethanolic floral extract of *C. officinalis* administrations. In diabetic rats significant ($P < 0.05$) reduction in the Hb and significant increased ($P < 0.05$) level of HbA1c were observed as compared to normal control. Treatment with glibenclamide in diabetic rats significantly ($P < 0.05$) lower the level of HbA1c than the diabetic rats. Similarly treatment with aqueous floral extract in diabetic rats significantly ($P < 0.05$) lowered the per cent HbA1c level as compared to diabetic rats, but these values were still significantly ($P < 0.05$) higher than the control animals. Aqueous floral extract was found to be more potent in restoring the concentration of HbA1c than the ethanolic floral extract of *C. officinalis*.

Effect on plasma lipids and proteins profile

Levels of TC, TG and LDL were significantly ($P < 0.05$) increased and reduction ($P < 0.05$) in level of HDL were seen as compared to control values. Treatment with glibenclamide significantly ($P < 0.05$) reduced the levels of TC, TG and LDL as compared to diabetic rats, but these values were significantly ($P < 0.05$) higher than the control group. Similarly repeated administration of either aqueous or ethanolic extract in diabetic rats significantly ($P < 0.05$) reduced the levels of TC, TG, LDL and significantly ($P < 0.05$) increased HDL. Aqueous extract of *C. officinalis* was more effective than ethanolic extract in lowering triglycerides, LDL and increasing HDL and these values were not statistically different from the normal control (Table 2). In diabetic rats concentrations of total plasma proteins, albumin and globulin were significantly ($P < 0.05$) reduced than their respective normal control. Administration of glibenclamide significantly ($P < 0.05$) increased level of total plasma proteins but the values were significantly ($P < 0.05$) lower from the control group whereas level of albumin was not differ significantly. Repeated administration of either aqueous or ethanolic floral extract of *C. officinalis* in diabetic rats didn't restore the values of total plasma proteins and albumin in diabetic rats (Table 3).

Effect on biochemical parameters

Activities of ALP, LDH, AST and ALT were significantly ($P < 0.05$) higher in diabetic rats as compared to their respective activities in normal control. Treatment with glibenclamide significantly ($P < 0.05$) reduced the AST, ALT, ALP and LDH activities as compared to diabetic control but values were significantly ($P < 0.05$) higher from the normal control with the exception of ALP and ALT non-significantly different from normal control. Treatment with either aqueous or ethanolic floral extract of *C. officinalis* significantly ($P < 0.05$) reduced the activities of AST, ALT, ALP and LDH as compared to diabetic rats but these activities excepting ALT were still significantly ($P < 0.05$) higher than the normal control in rats administered ethanolic extract of *C. officinalis*.

Table 2: Effect of aqueous and ethanolic floral extracts of *C. officinalis* administrations on plasma lipid profile i.e. total cholesterol (TC), triglycerides (TG), High and low density lipoproteins (LDL and HDL) of diabetic rats

Groups	TC	TG	HDL	LDL
Normal control	$72.49^{b} \pm 1.06$	$43.42^{a} \pm 3.12$	$38.65^{c} \pm 1.85$	$25.16^{a} \pm 2.98$
Aqueous extract	$82.13^{c} \pm 3.03$	$71.66^{b} \pm 4.74$	$42.85^{c} \pm 1.93$	$24.95^{a} \pm 3.27$
Ethanolic extract	$80.47^{bc} \pm 3.11$	$74.10^{b} \pm 3.87$	$41.07^{c} \pm 1.01$	$24.57^{a} \pm 2.55$
Diabetic control	$104.97^{d} \pm 2.72$	$117.57^{d} \pm 5.99$	$20.32^{a} \pm 1.56$	$61.13^{c} \pm 4.35$
Diabetic + Glibenclamide	$84.70^{c} \pm 3.63$	$101.97^{c} \pm 7.73$	$27.66^{b} \pm 1.53$	$36.64^{b} \pm 4.29$
Diabetic + Aqueous extract	$62.67^{a} \pm 1.11$	$41.89^{a} \pm 3.74$	$37.72^{c} \pm 3.24$	$22.56^{a} \pm 2.88$
Diabetic + Ethanolic extract	$78.96^{bc} \pm 3.63$	$64.64^{b} \pm 4.30$	$30.18^{b} \pm 1.46$	$35.85^{b} \pm 3.98$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a, b, c & d) in a column are statistically different from one another at 5 % level of significance
Values of TC (total cholesterol), TG (triglycerides), HDL (high density lipoproteins) and LDL (low density lipoprotein) are expressed in mg/dl

Significant ($P < 0.05$) increased levels of BUN and CR were observed in diabetic rats as compared to normal rats. Daily treatment with glibenclamide significantly ($P < 0.05$) reduced BUN and CR levels as compared to diabetic rats whereas value of BUN was still significantly higher than normal control. Either of the floral extract of *C. officinalis* used for daily treatment failed to reduce the levels of BUN, CR in any significant manner from their counterpart in the diabetic group (Tables 3 and 4).

Antioxidant parameters in blood

Increased ($P < 0.05$) levels of TOS, OSI and significant ($P < 0.05$) reduction in TAS were observed in diabetic rats as compared to normal rats. Repeated treatments with glibenclamide normalize the levels of TAS, TOS and OSI in diabetic rats. Treatment with aqueous floral extract of *C. officinalis* in diabetic rats significantly ($P < 0.05$) increased the TAS level but this level was still significantly ($P < 0.05$) lower than the normal rats. However, treatment didn't lower the increased levels of TOS and OSI as compared to normal rats although the levels were significantly ($P < 0.05$) reduced than the levels of diabetic group. Treatment with ethanolic floral extract of *C. officinalis* in diabetic rats restored the TOS and OSI levels and significantly ($P < 0.05$) improved the level of TAS as compared to control group. GSH and TTH were significantly ($P < 0.05$) reduced in diabetic rats but treatment with glibenclamide for 21 days these were significantly ($P < 0.05$) increased bringing TTH levels similar to control group. Treatment with aqueous floral extract of *C. officinalis* non-significantly increased GSH and TTH as compared to diabetic rats but daily administration of ethanolic floral extract of *C. officinalis* in diabetic rats restored GSH and TTH levels were similar to the normal control (Figure 1).

Activities of antioxidant enzymes like CAT, SOD, GPx, GST and G6PDH were significantly ($P < 0.05$) reduced in untreated diabetic rats as compared to normal rats. Treatment with glibenclamide in diabetic rats restored the activities of CAT, SOD, GST, G6PDH with activity of GPx being significantly increased ($P < 0.05$) from control rats. Aqueous floral extract administrations in diabetic rats restored the G6PDH and GPx activities, however activities of CAT, SOD and GST were significantly ($P < 0.05$) lower from their respective control. Treatment of diabetic rats with ethanolic floral extract of *C. officinalis* restored the activities of SOD, CAT, GST and significantly ($P < 0.05$) elevated activities of GPx and G6PDH than the normal control group (Figure 2).

Effect on Malondialdehyde (MDA) level

Malondialdehyde, end product of lipid peroxidation is used as an indicator of cellular damage on administration

Table 3 Effect of aqueous and ethanolic floral extracts of *C. officinalis* treatments on total plasma proteins (TPP), albumins, blood urea nitrogen (BUN), and creatinine (CR) of diabetic rats

Groups	BUN	CR	TPP	Albumins
Normal control	$51.92^{a} \pm 3.00$	$0.55^{a} \pm 0.03$	$7.81^{c} \pm 0.31$	$3.59^{b} \pm 0.13$
Aqueous extract	$55.05^{a} \pm 3.73$	$1.02^{b} \pm 0.08$	$6.92^{c} \pm 0.30$	$4.11^{c} \pm 0.20$
Ethanolic extract	$54.93^{a} \pm 4.37$	$0.88^{b} \pm 0.05$	$7.19^{bc} \pm 0.29$	$3.96^{bc} \pm 0.20$
Diabetic control	$97.27^{c} \pm 5.56$	$1.20^{c} \pm 0.10$	$5.94^{a} \pm 0.30$	$2.70^{a} \pm 0.11$
Diabetic + Glibenclamide	$74.17^{c} \pm 4.78$	$0.60^{a} \pm 0.04$	$6.33^{b} \pm 0.26$	$2.80^{a} \pm 0.11$
Diabetic + Aqueous extract	$83.31^{bc} \pm 2.86$	$0.59^{a} \pm 0.03$	$6.08^{a} \pm 0.38$	$2.38^{a} \pm 0.18$
Diabetic + Ethanolic extract	$94.42^{c} \pm 6.97$	$0.62^{a} \pm 0.03$	$5.97^{a} \pm 0.26$	$2.57^{a} \pm 0.16$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a, b, c & d) in a column are statistically different from one another at 5 % level of significance
Values of BUN, CR are expressed in mg/dl
TPP (total plasma proteins), albumin, are expressed in g/dl.

Table 4: Effect of aqueous and ethanolic floral extracts of *C. officinalis* treatments on plasma activitie of transferases (AST & ALT), phosphatase (ALP) and dehydrogenase (LDH) in diabetic rats

Groups	ALT	AST	ALP	LDH
Normal control	$54.53^{a} \pm 3.20$	$68.04^{a} \pm 5.82$	$336.97^{a} \pm 27.19$	$277.07^{b} \pm 18.89$
Aqueous extract	$89.87^{b} \pm 7.82$	$172.37^{c} \pm 8.61$	$434.58^{a} \pm 35.94$	$204.35^{a} \pm 14.36$
Ethanolic extract	$94.03^{b} \pm 2.30$	$99.83^{b} \pm 7.10$	$460.53^{ab} \pm 45.44$	$233.42^{a} \pm 21.86$
Diabetic control	$109.70^{c} \pm 7.95$	$192.58^{c} \pm 9.28$	$640.55^{c} \pm 53.80$	$388.18^{bc} \pm 29.11$
Diabetic + Glibenclamide	$59.21^{a} \pm 2.51$	$101.88^{b} \pm 5.51$	$343.92^{a} \pm 26.37$	$345.63^{c} \pm 13.48$
Diabetic + Aqueous extract	$74.44^{b} \pm 7.26$	$106.60^{b} \pm 5.94$	$453.00^{a} \pm 64.30$	$266.97^{a} \pm 27.96$
Diabetic + Ethanolic extract	$75.43^{b} \pm 6.57$	$66.91^{a} \pm 3.02$	$574.35^{bc} \pm 42.63$	$222.38^{a} \pm 19.17$

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a, b, c & d) in a column are statistically different from one another at 5 % level of significance
Values of AST & ALT (aspartate & alanine aminotransferase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase) are expressed in U/L

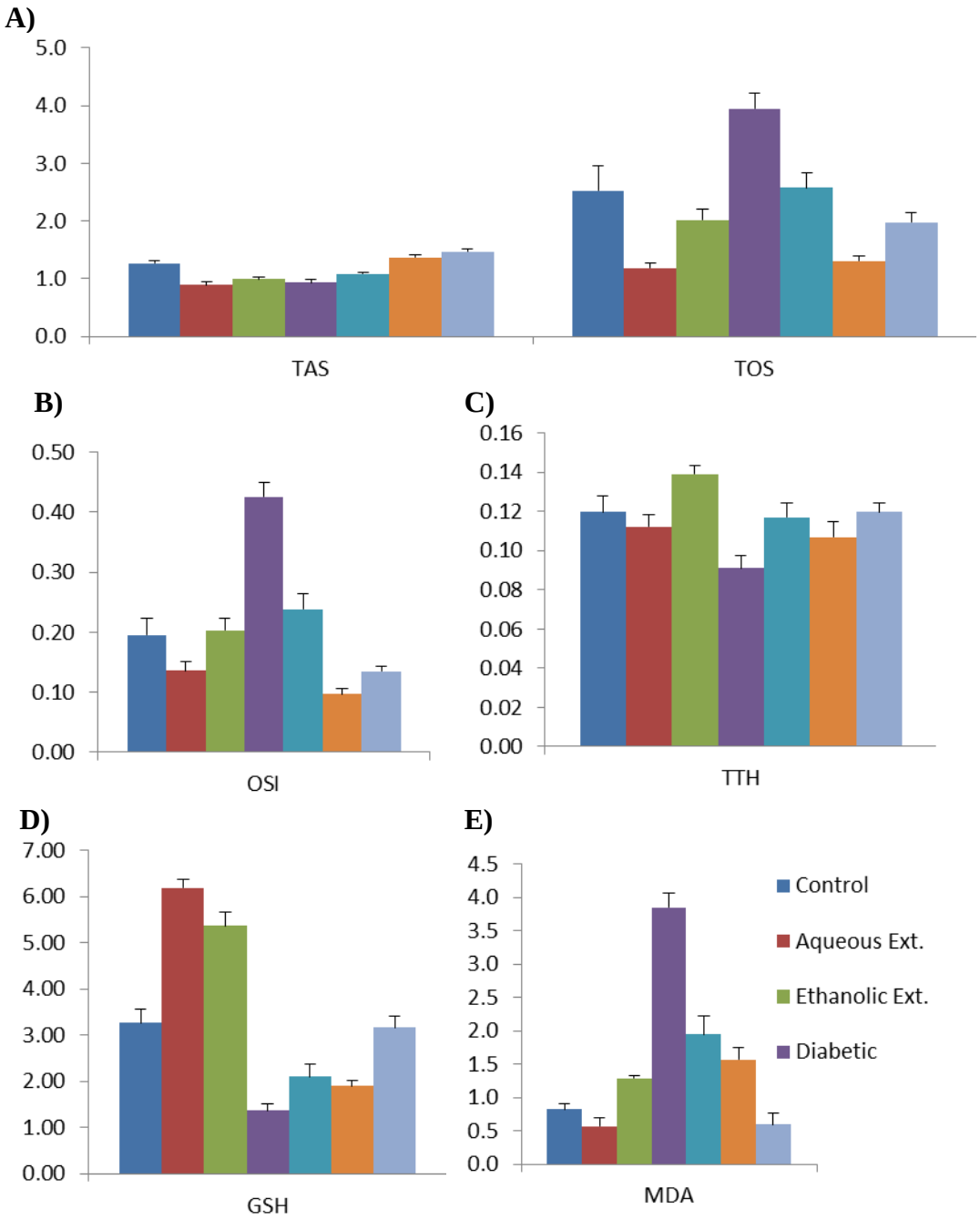

Figure 1 Effect of aqueous and ethanolic floral extracts of *C. officinalis* administrations on blood level of (A) TAS (mM), TOS ((µmol H_2O_2Equiv/ L), (B) OSI, (C) TTH (mM), (D) GSH (mM) and (E) MDA levels (nmole of MDA formed/ml/h) in diabetic rats.

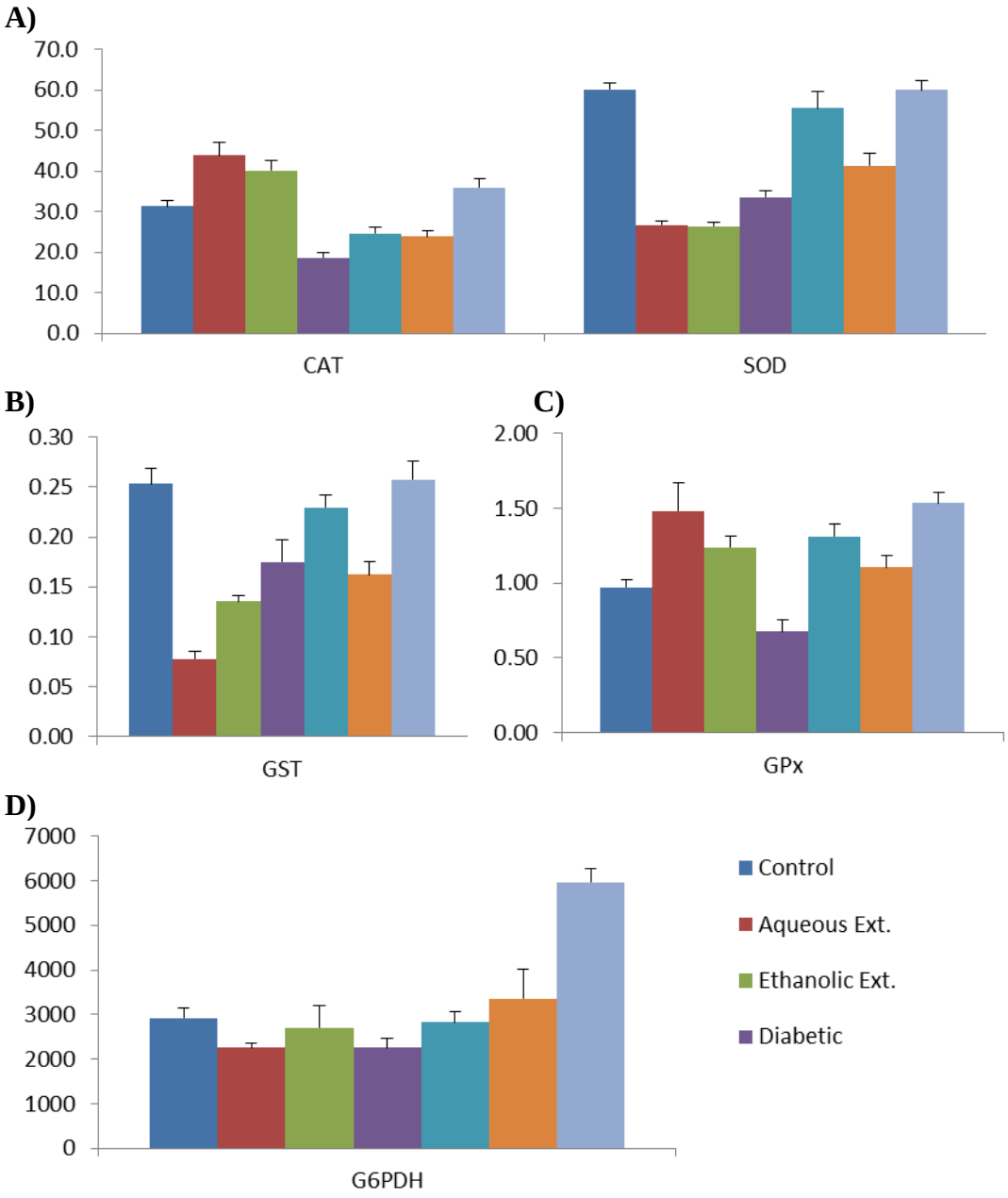

Figure 2 Effect of aqueous and ethanolic floral extracts of *C. officinalis* administrations on erythrocyte activities of (A) CAT (µmol H_2O_2 decomposed/ min/ mg of Hb), SOD (Unit/ mg of Hb), (B) GST (µmol of CDNB conjugate formed/ min/ mg of Hb) and (C) GPx (Unit/ mg of Hb) and (D) G6PDH activities expressed in U/L in diabetic rats

of the toxicant and/disease condition. MDA level in diabetic rats was significantly ($P < 0.05$) higher from the non-diabetic rats. Treatment of diabetic rats with glibenclamide significantly ($P < 0.05$) reduced the level of MDA as compared to diabetic rats and values are still significantly ($P < 0.05$) higher from the control animals. Diabetic rats exhibited significant ($P < 0.05$) reduction in MDA level on treatment with aqueous floral extract of *C. officinalis* but with ethanolic floral extract significantly ($P < 0.05$) reduced the MDA level as compared to diabetic rats and level was not significantly different from the normal control (Figure 2).

Discussion

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia with impaired carbohydrate, fat and protein metabolism (33). In the last few decades, increasing attention has been paid to the development of herbal medicines as a newly emerging treatment for diabetic complications. Single intra peritoneal administration of STZ significantly increased the level of MBG primarily due to oxidative damage in ß-cells of pancreas (34, 35). Persistent increased MBG causes glycation of proteins leading to increased level of HbA1c. International expert committee guidelines have also recommended monitoring the level of HbA1c fraction to assess the intensity of diabetic complications (36). Evidence showed that glycation itself may induce the formation of oxygen-derived free radicals and is considered a very sensitive index for glycemic control and degree of oxidative stress in diabetes (3). Glycation of major cellular proteins of visceral organs are the major factor in the pathogenesis of diabetes associated complications (3, 37). Apart from this, AGEs proteins also get accumulated as persisting molecules in tissues and generate abnormalities in cellular functions (38). Repeated oral administrations of either aqueous or ethanolic floral extract of *C. officinalis* in diabetic rats restored the level of MBG and per cent HbA1c indicating the floral parts of plant has hypoglycemic potential in STZ induced diabetes. The exact mechanisms of lowering MBG and per cent HbA1c by plant extracts is not clear. Studies have reported that alkaloids and flavonoids present in the various plant extracts produce hypoglycemic action by restoring insulin levels either from pancreatic tissue or extra-pancreatic mechanisms and/or decrease in the intestinal absorption of glucose or stimulation of peripheral glucose utilization (39, 40). Rao *et al.* (41) reported terpenoids present in the plant extract reduce diabetic complications by inhibiting aldose reductase and formation of AGEs complex.

Elevated HbA1c in diabetic and non-diabetic subject has been regarded as an independent risk factor for cardiovascular disorders (42, 43). Alterations in lipid profile is the major risk for the atherosclerotic heart disease and in present study increased levels of TC, TG, LDL and reduced HDL level indicate the cardiovascular disorders associated with diabetes. Repeated administrations of aqueous or ethanolic floral extracts of *C. officinalis* reduced the levels of TC, TG and maintained the levels of HDL and LDL in diabetic rats showing that floral extracts have potential to correct the lipid profile in diabetic rats also. The similar observations have been reported from leaf fractions of *C. officinalis* (44). High concentrations of lycopene, coumarins and carotenoids present in floral extract of *C. officinalis* (11, 45) may contribute to hypolipidemic potential (44).

Effect on renal parameters

In diabetes, nephropathy, retinopathy, neuropathy etc are the major complications developed mainly due to persistent rise in blood glucose level. Intensity of diabetic complications in different organs depends on the duration of diabetes and glycemic control. Significantly increased activities of phosphatases, transferases and dehydrogenases and increased BUN and CR levels in the present study indicated altered renal functions. Studies have shown the increased glycation (AGEs) of functional and structural proteins is involved in the pathogenesis of renal and other cellular dysfunctions during diabetes (46, 47). Repeated oral administrations of either aqueous or ethanolic floral extract in diabetic rats restored the activities of ALP, LDH, ALT and CR level, whereas BUN level remains high. The restoration of blood glucose and correction muscular tissue functions may be responsible for the correction of CR levels on repeated oral administrations of floral extracts in diabetic rats. But, reduced level of plasma proteins in diabetic rats on treatment with extracts indicates increased protein catabolism which may be responsible for increased BUN level. The floral

extract of *C. officinalis* is a rich source of polyphenolic compounds especially alkaloids, flavonoids and carotenoids (11). The flavonoids present in the floral extract reduce renal and muscular damage may be due to detoxifying ROS/RNS products by increasing the availability of GSH (48) and/or inhibitory effects on nitric oxide production in diabetic animals (49).

Antioxidant indices

Number of studies indicated that increased glycation of functional and structural proteins (AGEs formation) associated oxidative damage is primarily responsible for the diabetic complications (50). Although, mammalian cells are endowed with strong antioxidant defense comprising of enzymatic components like CAT, SOD, GPx, GST, G6PDH and non-enzymatic components (GSH, TTH) to scavenge these radicals for restoration of the antioxidant balance. In the present study increased plasma levels of TOS, OSI and reduced TAS, TTH, GSH levels indicate imbalance in antioxidant and oxidant ratio leading to oxidative stress in diabetic rats. Increased production of oxidants with reduced cellular antioxidant status in diabetic rats increased free radicals induced cellular damage as indicated by increased plasma levels of muscular and renal indices (51). Repeated administrations of aqueous floral extract of *C. officinalis* in diabetic rats significantly increased the TAS and restored the TOS and OSI to values of control group. However, treatment with ethanolic extract significantly increased TAS but failed to restore the levels of TOS and OSI in diabetic rats. Increased TAS values on repeated extract administration enhanced antioxidant defense either by direct scavenging the ROS/RNS radicals or increased levels of total thiols (TTH) and GSH levels (17).

The reduction in activities of CAT, SOD, GST, GPx and G6PDH in diabetic rats in present study indicates reduced scavenging mechanism leading to accumulation of these radicals in intra and extra cellular medium. The consequence of reduced activity of G6PDH leads to reduced cytosolic concentration of NADPH which is normally required as critical cofactor for CAT and glutathione reductase (52, 53) and makes cell vulnerable to oxidant damage (54). Repeated administration of ethanolic extract in diabetic rats restored the level of G6PDH which may be responsible for normalizing the activity of CAT and level of GSH by providing cytosolic concentration of NADPH. In mammalian tissues, thiols (protein and non-protein thiols) plays a pivotal role in scavenging small oxidants by directly interacting with them and provide protection against oxidative stress (cofactor for CAT, GPx) induced by reactive oxygen/nitrogen species (ROS/RNS). In the present study levels of total thiols (non-protein and protein -SH) reduced significantly in diabetic rats as compared to control group. Reduced GSH levels adversely affect the activities of SOD and GPx in diabetic rats (55).

Repeated administration of either aqueous or ethanolic extract of *C. officinalis* in diabetic rats normalized the activities of G6PDH, GST, GPx and CAT which catalyses/metabolizes the free radicals/ROS generated during hyperglycemia. Normalization of various parameters in diabetic rats by repeated administration of either aqueous or ethanolic floral extracts of *C. officinalis* may be due to presence of high polyphenolic and flavonoids constituents. Further *in vitro* studies have shown that floral extract have high total antioxidant capacity, free radicals, superoxide radicals, hydroxyl, and nitric oxide radicals scavenging potential (11, 57). Further presence of flavonoids, triterpenoids and alkaloids were identified in *C. officinalis* plants which may contribute to antidiabetic potential (58, 59). The flavonoids and triterpenoids posses direct antioxidant effect and also by induction of nuclear factor 2- antioxidant response element (Nrf2-ARE) involved in stimulating cellular defense (60, 61).

Conclusion

Hypoglycemic, hypolipidemic, restored antioxidant level, and reduced altered renal functions by the repeated administrations of floral extract of *C. officinalis* in diabetic rats. Further, modulatory effect was better in aqueous as compared to ethanolic floral extract of *C. officinalis* administration. Oxidative stress plays a central role in diabetes and its complications, thus supplementation with phytochemical ingredients endowed with high antioxidant potential could be of interest, by allowing a delay in the appearance or in the development of diabetic complications in animals.

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Conflict of Interest

Authors declare there are no conflicts of interest.

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