

Modulatory effect of baicalein on gene expression and activity of antioxidant enzymes in streptozotocin-nicotinamide induced diabetic rats

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Oxidative stress plays the central role in the pathogenesis and progression of diabetic complications. The present study aims to investigate the beneficial effect of oral administration of flavone baicalein in streptozotocin-nicotinamide (STZ-NA) induced diabetic rats by measuring oxidative stress markers, antioxidant enzyme activities and expression analysis of antioxidant genes. Experimental diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (55 mg/kg b.wt), 15 min after the i.p. administration of NA. At the end of the experimental period, thiobarbituric acid reactive substances (TBARS), activities of antioxidant enzymes and expression levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx) were measured in diabetic rats along with serum biochemical parameters namely total cholesterol (TC), total triglyceride (TG), aspartate transaminase (AST) alanine transaminase (ALT) and glycosylated hemoglobin (HbA1c). Oral administration of baicalein (40 mg/kg b.wt/day) demonstrated a significant ameliorative effect on all studied biochemical and oxidative stress parameters. Biochemical findings were corroborated by qPCR expression analysis which showed significant upregulation of antioxidant genes in diabetic rats. These results suggest that baicalein supplementation may reduce diabetes and its complications by suppressing oxidative stress and enhancing gene expression and antioxidant enzyme activities in diabetic rats.

Keywords: Diabetes. Streptozotocin/pharmacology. Baicalein/pharmacology. Nicotinamide/pharmacology. Glibenclamide. Diabetes Mellitus/ Experimental/prevention and control. Gene Expression/ drug effects. Antioxidants/pharmacology. Glyburide/pharmacology. Rats.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated levels of glucose in the blood (hyperglycemia) and insufficiency of production or action of insulin produced by the pancreas in our body (Maritim, Sanders, 2003). Diabetes is currently affecting more than 100 million people worldwide. Oxidative stress plays a pivotal role in the onset and progression of diabetic complications (Ayepola, Brooks, Oguntibeju, 2014). Oxidative stress results from an imbalance between the production and neutralization of reactive oxygen species (ROS) such as highly reactive hydroxyl radicals, superoxide anion, peroxy radicals, singlet oxygen, peroxynitrite, and hydrogen peroxide (Sellamuthu *et*

al., 2013). Oxidative stress-induced complications from diabetes include neuropathy, coronary artery disease, nephropathy, retinopathy (Phillips *et al.*, 2004). There are several reports on altered antioxidant defenses and the role of free radicals in the etiology of diabetes and its complications (Raza *et al.*, 2011). While the external supply of insulin and other medications can control many aspects of diabetes, numerous complications that affect the kidney, peripheral nerves, vascular system, retina, lens, and skin are common and are extremely important factors in terms of longevity and quality of life (Maritim, Sanders, 2003). Increased oxidative stress is a key participant in the development and progression of diabetes and different diabetes-related complications (Giacco, Brownlee, 2010). It is very important to explore the relationship between free radicals, diabetes, and its complications, and to reveal the mechanisms by which increased oxidative stress accelerates the development of diabetic complications, in an effort to find effective treatment options.

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Neutralization of oxidants by increased antioxidant availability may mitigate the effects associated with oxidative stress in DM. The antioxidant systems that fight free radical-mediated damage involves enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) as well as non-enzymatic substances such as vitamins A, C and E, glutathione, α -lipoic acid, carotenoids, trace elements like copper, zinc and selenium, as well as coenzyme Q10 (Dehghan, Gargari, Asgharijafarabadi, 2013). As a traditional medicine, plants represent a valuable alternative to control and treat diabetes mellitus with minimal or no side effects in clinical settings, plants also entail relatively lower costs than oral synthetic hypoglycemic agents (Gupta *et al.*, 2005). According to ethnobotanical reports, approximately 800 plants exhibit antidiabetic properties (Patel *et al.*, 2012), and many plants have been recommended for treatment of diabetes (Yajnik, 2001). The principal chemical compounds isolated and identified from these plants are glycans, proteins, and mucilages. Other compounds, such as phenolics, flavonoids, steroids, triterpenoids, and alkaloids, with hypoglycemic activity, have been extracted from these traditionally used medicinal plants by using different organic solvents (Gupta *et al.*, 2006).

One such flavonoid found in the plant *Scutellaria baicalensis* Georgi is baicalein which shows antioxidant (Gao *et al.*, 1999), anti-inflammatory (Chi *et al.*, 2003), and anti-cardiovascular effects (Wang, Mehendale, Yuan, 2007). *S. baicalensis* is a medicinal plant which is cultivated in Siberia, Mongolia, the Russian Far East, China, and Korea. The root of *S. baicalensis*, also known as Huangqin in Chinese, has been used as an ingredient in traditional Chinese medicine formulations for thousands of years. There are more than 40 flavonoids identified from Huangqin that are primarily conjugated with a glucose molecule to form glycosides. Baicalein, baicalin, wogonin, and wogonoside are the main bioactive components found in Huangqin (Li-Weber, 2009). Studies on whether baicalein has an anti-diabetic effect are scarce. But the evidence suggests that baicalein have effects on diabetes-related complications. Previous studies of Fu *et al.* (2014) indicated that baicalein reduces blood glucose levels, improves circulating insulin concentration and preserves islet β -cell mass in obese diabetes mice, suggesting that this compound may have an anti-diabetic effect as loss of β -cell mass and function is central to the development of diabetes.

Streptozotocin-nicotinamide (STZ-NA) has been extensively used to induce diabetes in experimental

animals (Aboonabi, Rahmat, Othman, 2014). STZ causes pancreatic B-cell damage, while NA is administered to partially protect insulin-secreting cells against STZ (Szkudelski, 2012). The cytotoxic action of STZ is associated with the production of ROS which causes oxidative stress and oxidative damage in the cells (Lenzen, 2008). Lipid peroxidation is the main marker for oxidative stress determination. Increased oxidative stress denoted by indices of increased lipid peroxidation, depletion of endogenous antioxidant enzyme activities in plasma etc. is commonly observed in the STZ-NA induced diabetic rats (Nizamutdinova *et al.*, 2009).

Glibenclamide (GC) is a Sulfonylurea anti-diabetic drug used in the treatment of diabetes mellitus. Several antioxidant studies suggest that GC (Elmali, Altan, Bukan, 2004) has the potential to counteract the ROS-mediated oxidative stress.

The present study was undertaken to evaluate the potential effect of baicalein to protect the antioxidant status and ameliorate oxidative stress in STZ-NA diabetic rats. Additionally, the effects of baicalein on the expression levels of antioxidant genes in liver tissues of STZ-NA induced diabetic rats using qPCR system were studied and compared to glibenclamide, a hypoglycemic drug used for type 2 diabetes treatment. As diabetes is a growing public health problem, it is very important to search for novel, low side effects and affordable compounds which can attenuate induced oxidative stress and provide effective treatment for this disease. The present work aims towards this goal.

MATERIALS AND METHODS

Animals

Thirty adult male albino Wistar rats weight (180 - 200 g) were purchased from College of Veterinary Sciences, Khanapara, Assam, India. The animals were housed in standard polypropylene cages with 12 h dark/light cycles and were maintained at 25 ± 2 °C. Animals were allowed to acclimatize for one week, prior to the experimental procedure. An ethical clearance approval for animal use and the experimental protocol was obtained from Gauhati University Institutional Ethical committee (Animal ethics approval number: IAEC/PER/2016/2017-2 dated 30/01/2017). The rats were fed a commercial standard pellet diet (Lab 030525, Hindustan Animal Feeds, Gujrat, India), with the chemical composition of protein 24.6 %, fat (Ether extract) 4.8 %, fat (Acid hydrolysis) 5.5 %, crude fiber 4.1 %, nitrogen-free extract 50.1%.

Chemicals and drugs

Baicalein, Glibenclamide, Nicotinamide, and Streptozotocin were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA) and were stored at 2 - 4 °C for further use. All the other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Experimental design

All the experimental rats were divided into six groups, each comprising of five rats designated as follows: Group 1 (NC): Normal control rats receiving 0.1 M citrate buffer (pH 4.5).

Group 2 (NC+BC): Normal control rats treated with baicalein (40 mg/kg b.wt/day) for 30 days.

Group 3 (NC+GC): Normal control rats treated with glibenclamide (0.6 mg/kg b.wt/day) for 30 days.

Group 4 (DC): Diabetic control treated with STZ+NA (55 and 120 mg/kg b.wt/day respectively).

Group 5 (DC+BC): Diabetic rats treated with baicalein (40 mg/kg b.wt/day) for 30 days.

Group 6 (DC+GC): Diabetic rats treated with glibenclamide (0.6 mg/kg b.wt/day) for 30 days.

At the end of the total experimental period, the animals were fasted overnight, recorded the final body weight and sacrificed, by cervical dislocation. The serum samples of each rat were obtained by centrifuging blood samples at 4000 x g at 25 °C for 4 min and stored at -20 °C for further biochemical parameters analysis.

Induction of diabetes

Overnight fasted rats were injected with a single intraperitoneal (i.p.) injection of freshly prepared STZ (55mg/kg b.wt.) in 0.1 M cold citrate buffer (pH 4.5), and control rats were injected with citrate buffer alone. Nicotinamide was dissolved in normal saline and administered (120 mg/kg b.wt.) 15 min before STZ administration (Masiello *et al.*, 1998). The rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The development of diabetes was confirmed by the elevated glucose levels in the blood, which was determined at 72 h by means of a glucometer (Roche Diabetes Care India Pvt. Ltd. Mumbai, India). The rats with fasting blood glucose level higher than 180 mg/dl were considered to be diabetic and used in the experiment. The treatment was started on the 4th day after STZ injection, considering that as the 1st day of treatment, which was continued for 30 days.

Preparation of baicalein

Baicalein (40 mg/kg b.wt/day) was dissolved in dimethyl sulfoxide (DMSO) before it was administered.

Preparation of glibenclamide

Glibenclamide (0.6 mg/kg b.wt/day) was dissolved in dimethyl sulfoxide (DMSO) before it was administered.

Preparation of tissue homogenate for biochemical parameters

For tissue preparation, the animals were fasted overnight, anesthetized and sacrificed by cervical dislocation. The liver tissues from all the rats were excised and rinsed with ice-cold saline. The preparation of tissue homogenates was done homogenizing a known amount of the liver in 0.1 M Tris-HCl buffer, pH 7.4 at 4 °C, employing a mortar and a pestle. The homogenates were centrifuged at 3000 x g for 10 min at 4 °C using a centrifuge. The supernatant was collected as tissue homogenate, and the same was used for the assay of oxidative stress markers.

Biochemical analysis

The biochemical parameters for total cholesterol (TC), total triglyceride (TG), aspartate transaminase (AST) and alanine transaminase (ALT) was done by using commercial kits (Span Diagnostics Ltd., India).

Estimation of thiobarbituric acids content

Tissue homogenates were used for the oxidative stress marker estimations. The thiobarbituric acids content was assayed by measuring thiobarbituric acid reacting substances (TBARS) according to Ohkawa, Ohishi, Yagi (1979). Briefly, to 0.2 mL of tissue homogenate, 0.2 mL of SDS (8.1 %), 1.5 mL of acetic acid (20 %, pH above 3), and 1.5 mL of TBA (0.8 % aq sol) were added. The mixture was taken to a final volume of 4 mL with water and then heated in a water bath at 95 °C for 60 min. After cooling with tap water, 1 mL of water and 5 mL of n butanol/pyridine (15:1 v/v) mixture were added and shaken vigorously. After that it was centrifuged at 4000 x g for 10 min; then, the organic layer was taken and measured at 532 nm absorbance. The 1,1',3,3'-tetramethoxypropane was used as a standard. The level of lipid peroxides was expressed as moles of TBARS / 100 g of tissue.

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase was assayed following the method of Misra, Fridovich (1972). The tissue homogenate (0.1 mL) was mixed by reaction mixtures that contained sodium carbonate (1 mL, 50 mM), nitroblue tetrazolium (0.4 mL, 25 μ M), and hydroxylamine hydrochloride (0.2 mL, 0.1 mM). The absorbance of the samples was measured at 560 nm.

Determination of catalase (CAT) activity

The activity of catalase was assayed according to the method of Takahara *et al.* (1960). Phosphate buffer (1.2 mL) and 0.2 mL of tissue homogenate were mixed, and the reaction was started by the addition of 1.0 mL of H₂O₂ solution. The decrease in the absorbance is measured at 240 nm at 30 s intervals for 3 min.

Determination of reduced glutathione (GSH) activity

Reduced glutathione was determined by the method of Sedlak, Lindsay (1968). 0.5 mL of tissue homogenate was mixed with 1.5 mL of 0.2 M Tris buffer with pH 8.2 and then contents were mixed with 0.1 mL of 0.01 M Ellman's reagent, (5,5'-dithiobis-2-nitro-benzoic acid or DTNB), then centrifuged at 3000 x g for 15 min at RT. The absorbance was read at 412 nm. The GSH content was calculated as mole GSH conjugate formed/gram tissue using molar extinction coefficient of $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Determination of glutathione peroxidase (GPx) activity

The activity of glutathione peroxidase was assayed by the method of Rotruck *et al.* (1973). The reaction mixture containing 0.2 mL of EDTA, 0.1 mL of sodium azide, 0.1 mL of H₂O₂, 0.2 mL of reduced glutathione, 0.4 mL of phosphate buffer and 0.2 mL tissue homogenate was incubated at 37 °C for 10 min. The reaction was arrested by the addition of 3 mL of disodium hydrogen phosphate and 1.0 mL DTNB was added to the supernatant, and the colour developed was read at 420 nm immediately. The enzyme activity was calculated using the molar extinction coefficient.

Extraction of RNA and cDNA Conversion from liver tissue for qPCR analysis

To study the expression pattern of oxidative stress

genes, approximately 300 mg of each rat liver tissue was cleaned with saline. Total RNA was isolated using TRIzol reagent according to the manufacturer's protocol (Life Technologies, Carlsbad, USA). RNA purity and concentration were measured by the NanoDrop quantifier (ThermoFisher Scientific, Waltham, Massachusetts, USA). For cDNA conversion, two micrograms of total RNA was reversed transcribed in a total reaction volume of 20 μ L by using Superscript III Reverse Transcriptase (Invitrogen, Life Technology, Carlsbad, USA) enzyme following manufacturer's protocols. cDNA produced was further amplified by the qPCR system using the designed set of primers listed in Table I. qPCR was performed with Rotor-Gene Q (Qiagen, Hilden, Germany) PCR System using SYBR *PremixEx* Taq II master mix according to the protocols provided by the manufacturer (TAKARA BIO INC. Shiga, Japan). Briefly, PCR was performed in a final volume of 20 μ L including 2 μ L of 100 ng sample cDNA, 0.2 μ L of 100 nM forward and reverse primers, and 10 μ L Power SYBR green PCR Master Mix. The following thermal cycling profile was used (40 cycles): 94 °C for 20 s, and 56 °C for 20 s, 72 °C for 20 s. Standard curves for each gene were generated by 1:5 dilutions of cDNA to determine the PCR efficiency of target and reference gene. Standard curves were established by plotting the threshold cycle Ct vs. Log₁₀ (cDNA). The slope (S) obtained from the standard curve was used to calculate PCR efficiency by the following equation: PCR efficiency (E) = $(10^{-1/\text{slope}} - 1) \times 100$. Using the Ct comparative method, the relative abundance of the target transcript was calculated from triplicate samples after normalization

TABLE I - Primers used in qPCR

Gene	Primer (5'-3')	
SOD	Forward	GTCCACGAGAAACAAGATGA
	Reverse	ATCCCAATCACACCACAAG
CAT	Forward	GAATGAGGAGGAGAGGAAAC
	Reverse	GGTGGACGTCAGTGAAAT
GSH	Forward	CCCAATGGAGTAACAAGTAGG
	Reverse	GAGCAGGAAGGAGAGAGAA
GPx	Forward	GGGACTACACCGAAATGAA
	Reverse	CCTGATGTCCGAACTGATT
GAPDH	Forward	TGCACCACCAACTGCTTAGC
	Reverse	GGATGCAGGGATGATGTTCT

against a housekeeping gene. The housekeeping gene *GAPDH* was chosen in this study specifically as the most stably expressed reference gene for normalization to ensure reliable results and highest accuracy of the analysis. The mRNA levels (arbitrary units) are expressed in relation to those of the control rats. Relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct = (Ct, \text{target} - Ct, \text{GAPDH}) \text{ treated sample} - (Ct, \text{target} - Ct, \text{GAPDH}) \text{ control sample}$. qPCR results are presented as the mean and SD of the relative fold changes to $2^{-\Delta\Delta Ct}$ (Livak, Schmittgen, 2001).

Statistical analysis

All data are expressed as means and standard deviation. Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by Tukey's Multiple Comparison Test using GraphPad Prism Software and $P < 0.05$ was considered as significant. ** $P < 0.05$; *** $P < 0.01$ when compared with control group and ## $P < 0.05$; ### $P < 0.01$ when compared with diabetic group.

RESULTS

Effect of the baicalein treatment on blood glucose level, body weight, HbA1c and TBARS level

Measurement of blood glucose levels and body weight are important factors in the diabetic study (Pournaghi *et al.*, 2012). Diabetic rats showed a significant ($P < 0.05$) increase in blood glucose levels and significant ($P < 0.05$) decrease in body weight when compared to normal control rats. Administration of baicalein (40 mg/kg b.wt.day) in diabetic rats significantly ($P < 0.05$) lowered the blood glucose level (Figure 1A) and increased body weight (Figure 1B) when compared to untreated diabetic rats. Similarly, HbA1c level in diabetic rats receiving baicalein treatment was significantly ($P < 0.05$) lower than for untreated diabetic rats (Figure 1C). The level of TBARS has been demonstrated to be a reliable marker for oxidative stress (Al-Dosari *et al.*, 2017). There was a significant ($P < 0.05$) increase in TBARS in the liver of diabetic rats compared to normal control rats. Baicalein

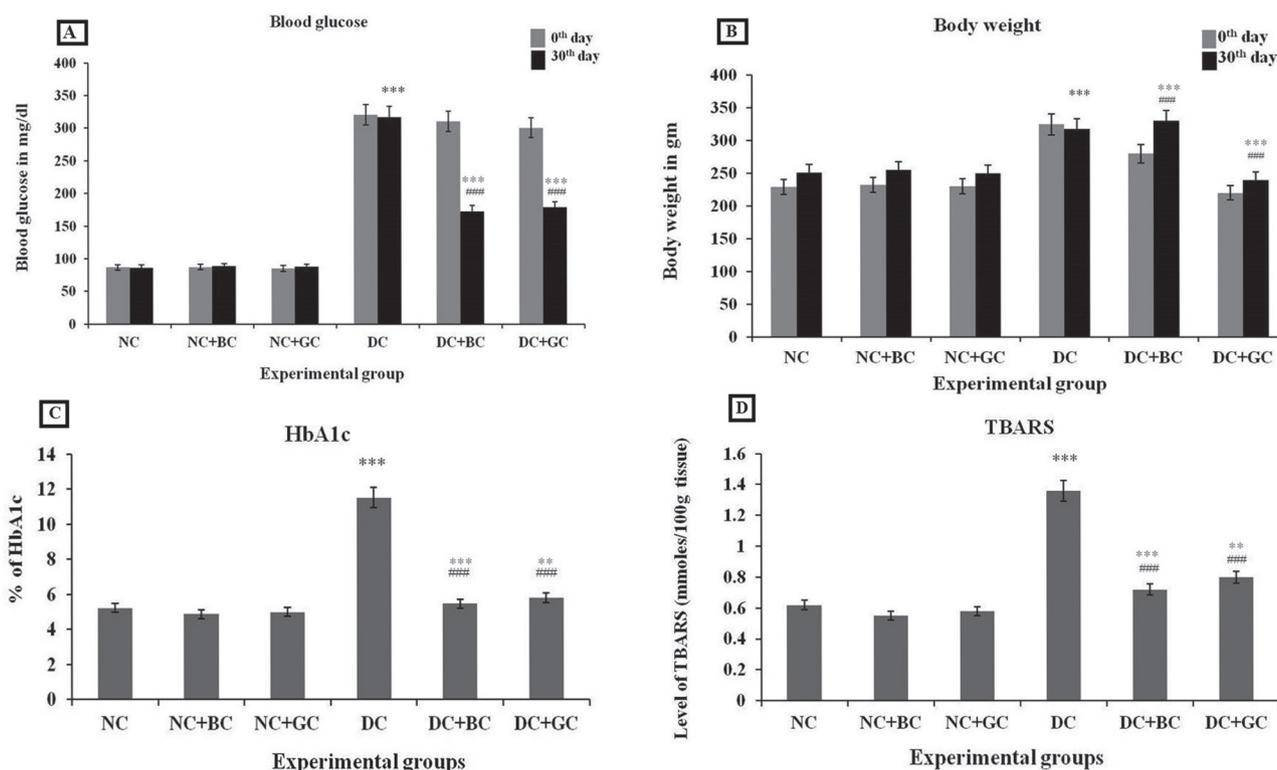


FIGURE 1 (A-D) - Effect of baicalein supplementation on blood glucose, body weight, % HbA1C and TBARS levels of rats in different experimental groups. Data were expressed as mean \pm SD and analyzed using one-way ANOVA followed by Tukey's Multiple Comparison Tests. Values are statistically significant at $P < 0.05$; ** $P < 0.05$; *** $P < 0.01$ when compared with control group and ## $P < 0.05$; ### $P < 0.01$ when compared with diabetic group. NC: normal control, NC+BC: normal control treated with baicalein, NC+GC: normal control treated with glibenclamide, DC: diabetic control, DC+BC: diabetic rats treated with baicalein, DC+GC: diabetic rats treated with glibenclamide.

treatment significantly ($P < 0.05$) inhibited the formation of TBARS in liver tissue and reduced TBARS level in the treatment group when compared to diabetic control rats (Figure 1D). Statistical differences ($P < 0.05$) were also found in (DC+BC) and (DC+GC) rats as compared to normal control rats in all the above studied parameters.

Effect of the treatment on biochemical parameters

The aspartate transaminase (AST) and alanine transaminase (ALT) activities were measured for the detection of possible liver damage. Activities of serum AST, ALT in the control and experimental groups are illustrated in Figure 2A and Figure 2B. There was a significant ($P < 0.05$) increase in the activities of AST, ALT in the serum of diabetic rats when compared to normal control rats. Treatment with baicalein to diabetic rats significantly ($P < 0.05$) decreased the enzymatic activities of AST and ALT. Significant ($P < 0.05$) reduction in triglyceride levels was observed in the treatment group compared to the diabetic control group (Figure 2C), which

indicates that baicalein possesses a hypotriglyceridemic effect. A similar trend was observed in total cholesterol levels (Figure 2D). Reduction of circulating triglyceride and total cholesterol is an important aspect of the treatment of diabetes (Bitzur *et al.*, 2009).

Effect of the treatment on activities of antioxidant enzymes and gene expression

The activities of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx) in the liver tissues of control and treatment group of rats are depicted in Figure 3(A-D). A significant ($P < 0.05$) reduction in the activities of SOD, CAT, GSH and GPx in the liver of STZ-NA administered rats was observed. However, treatment with baicalein and glibenclamide increased the level of enzymatic antioxidant in the liver of diabetic rats, this increase was more significant ($P < 0.01$) in baicalein than glibenclamide ($P < 0.05$) treatment groups. qPCR results showed that gene expression levels of main antioxidant enzymes *SOD*, *CAT*, *GSH*, and *GPx* were suppressed

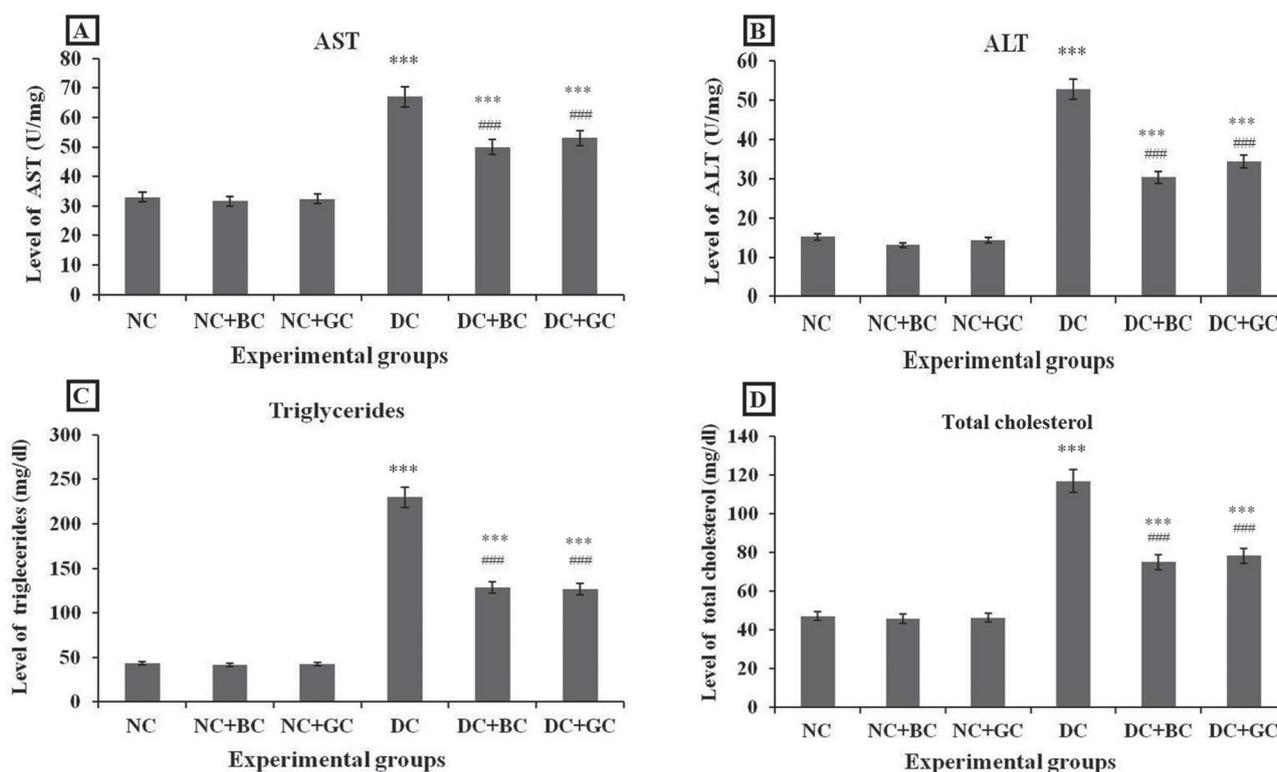


FIGURE 2 (A-D) - Effect of baicalein supplementation on AST, ALT, Triglycerides and Total cholesterol in different experimental groups. Data were expressed as mean \pm SD and analyzed using one-way ANOVA followed by Tukey's Multiple Comparisons Tests. Values are statistically significant at $P < 0.05$; ** $P < 0.05$; *** $P < 0.01$ when compared with control group and ## $P < 0.05$; ### $P < 0.01$ when compared with diabetic group. NC: normal control, NC+BC: normal control treated with baicalein, NC+GC: normal control treated with glibenclamide, DC: diabetic control, DC+BC: diabetic rats treated with baicalein, DC+GC: diabetic rats treated with glibenclamide.

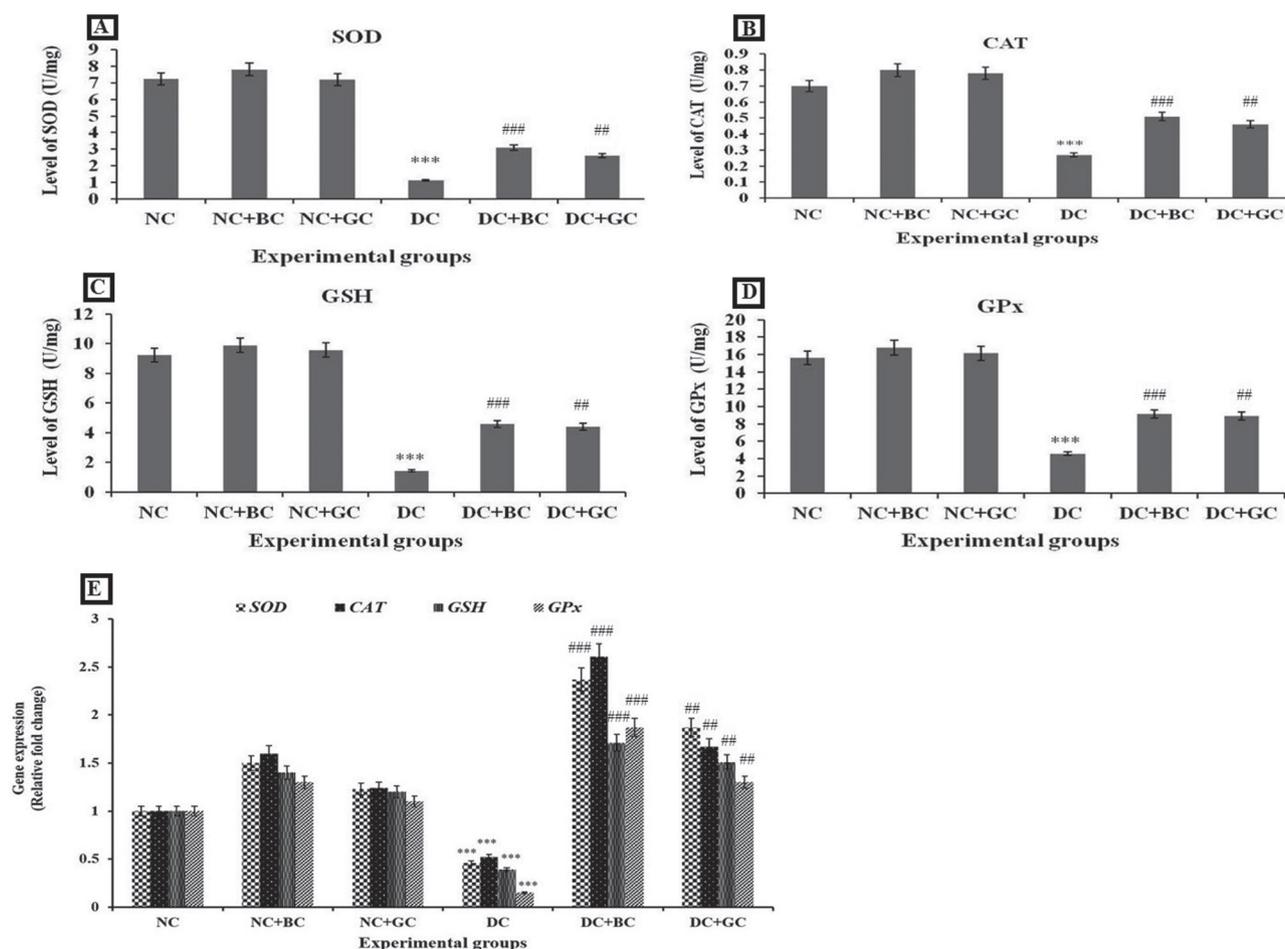


FIGURE 3 (A-E) - Effect of baicalein supplementation on activities of antioxidant enzymes and gene expression in different experimental groups. Data were expressed as mean \pm SD and analyzed using one-way ANOVA followed by Tukey's Multiple Comparisons Tests. Values are statistically significant at $P < 0.05$; $**P < 0.05$; $***P < 0.01$ when compared with control group and $##P < 0.05$; $###P < 0.01$ when compared with diabetic group. NC: normal control, NC+BC: normal control treated with baicalein, NC+GC: normal control treated with glibenclamide, DC: diabetic control, DC+BC: diabetic rats treated with baicalein, DC+GC: diabetic rats treated with glibenclamide.

in diabetic rats (Figure 3E). Treatment with baicalein significantly ($P < 0.05$) increased the expression of antioxidant genes in diabetic rat liver tissues. Expression levels were seen to be greater in baicalein treated rats than glibenclamide treated rats.

DISCUSSIONS

Diabetes mellitus is a severe health problem worldwide. An aging population, increased urbanization and more sedentary lifestyles have been linked to increase in the prevalence of diabetes (King, Aubert, Herman, 1998). The absence of a perfect cure for diabetes as well as the potential side effects of most of the antidiabetic medications has led many researchers to identify natural substances that show potent hypoglycemic activity with fewer side effects (Saxena, Vikram, 2004). As oxidative

stress plays the central role in the pathogenesis and progression of diabetic complications, therefore, it is believed that antioxidant compounds would prevent and/or delay the progression of diabetic complications (Brownlee, 2001). The present study was conducted to assess the effects of baicalein on diabetes-induced oxidative stress by evaluating antioxidant enzyme activities and expression of antioxidant genes in the STZ-NA induced diabetic rats. Administration of STZ partially damages pancreatic cells (Szkudelski, 2012). The induction of diabetes with STZ in rat models caused a significant increase in blood glucose level, showing type II-like features as compared to the control group of rats, thus developing a state of oxidative stress. Baicalein, a flavonoid obtained from the roots of medicinal herb *Scutellaria baicalensis* Georgi, has been reported to have beneficial effects in diabetes-associated health complications (Ndisang, 2010). However, Fox *et al.*

(2012) have demonstrated baicalein as an inducer of DNA damage and cell death by high-throughput genotoxicity assay in human ATAD5 cells. They showed that despite having genotoxic effects, baicalein did not cause any mutagenesis, further they observed that baicalein rapidly killed dividing cancer cells without causing potentially detrimental genomic instabilities and proved to be a chemotherapeutic agents. Recently, much interest has been focused on the antidiabetic effects of baicalein. In particular, it has been demonstrated that baicalein improves hyperglycemia and glucose intolerance, and promotes insulin secretion by inhibiting islet cell apoptosis in streptozotocin-induced diabetic mice (Fu *et al.*, 2014). Baicalin, the glucuronide form of baicalein, also exerts beneficial effects on diabetes mellitus and its complications according to recent studies (Pu *et al.*, 2012).

Lipid peroxide-mediated damage has been observed in the development of type 1 and type 2 diabetes. TBARS is the most commonly used indicator of lipid peroxidation (Lyons, 1991). In the present study, levels of TBARS were significantly elevated in liver, indicating increased oxidative stress and such results have also been represented by Jin *et al.* (2008) who reported elevated levels of TBARS in STZ-induced diabetic rats. Administration of baicalein significantly lowered the TBARS levels in diabetic treated groups compared with the untreated diabetic group, indicating inhibition of diabetes-induced oxidative stress. The present findings were in agreement with Sellamuthu *et al.* (2013) who reported reduced levels of TBARS upon oral administration of mangiferin and glibenclamide in diabetic rats in the liver and kidney tissues.

In general, increased blood glucose levels and severe loss of body weight characterize STZ-induced diabetes. The results of the present study are consistent with the observations of Gandhi, Sasikumar (2012). The spectrum of biochemical changes that typically occur in DM resembles those of liver diseases, from the secretion of abnormal liver enzymes to the end-stage liver failure (Moscatiello, Manini, Marchesini, 2007). The initial and most important indicators in assessing liver injury are levels of AST and ALT in *in vivo* (Hwang *et al.*, 2005). Baicalein reduced the plasma triglycerides and total cholesterol contents which is an important aspect of the treatment of diabetes. AST and ALT levels are also showed improvement in the baicalein treated group as compared to diabetic control group. In diabetes, renal and hepatic failures are known to be the long-term consequences, therefore possible effects of the treatment with antioxidant on the hepatic tissue can be demonstrated by comparing the plasma levels of AST and ALT activities (Nathan, 2014). In our study, the AST and ALT levels of the

baicalein treated group of rats were found to be within the normal range.

Diabetes-induced oxidative stress results in alteration of both enzymatic and non-enzymatic antioxidant systems. The liver is equipped with potent antioxidants systems such as SOD, CAT, and GSH enzyme family, including GST and GPx, not only to neutralize free radicals but also to protect the liver cells from oxidative damage (Parveen *et al.*, 2010). Hence, we assessed the activity of the most important antioxidant enzymes such as SOD, CAT, GSH, and GPx in liver tissue of diabetic rats upon baicalein treatment. The relative changes in activity in diabetic tissues compared with control tissues indicate an altered antioxidant system. In this context, treatment with baicalein appears to have resulted in a considerable reversal of antioxidant enzymes that were altered in diabetic tissues. In the present study, treatment with baicalein increased the activity of antioxidant enzymes as compared to diabetic rats.

Previous research has proven that a decrease in SOD and CAT activities within a hyperglycaemic state leads to an increase in ROS, which eventually contributes to oxidation-induced liver damage (Han *et al.*, 2006). The enzymatic activities of SOD, CAT, GSH, and GPx are significantly decreased in diabetes due to increased oxidative stress. In addition, to measure the activities of these enzymes, quantitative polymerase chain reaction (qPCR) was also used to study the expression levels of the antioxidant genes. In the present study, we found that the expression of these genes was decreased in the diabetic control group of rats as compared to the control group of rats. Induction of diabetes using STZ-NA was found to alter the expression of *SOD*, *CAT*, *GSH*, and *GPx*. The relative fold change of all the genes was observed to be significantly downregulated in diabetic control group and treatment with baicalein significantly upregulated the expression of all the tested genes. The present finding was in agreement with a recent study reporting that resveratrol administered to STZ-diabetic rats increased the gene expression of the antioxidant enzymes (Sadi, Baloglu, Pektas, 2015).

The importance of maintaining the redox balance and reducing the blood glucose levels plays important role in combating diabetic micro and macrovascular complications (Rask-Madsen, King, 2013). The present study showed that baicalein has the ability to ameliorate oxidative stress in liver of STZ-NA induced diabetic rats which were ascertained by improved glycemic and reduced lipid peroxidation along with improved antioxidant enzymatic activities. Moreover, it increased the expression pattern of the antioxidant genes in liver of

STZ-NA induced diabetic rats. Antioxidant therapy is a potential future therapeutic strategy; increasing antioxidant levels in patients with DM-induced oxidative damage that may hopefully counter the effects of oxidative stress and inflammation, thereby reducing the severity of diabetic complications. The intervention for oxidative stress reduction is necessary for the overall therapy of diabetes which highlights the importance of antioxidants such as baicalein in the treatment of diabetes. Furthermore, the present study may contribute for future studies to establish pharmacological targets for novel therapies to prevent, reverse, or delay the onset of diabetic complications.

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CONFLICT OF INTEREST

Authors declared that there is no conflict of interests.

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