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Evaluation of Antioxidant activity of Different parts of *Bauhinia Purpurea L.* in STZ-Induced Diabetic Rat

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ABSTRACT

Reactive oxygen species are increased by hyperglycemia. Hyperglycemia, which occurs during diabetes (both type 1 and type 2) and, to a lesser extent, during insulin resistance, causes oxidative stress. Oxidative stress may be important in diabetes because of its role in the development of diabetic complications. The present study was undertaken with the objective to evaluate and compare the antioxidant activity of crude ethanol extract of the two different plant parts (Barks and Leaves) of *Bauhinia purpurea* L. *in vivo* in order to select plant part with better efficacy to continue further studies with semi purified and purified fractions. Antioxidative effect *in vivo* was evaluated in Type 1 diabetes in albino wistar rats made diabetic by single i.p injection of streptozotocin at a dose level of 60mg/Kg BW. Diabetic rats were treated with both the extracts ($1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of respective LD_{50}) for twenty eight days orally once daily. On 28^{th} day animals were sacrificed under ether anesthesia and blood and tissues were collected and analysed for related biochemical parameters. Significant decrease in fasting blood glucose level, reduction in glycosylated hemoglobin in blood, and increased catalase level in packed RBC, reduced lipid peroxidation and increased cellular glutathione level(GSH/NPSH) in packed RBC and liver were observed with both the crude extracts. The results validate the *in vivo* antioxidant efficacy of both the extracts under our study. The efficacy of bark extracts came out better than the leaves extract on the basis of data accrued.

Keywords: *Bauhinia purpurea*; Barks and leaves; antioxidant activity; diabetic rats, Lipid peroxidation, GSH, NPSH.

INTRODUCTION

Bauhinia purpurea L. (BP) belonging to the family *Caesalpiniaceae*, is such a plant locally known as Kanchan,Rakta Kanchan etc., is native of Southern Asia,Southeast Asia, Taiwan, and China. The different plant parts are reported to be traditionally used for treatment of a variety of illness (Chopra *et al.*, 1956; Asolkar *et al.*,2000; Parrota, 2001; Janardhanan *et al.*, 2003,Kirthikar and Basu,2001).The leaves, stems, and roots are widely used to treat infections, pain, diabetes, jaundice, leprosy, and cough (Morais *et al.*, 2005).The *in vitro* antioxidant activity of different plant parts has been reported previously *(*Zakaria et al, 2011; Shajiselvin *et al.* 2011; Chew *et al.* 2009; Krishnaveni, *2014*,). The literature survey shows that there are a few numbers of *in vivo* studies on the effect of these extracts on oxidative biomarkers in animal model. In milieu of these observations, the present study had been undertaken to evaluate the antioxidative property of this plant's bark and leaves crude ethanolic extracts *in vivo.*

MATERIALS AND METHODS

Collection of Plant Materials:

The barks and leaves of *B. purpurea* (BP) were together collected at Shantiniketan campus of Visva-Bharati University, Birbhum district, India, in May 2013 and authenticated by botanist Professor DR Sudhendu Mandal, UGC Professor of Botany, Visva-Bharati Shantiniketan, India. The herbarium for future reference has been kept in Department of Veterinary Pharmacology and Toxicology, WBUAFS, Belgachia, Kolkata.

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Chemicals and Reagents:

Glucose estimation kits were obtained from Span Diagnostics Ltd. India. Glycosylated hemoglobin kit was procured from Lab care Diagnostics (India) Pvt. Ltd. Streptozotocin (STZ was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Standard drug glibenclamide pure samples were procured from M/S Hindustan Chemicals & Pharmaceuticals, West Mumbai, India. All other chemicals (Analytical Grade) were procured from Hi-Media Laboratories Ltd, India and Merck India Ltd. All organic solvents were of analytical grade purchased from local companies.

Animals:

The study was conducted on Albino Wistar Rats of either sex, five to six weeks of age weighing between 150–200 g, housed in polypropylene cages at an ambient temperature of 25 ± 2^0C with 12 h light and 12 h dark cycle. The rats were fed standard diets and water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for 1 week. All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (IAEC), West Bengal University of Animal and Fishery Sciences, West Bengal, India.

Induction of Type I diabetes:

The rats were fasted overnight and administered freshly prepared STZ solution (60 mg/kg body weight dissolved in cold citrate buffer, (0.1M, pH 4.5) intraperitoneally. Diabetes was identified by polydipsia, polyuria, and by measuring non fasting plasma glucose levels. Animals with postprandial glycemia over 225 mg/dL, 3 days after STZ administration, were considered diabetic. Whole blood was collected from the tail vein of the rats for

glucose estimation in serum. Control rats received only citrate buffer. (Mondal *et al*, 2012).

Experimental Design:

The *in vivo* antioxidative effect of the extracts was studied in STZ- induced diabetic rats. Diabetes generates detrimental ROS inducing exhaustion of cellular anti oxidative systems that leads to liver and kidney damage and other diabetic complication. Hence drugs having hypoglycemic and anti oxidative effects are helpful under such situation. The rats (n = 3 per group) were divided into 9 groups: group 1 (NC): normal rats treated with vehicle alone; group 2 (DC): diabetic control (DC) treated with STZ solution (60 $mgkg^{-1}$ body weight dissolved in cold citrate buffer,0.1M, pH 4.5) intraperitoneally; group 3 (PC): diabetic rats treated orally with glibenclamide @0.5mg/Kg BW; group 4,5 and 6 diabetic rats were administered BPBE@$1/5^{th}$ $1/10^{th}$ and$1/20^{th}$ of its LD50 respectively (BPBE105,BPBE210 and BPBE420); groups 7,8 and 9 diabetic rats were administered BPLE@$1/5^{th}$,$1/10^{th}$ and$1/20^{th}$ of its LD50 respectively (BPLE115, BPLE230 and BPLE460); the vehicle, standard drug, and extracts were administered orally to respective groups once daily for 28 days. On day 28, the fasted animals were euthanized under ether inhalation. The fasted blood was collected by cardiac puncture with anticoagulant and processed for estimation of catalase (Bergmeyer, 1974) reduced glutathione (GSH) (Prins and Loos, 1969) and malondialdehyde (MDA) in packed RBC (Jain, 1988). Liver tissue was dissected out, washed with ice cold NSS and a 10% homogenate (w/v) was prepared in 0.1M, pH 7.4 PBS and stored at -20^0C before analysis for MDA (Placer et. al, 1966) and cellular glutathione (NPSH) (Sedlak and Lindsay, 1969).

Statistical Analysis:

Statistical analysis was performed using Graph Pad Prism software package Version 6.0. The values were analyzed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, Tukey's multiple comparison test or Sidak's multiple comparison test as applicable for the data.

RESULTS AND DISCUSSION

The detailed study of plant constituents for two crude ethanolic extracts have been done by standard natural products chemistry methods (Brahmachari, 2009) and reported earlier (Brahmachari *et al*, 2015). The results of acute toxicity studies of the plant extracts had also been reported earlier (Brahmachari *et al*, 2015). The oral glucose tolerance test conducted and reported in our earlier publication (Brahmachari *et al*, 2015) revealed promising results. The oxidative stress biomarkers include superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins, and hyperglycemia (Matrim *et al*, 2003). Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. Clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. These consequences of oxidative stress can promote the development of complications of diabetes mellitus (Matrim *et al*, 2003). Lipid peroxidation measured as MDA (Malondialdehyde) was seen increased ($P<0.01$) in RBC and liver of streptozotocin-diabetic rats in this study. Diabetic rats when treated with the plant extracts at various dose levels, both drugs exhibited a significant ($P<0.01$) effect on MDA production in these cells and tissues and decreased their levels when compared with diabetic control rats (Fig. 1 and 2). No significant difference ($P>0.01$) in reducing lipid peroxidation was found between same dose levels of the bark and leaves extracts in RBC system. In case of liver tissue, effect of BP bark extract at all dose levels was significantly higher than BP leaves extract ($P<0.01$). MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress (Shodehinde and Oboh, 2013). Lipid peroxidation is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants (Korkina & Afanas'ev, 1997). Drugs with antioxidant properties may supply endogenous defense systems and reduce both initiation and propagation of reactive oxygen species (Bergendi *et al.,* 1999). Elevated levels of MDA in these cells and tissue in our study is a clear manifestation of excessive formation of free radicals resulting tissue damage. Karpan *et al* (1982) observed an elevated level of lipid peroxides in plasma of STZ-induced diabetic rats. Yang *et al*. (2009) observed greater serum lipid peroxidation evaluated in terms of MDA in hyperglycemic mice and proposed that the increase in lipid peroxidation exacerbated the occurrence of myocardial infraction through NADPH oxidase activation (Yang et al, 2009). Significant decline in the concentration of MDA in RBC and liver of BP bark and leaves extract treated diabetic animals indicates the potential use of these extracts *in vivo* to counteract the oxidative stress induced changes in diabetes. Recently, a clinical study performed by Bandeira and coworkers (2012) aimed at characterizing blood oxidative stress in diabetic patients reported a significant higher lipid peroxidation which showed a close relationship with high glucose levels as observed by the fasting glucose and HbA1c levels (Bandeira et al, 2012). The non protein thiols measured in RBC and liver (Fig. 3, 4) decreased significantly ($P<0.01$) in diabetic rats as compared to normal control. No significant difference ($P>0.01$) was found between same dose groups of the bark and leaves extracts in increasing cellular glutathione content in RBC and liver tissue**.** The crude extracts of BP barks and leaves increased the cellular glutathione level in the present study as compared to diabetic control significantly ($P<0.01$). However no significant difference ($P>0.01$) was observed between two crude drugs types at same dose levels. GSH can maintain SH groups of proteins in a reduced state, participate in amino acid transport, detoxify foreign radicals, act as coenzyme in several enzymatic reactions, and also prevent tissue damage (Tsai et al, 2012). Cellular reduced glutathione plays a major role in coordinating the body's antioxidant defense processes. It is present in all mammalian tissues as the most abundant nonprotein thiol that defends against oxidative stress (Lu, 2013). It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level (Rizvi and Chakravarty, 2011). There are several reports that claim reduced level of GSH in diabetes (Calabrese et al, 2012). Perturbation of GSH/NPSH status of a biological system can lead to serious consequences. Hyperlipidemia, inflammation, and altered antioxidant profiles are the usual complications in diabetes mellitus as results decreased GSH/GSSG ratio (Das et al, 2012). Glutathione depletion is a sign of cellular toxicity. Glutathione serves as a substrate for the enzyme glutathione peroxidase which catalyses the reaction of endogenously formed toxic hydroperoxides with reduced glutathione to produce oxidized glutathione (GSSG) and hydroxy derivatives (Christopherson, 1969). In the present study significant decrease in level of GSH in diabetic animals and attainment of significant improvements, particularly at highest dose levels of both the extracts, indicate that oxidative stress elicited by STZ was significantly reduced by these extracts. The results of the present study show that catalase activity (Fig. 5) decreased significantly in diabetic rats as compared to control. Catalase protects pancreatic β-cells from damage by hydrogen peroxide (Tiedge *et al*, 1998). The BP bark and leaves extracts at dose levels of 420 and 460$mgKg^{-1}$ B.W. increased the catalase activity ($P<0.01$) as compared to diabetic control rats and their effect is significantly comparable ($P>0.01$) with positive control. The lower doses had no significant effect ($P>0.01$). Catalase is an antioxidative enzyme present nearly in all living organisms. It plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases (Chelikani et al, 2004). Catalase acts as main regulator of hydrogen peroxide metabolism. Hydrogen peroxide is a highly reactive small molecule formed as natural by-product of energy metabolism. Excessive concentration of hydrogen peroxide may cause significant damages to proteins, DNA, RNA, and lipids (Takemoto *et al,* 2009). Catalase enzymatically processes hydrogen peroxide into oxygen and water and thus neutralizes it. The deficiency of this enzyme leads, in the pancreatic β-cell, to an increase in oxidative stress and ultimately to a failure of this cell type. β-cells are rich in mitochondria, and thus this organelle might be a source of ROS (Góth and Eaton, 2000**)**.

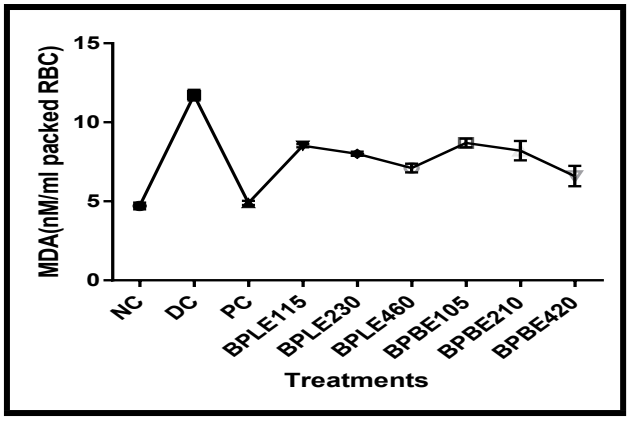

Fig. 1: Comparative effects of BP bark and leaves extracts on MDA production in RBC in diabetic albino wistar rats.

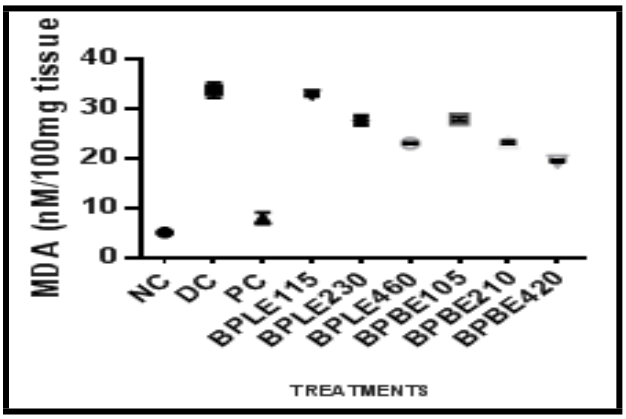

Fig. 2: Comparative effects of BP bark and leaves extracts on MDA content in liver in diabetic albino wistar rats.

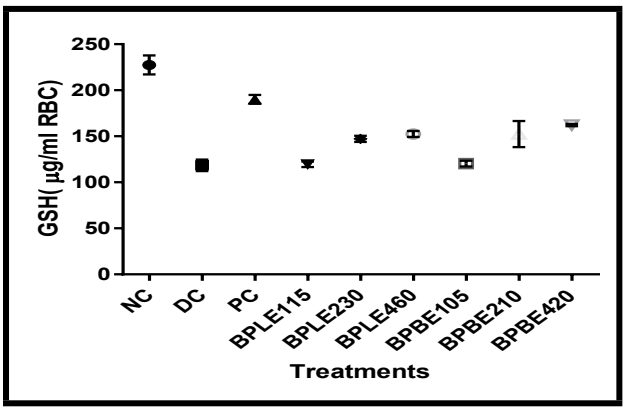

Fig. 3: Comparative effects of BP bark and leaves extracts on GSH content in blood in diabetic albino wistar rats.

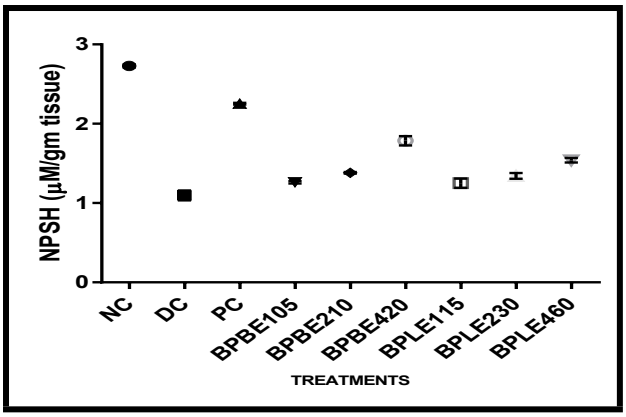

Fig. 4: Comparative effects of BP bark and leaves extracts on NPSH content in liver in diabetic albino wistar rats.

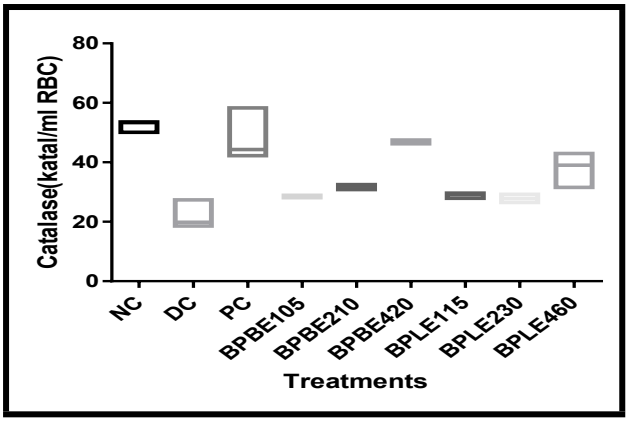

Fig. 5: Comparative effects of BP Bark and leaves extracts on catalase activity in RBC in diabetic albino wistar rats

CONCLUSION

On the basis of experimental observations and data accrued it may be assumed that BP bark and leaves CEE possess antioxidative potential and BPBE being superior to BPLE. The actual mechanism of action of the antioxidative actions exerted by these two types of crude extracts at different dose levels is not clear, as it is a preliminary study. However, activities such as reduction of lipid peroxidation in tissues, enhancement of cellular glutathione and catalase activity in cells and as earlier reported in our previous publication, reduction of hyperglycemia and hemoglobin glycation make them potential candidates for antioxidant activities which may partly be attributed to their phenolic, flavonoids, glycosides and terpenoids content. Further studies with semipurified and purified fractions are necessary to explore the underlying mechanism.

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