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Research Paper

EFFECT OF BAUHINIA VARIEGATA STEM BARK AQUEOUS EXTRACT ON HYPERGLYCEMIA, NEUROPATHY AND DIABETIC COMPLICATIONS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Protective activity of *Bauhinia variegata* aqueous extract in diabetic complication on streptozotocin (STZ)-induced diabetic rats was done using Male Wistar albino rats (150–200 g) by single intraperitoneal injection of STZ (65 mg/kg b.w i. p). Rats (*n* = 24) were divided into four groups of six animals each. Group 1 (normal control) and Group 2 (diabetic control) received normal saline (10 ml/kg/day p. o). Group 3 (Standard, insulin 6 U/kg/day s. c) and Group 4 (received *Bauhinia variegata* aqueous extract in dose of 1000 mg/ kg) for 28 days. *Bauhinia variegata group animals* exhibited substantial decrease in blood glucose, SGOT, SGPT, creatinine, urea, triglycerides, LDL and glycated hemoglobin. It particularly had protective effect in diabetic complications like neuropathy and learning and memory dysfunction evaluated by elevated plus maze, object recognition, open field test, rotarod, hot plate, tail flick, immersion in hot and cold water etc. Hence it can be concluded that *Bauhinia variegata* aqueous extract has potential antidiabetic and protective activity in diabetic complications in STZ-induced diabetic rats and can be considered as an alternative in Diabetes management therapy.

Keywords: *Bauhinia variegata*, Diabetes, Neuropathy, Hyperglycemia, Streptozotocin

INTRODUCTION

Currently Diabetes mellitus is the most common metabolic disorder worldwide accelerated by modern lifestyle, stress, diet etc. India is leading in diabetes patients and because of inadequacies of conventional modern drugs in managing diabetes and particularly in averting its long term complications there is renewed interest in alternative medicines for their proved efficacy and few adverse effects. *Bauhinia variegata* is one such drug which has been used traditionally in management of diabetes and other diseases. It is official in Ayurvedic system and its antidiabetic and antioxidant efficacy has been established. Present study was undertaken with the assumption that being antihyperglycemic and

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antioxidant it will be effective particularly in diabetic complications which are a result of reactive oxygen species. Complete aqueous extract was used as the phytotherapeutic effects of plant materials are unique to the particular plant species and its medicinal effects are due to the combination of secondary products present in the plant (Uddin et al., 2012).

Bauhinia variegata is a tropical plant also called as Mountain Ebony, Kachnar commonly seen on roadside, gardens, residential areas etc in India. In microscopial studies uppermost zone of the bark is cork cells which is 12-20 layered, below it there is a single layer of phellogen which is followed by wide zone of phelloderm, tangentially elongated to isodiametric cells. This zone also contains lignified fibres and stone cells. The pericyclic fibres found in the bark have narrow lumen, thickened, lignified, broad walled and tapering ends. The phloem part of the bark is characterized by sieve tubes, companion cells, crystal fibres, phloem fibres, phloem parenchyma and stone cells, transverse by uni-bivariate medullary rays (Vipin et al., 2014). Though the plant has been used traditionally in diabetes it is essential to establish its efficacy scientifically so that it can be developed as an alternative drug in comprehensive diabetes management.

Botanical name: *Bauhinia variegata,*

Common name: *Mountain ebony, orchid tree*

Kingdom: *Plantae*

Family: *Fabaceae*

Genus: *Bauhinia*

Species: *variegata*

MATERIALS AND METHODS

Plant Material

Stem bark of *Bauhinia variegata* was collected from botanical gardens in Aurangabad region. Bark was authenticated by Taxonomist, Department of Botany, Maulana Azad College Aurangabad, India with herbarium number MACH – 012451. Bark was shade dried and coarsely powdered. It was then powdered in mixer and 2 kg bark powder was packed in batches in soxlet apparatus and defatted with Pet Ether. Aqueous extract was obtained using distilled water and heating for 36 hours. The crude extract was vacuum dried and concentrated in desiccator and percentage yield was 15.4%. Dark brown to maroon colored product was obtained which was kept in refrigerator for further use. Fresh extract was prepared daily in distilled water for administering to diabetic rats.

Experimental Animals

Male Wistar rats were obtained from Wockhartd Ltd, Aurangabad, India. Animals were housed under standard environmental conditions (25 ± 2°C temperature, 50 ± 5 % humidity with a 12 h each of dark and light cycle) and maintained with free access to water and standard laboratory diet *ad libitum*. The study was approved by the Institutional Ethics Committee with Ref number CPCSEA /IAEC/pharm-chem-26/2015-16/116. Experiment was performed on 8-10 weeks old, healthy, male Wistar albino rats of body weight ranging from 150-200 grams.

Induction of Diabetes

Diabetes was induced by a single intraperitonial injection of freshly prepared Streptozotocin (purchased from Sigma Aldrich Chem. Co. USA.) at a dose of 65 mg/kg b.w in 0.1 M citrate buffer (pH 4.5) to a group of overnight fasted rats. After 3 days of STZ administration, fasting blood glucose level was estimated and postprandial glucose (PPG) was checked regularly till stable

hyperglycemia, generally 1 week after STZ injection was achieved. Animals having marked hyperglycemia (> 250 mg/dl) were selected for the study.

Experimental Design

The experiment was carried out on four groups (1, 2, 3 and 4) of six rats each. Group 1: normal (control) treated with *vehicle,* Group 2: severely diabetic (control) treated with *vehicle*, Group 3 severely diabetic treated with Insulin (6 U/kg/day/ s. c) (Borah and Das, 2017) and Group 4 with aqueous extract of *Bauhinia variegata* in a dose of 1000 mg/kg for 28 days. Control rats (group 1 & 2) received vehicle (distilled water only) orally regularly once a day up to 28 days.

PHYTOCHEMICAL SCREENING OF *BAUHINIA VARIEGATA*.

Preliminary Phytochemical analysis of *Bauhinia variegata* bark shows presence of Tannins, Alkaloids, Saponins, Cardiac Glycosides, Steroids, Terpenoids and Flavonoids (Jigna et al., 2006). The stem bark contains 5, 7 dihydroxy and 5, 7 dimethoxy flavanone-4-O—L rhamnopyrosyl-β-D glycopyranosides, kaempferol-3-glucoside, lupeol, and beta sitosterol (Mopuru et al., 2003; Zhao et al., 2005; Rajani and Ashok, 2009).

Acute Toxicity Studies

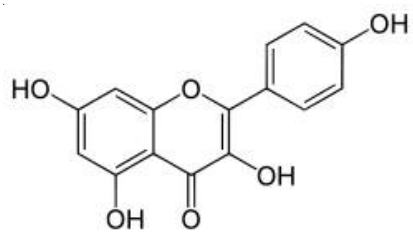
The extract was found to be safe till a dose of 2000 mg/kg in rats as per OECD guidelines no 425.

Statistical Analysis

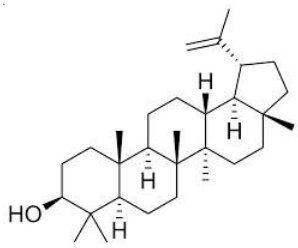
Data were statistically evaluated using one-way ANOVA, followed by Dunnet's test.

DISCUSSION

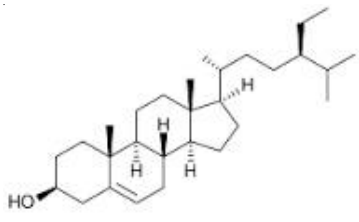
Effect of aqueous extract of *Bauhinia variegata* was evaluated on blood glucose, various biochemical parameters, behavioral models of learning and memory dysfunction and neuropathy. In prolonged hyperglycemia there are functional changes in many tissues or organs like pancreas, liver, heart etc which are reflected as alterations in some of the metabolic pathways and many blood parameters (Prashant et al., 2008). *Bauhinia variegata* significantly normalized the biochemical parameters like blood glucose, urea, creatinine, SGOT, SGPT, LDL, CRP, Triglycerides, serum insulin and glycated hemoglobin at the end of 28 days treatment as shown in Figure 1, Figure 4a and Figure 4b compared to untreated diabetic animals.



Lupeol



Kaempferol



β Sitosterol

In behavioral models of neuropathy and learning and memory dysfunction the drug exhibited significant protective activity. Paw withdrawal latency in hot plate, tail flick, immersion in hot and cold water and rotarod indicated that drug was very effective in preventing diabetic neuropathy as the readings are comparable with standard drug insulin in Figure 2a and Figure 2b. *Bauhinia variegata* extract was very effective in a very critical complication of diabetes i.e. learning and memory dysfunction as indicated by the results of object recognition, open field and elevated plus maze test in Figure 3a, Figure 3b and Figure 3c.

The aqueous extract of *Bauhinia variegata* shows presence of alkaloids, flavonoids, carbohydrates, proteins, fixed oils and phenolic compounds. As the extract has potent antioxidant activity in vitro, it can be effective in diseases caused due to free radicals like diabetic complications (Sawhney et al., 2012). The efficacy of *Bauhinia variegata* extract might be because the stem bark has a hentriacontane, octacosanol, stigmasterol,5,7-dihydroxyflavanone-4'-O-L-rhamnopyranosyl-D glucopyranoside, sitosterol, lupeol, kaempferol-3-glucoside, 2, 7-dimethoxy-3-methyl-9, 10-dihydrophenanthrene - 1,4-dione on the basis of spectroscopic analysis and also glycosides, reducing sugars and nitrogenous substances (Zhao *et al*., 2005).

These hypoglycemic effects of *Bauhinia variegata* may also be due to potentiation of insulin release, insulin secretagogue activity from INS- 1 cells, presence of insulin-like protein and due to regeneration of β-cells of Islets of Langerhans of pancreas which are destroyed by streptozotocin (Eliandra et al., 2004). This regeneration effect is supported by the results of plasma insulin level in Figure 4b. Diabetic patients have reduced antioxidant defenses and suffer from an increased risk of free radical–mediated diseases. Diabetic patients, both type 1 and 2 exhibit abnormal antioxidant status, auto-oxidation of glucose and excess glycosylated proteins. Prolonged oxidative stress in diabetes leads to tissue damage, lipid peroxidation, inactivation of proteins and protein glycation as intermediate mechanisms for complications including retinopathy, nephropathy and coronary heart disease. Flavonoids are polyphenolic phytochemicals found in almost all types of plants from stems to roots to fruit (Koti *et al*., 2009). Consumption of flavonoids having antioxidant properties of plant extracts may have protective effects against long-term complications of diabetes (Michael et al., 1999).

Bauhinia variegata has significant antioxidant activity (Rajani and Ashok, 2009). It is possibly because *Bauhinia* extract has kaempferol, lupeol and beta sitosterol which have proven antioxidant activities. Kaempferol glycosides and several kaempferol-containing plants have antidiabetic activity (Calderon et al; 2011) and may prevent diabetic complications (Asgary et al., 2002; Ghaffari and Mojab, 2007) The extract contains kaempferol and phosphatidylinositol 3-kinase. Glycogen synthase kinase 3 pathway and the MAPK-protein phosphatase 1 pathway are involved in the stimulatory effect of kaempferol on glycogen synthesis (Cazarolli et al., 2009) This flavonoid also induce a stimulatory effect on glucose and increase glycogen content in the muscle (Zanatta et al., 2008). Besides kaempferol potentially act on multiple targets to ameliorate hyperglycemia, including the peroxisome proliferator-agonist receptor ã (Fang et al., 2008). Kaempferol also prevent the onset of diabetes by preventing oxidative damage in pancreatic â

cells (Lee et al., 2010). Thus kaempferol, several glycosides of kaempferol and/or some kaempferol containing plants have potential to be developed as antidiabetic agents (Asgary et al., 2002).

Extract also contains lupeol which exerts its protective effect against STZ-induced diabetes due to stimulation of pancreatic regeneration through an improved synthesis of proteins and/ or its accelerated detoxification as well as minimizes the deleterious effects of free radicals including the peroxynitrate in combination with the inhibition of lipid peroxidation and nitric oxide, thus lupeol is a natural antioxidant (Rajnish et al., 2012). Also Beta sitosterol present neutralizes free radicals and reactive oxygen species and is effective in preventing protein damage caused by oxidative stress, which is thought to be involved in the degeneration of beta cells. Beta sitosterol exerts protective effects on serum and pancreatic tissue, as well as on antioxidant status, in streptozotocin-induced diabetes due to the stimulation of pancreatic regeneration through improved proteins synthesis and /or its accelerated detoxification, as well as minimization of the deleterious effects of free radicals. It has protective effect on pancreatic tissue with enhancement of pancreatic antioxidants (Rajnish et al., 2011). â-sitosterol promotes sensitivity to insulin also (Radika et al., 2013).

CONCLUSION

Bauhinia variegata aqueous extract possess remarkable hypoglycemic, antioxidant and protective activity in diabetic complications. Though it can be concluded that its activity is due to various phytoconstituents present which have hypoglycemic and antioxidant activity there is still scope to identify particular phytoconstituents responsible for activity and study the mechanism at molecular level.

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Nil.

CONFLICTS OF INTEREST

None.

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