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EVALUATION OF N-BUTANOLIC FRACTIONS OF *BUTEA MONOSPERMA* FLOWERS ON DEXAMETHASONE INDUCED HYPERGLYCEMIA AND HYPERLIPIDEMIA IN MICE

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ABSTRACT

To reveal the traditional claims of *Butea monosperma* in the treatment of diabetes, investigation was carried out to evaluate its effect on dexamethasone (Dexa) induced hyperglycemia in mice. In the present study mice were treated with pre standardized dose of Dexamethasone (1mg/kg body wt. i.m.) for 22 days and effect of n-butanolic (NBF) fraction of flowers of *B. monosperma* at three different doses (50,100,200 mg/kg body wt. p.o.) were studied. The diabetic mice showed increase in serum glucose level, total cholesterol level, LDL, VLDL, serum triglyceride level and Athrogenic index and decreased body weight. After the administration of different doses of NBF there was significant decreased in serum glucose level in groups receiving 100 mg/kg ($p<0.05$) and 200 mg/kg ($p<0.01$) as compare with control. The NBF also showed significant decrease in total cholesterol, LDL, VLDL, serum triglyceride, Athrogenic index, LPO and Significant increase in body weight and HDL. We can conclude that NBF of *Butea monospermous* may have Antidiabetic effect.

Keywords: *Butea monosperma*, Dexamethasone, hyperglycemia, lipid peroxidation.

INTRODUCTION

Diabetes mellitus (DM) is a major health problem worldwide in recent time and Asia and Africa are the most viable areas where the disease is feared to raise 2–3 folds. Many herbal products have been recommended for the treatment of diabetes in ancient literature of Ayurveda in India [1]. Diabetes mellitus is basically an endocrine disorder in which carbohydrate metabolism is impaired, leading to excess deposition of glucose in blood. Hormones such as catecholamine, glucagon, cortisol and thyroxin, either through directly or through their influence on other hormones, affect carbohydrate metabolism to elevate blood glucose level leading to diabetes mellitus [2]. This sustained hyperglycemia attacks both microvessels and macrovessels throughout the body and leads to various complications like blindness, neuropathy, end stage kidney disease, cardiovascular events in addition to significant psycho-social effects [3].

B. monosperma (Fabaceae) commonly known as 'flame of forest'. It is traditionally used in the treatment of diabetes, leprosy, gout, skin disease, eye disease, piles, aphrodisiac, laxative, anthelmintic and as per Ayurveda are given for Scorpion-sting bite [9]. *B. monosperma* reported to have antistress activity [10], anticonvulsive activity [11], Osteogenicactivity [12], Antimicrobial activity [13], anti-inflammatory [14], radical scavenging activity [15], anthelmintic [16]. Taking in to consideration the traditional claims and reported activities, the present study aims to reveal the possible role of these plant fractions in amelioration of the corticosteroid induced hyperglycemia using mouse as experimental model.

MATERIALS AND METHODS

Plant materials

Flowers of *B. monosperma* were collected from Toranmal region, Maharashtra, India in the month of April and May. The specimen was authenticated by Dr. D. A. Patil of PG department of Botany, SSVPS Science College, Dhule (M.S.), India. The specimen was deposited in our departmental herbarium for future reference.

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Preparation of fraction

The collected flowers were kept for shade drying for about 20 days. Petals separated from the dried flowers, and then material pulverized to coarse powder with the help of laboratory pulverizer (Drone 9500). This powder was then extracted with methanol using Soxhlet extractor. The extract was filtered and concentrated, further methanolic extract defatted with petroleum ether. This defatted aqueous methanolic extract fractionated with n-butanol (1:3). The n-butanolic fractions was concentrated. The yields was 5.5% (W/W).

Phytochemical screening

Methanolic extract of B. monosperma along with NBF was subjected to qualitative phytochemical screening [17].

Animals

Swiss albino mice of either sex, weighing 25-30g were obtained from Haffkine Bio Pharmaceuticals, Parel, Mumbai, India. Mice were maintained at 26±4^{0}C with relative humidity 44–56% along with light and dark cycles of 12h. Animals were provided with standard diet and water *ad libitum*. The experimental protocol was approved (Ref. No. IAEC/2009-10/10) by Institutional Animal Ethical Committee of R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India, registered under CPCSEA, India (Registration No.651/02/C/CPCSEA).

EXPERIMENTAL DESIGN

Acute oral toxicity study

Acute oral toxicity study of NBF of flowers of *B. monosperma* was carried out using innulliparous and non-pregnant female Swiss albino mice (22-25g); according to OECD guideline no 425. LD_{50} was calculated as per OECD guidelines 425 using computer software AOT425 statPgm [18].

Dexamethasone induced hyperglycemia in mice

All the mice were weighed before treatment. Group I (normal control) received equivalent amount of 1% gum acacia (1ml/kg, p.o.). Thirty six mice were rendered hyperglycemic by daily administration of a prestandardized dose of Dexamethasone (1mg/kg body wt., i.m.) for 7 consecutive days and then divided into Six groups of six each. Group II (Dexamethasone -control) continued to receive only Dexamethasone for next 15 days. Group III (Standard control) received glibenclamide (5mg/kg, p.o.) along with Dexamethasone , where as the other six groups were treated with equivalent dose of Dexamethasone along with 50,100,200mg/kg, p.o. doses of NBF (Group IV,V,VI) respectively. Fractions and glibenclamide were administered daily between 10:00 to11:00 h to avoid circadian variation. On the last day, all the animals were weighed, and after an overnight fast were sacrificed by cervical dislocation. Blood samples were collected from retero-orbital plexus, allowed to clot, and centrifuged to obtain clear serum. Obtained serum sample was used for estimation of glucose, total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein VLDL, Athrogenic index [2,4,8,9].

Biochemical estimation

Serum glucose was estimated by GOD/POD method using standard diagnostic kits from Span diagnostic Pvt. Ltd. India. CHOD-PAP method was used to estimate the serum cholesterol and HDL. It was estimated by using commercially available standard kit supplied by RFCL Limited, India. VLDL, LDL and Atherogenic index were calculated using *Friedewald's equation*. GPO-PAP method was used for the estimation of serum triglycerides using commercially available standard kit supplied by RFCL Limited, India. Dexamethasone from Cadila Healthcare, India was used.

Estimation of LPO

On the 22nd day liver samples were dissected out and washed immediately with ice cold saline to prevent contamination with blood. The concentration of malonaldehyde (MDA) measured spectrometrically at 530 nm [20,21].

Statistical analysis

The results were expressed as mean ± S.E.M. and statistically analyzed by ANOVA followed by Dunnett test, with level of significance set at $p<0.05$.

RESULTS

Phytochemical screening

Phytochemical screening of fractions of B. monosperma showed the presence of flavonoids, steroids, phenolic contents, glycosides.

Acute toxicity study

There was no mortality or any signs of behavioral changes or toxicity observed after oral administration of NBF of B. *monosperma* up to the dose level of 2000 mg/kg. The LD_{50} for the NBF was found to be a greater than 2000 mg/kg .

Effect of NBF on serum glucose, total cholesterol, HDL, LDL, VLDL

In Dexamethasone control group there was significant increase in serum glucose concentration ($p<0.01$) and total cholesterol ($p<0.01$) was observed when compared with normal control. LDL and VLDL also shows significant increase ($p<0.01$) in Dexa

control when compared with normal control, however significant decrease ($p<0.01$) in HDL concentration was observed. After the administration of NBF of *B. monosperma* there was significant decrease in serum glucose, total cholesterol, HDL, LDL, VLDL. Mice treated with Dexa and NBF significantly decrease serum glucose level, LDL and VLDL. There was more significant ($p<0.01$) results were observed at doses of 100mg/kg ($p<0.05$) and 200 mg/kg when compared with Dexa control, while mice treated with Dexa and NBF 50mg/kg showed nonsignificant changes. Significant reduction ($p<0.01$) in serum glucose, total cholesterol, LDL, VLDL and increase in HDL were observed in the mice treated with Dexa and glibenclamide when compared to Diabetic control.

Effect of NBF on serum Triglycerides, Atrogenic Index, LPO and Body weight

Significant increase ($p<0.01$) in serum Triglycerides, Atrogenic Index and LPO and significant reduction ($p<0.01$) in body weight were observed in Dexa control when compared to normal control. NBF and glibenclamide significantly ameliorate serum Triglycerides, Atrogenic Index, LPO and Body weight. NBF significantly decreases serum Triglycerides, Atrogenic Index and LPO at doses of 100mg/kg ($p<0.05$) and 200mg/kg ($p<0.01$) as compared to Dexa control. Significant increase ($p<0.01$) in body weight was observed in NBF treated as compared to Dexa control. Dexa and glibenclamide treatment significantly inhibit ($p<0.01$) the Dexa induced increase in body weight and significantly increase ($p<0.01$) serum Triglycerides, Atrogenic Index and LPO as compared to Dexa control.(Table 2.)

Table 1. Effect of NBF of *B. monosperma* And Glibenclamide on serum glucose, total cholesterol, HDL, LDL, VLDL levels in normal and dexamethasone induced hyperglycemic mice.

Groups	Glucose (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	57±5.5	73.00±3.30	37±1.4	19±3.0	17.00±0.83
Dexa control	81±3.0[a]**	129.00±4.70 [a]**	21±1.7 [a]**	79±4.6 [a]**	29.00±1.50 [a]**
Dexa+ glibenclamide	59±4.6**	79.00±3.90**	30±2.1**	30±2.4**	18.00±0.75**
Dexa + NBF (50mg/kg)	76±4.5	124.00±3.50	24±2.1	72±3.3	28.00±1.40
Dexa + NBF (100mg/kg)	65±3.4*	113.00±3.80*	25±1.8	63±5.1*	25.00±1.20
Dexa + NBF (200mg/kg)	60±2.6**	106.00±3.50**	30±1.3**	57±3.2**	22.00±0.70**

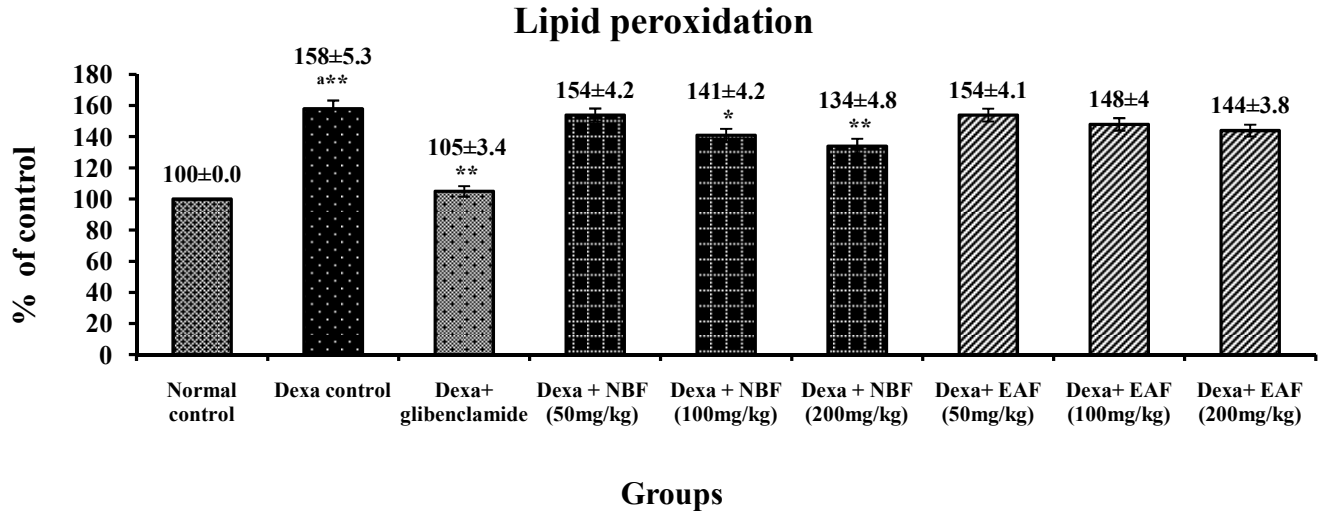
Values are expressed as mean±S.E.M., n =6, Dexa=Dexamethasone 1mg/kg, i.m, NBF= n-butanolic fraction, *P < 0.05, * * P < 0.01 when compared with Dexa control. [a]**p<0.01 when compared with normal control.

Table 2. Effect of NBF and EAF of flowers of *B. monosperma* And Glibenclamide on serum Triglycerides, Athrogenic Index and Body weight in normal and dexamethasone induced hyperglycemic mice.

Groups	**Triglycerides (mg/dl)**	**Athrogenic Index (mg/dl)**	**Body weight (gm)**
Normal control	86±4.2	2.8±0.43	28±1.3
Dexa control	144±7.4 [a]**	7.3±0.81[a]**	16±0.82 [a]**
Dexa+ glibenclamide	90±3.8**	3.4±0.24**	25±0.79**
Dexa + NBF (50mg/kg)	139±6.8	5.9±0.57	17±1.1
Dexa + NBF (100mg/kg)	127±6.2	5.2±0.45*	20±0.65*
Dexa + NBF (200mg/kg)	110±3.5**	4.4±0.35**	21±0.49**

Values are expressed as mean ± S.E.M., n=6, Dexa=Dexamethasone 1mg/kg, i.m, NBF= n-butanolic fraction, (+) and (−)sign indicates increase and decrease in body weight. * P < 0.05, * * P < 0.01 when compared with Dexa control. [a]**p<0.01 when compared with normal control.

Figure 1: Effect of NBF on lipid peroxidation in normal and Dexamethasone induced hyperglycemic mice



Values are expressed as mean ± S.E.M., n=6, Dexa=Dexamethasone 1mg/kg, i.m, NBF= n-butanolic fraction, * P < 0.05, * * P < 0.01 when compared with Dexa control.[a]**p<0.01 when compared with normal control.

DISCUSSION

Dexamethasone has ability to produce diabetes by affecting the posttransitional degradation mechanism [23]. Increased serum glucose level is major symptom of diabetes. The increase in blood glucose level in diabetic mice as compared to normal mice might be due to glycogenolysis or gluconeogenesis [24]. In the present study dexamethasone administration resulted in significant increase in serum glucose level, total cholesterol, serum triglyceride, LDL, VLDL, LPO and significant decrease in HDL and body weight.

In the present investigation we found that there was significant dose dependent reduction in the serum glucose concentration was observed in NBF treated mice. This effect NBF of *B. monosperma* may be due to the steroids or phenolic contents, as the NBF is reach in bothn [15]. Many steroidal compounds have ability to produce hypoglycemic effects [25]. The mechanisms underlying these effects may be inhibition of the intestinal absorption of glucose or by suppressing enzymes involved in gluconeogenesis or by stimulating glucose uptake in the peripheral tissues or due to increased insulin sensitivity or by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from the bound form [26].

Dexamethasone induced hyperglycemia is a result of pancreatic beta cell death mediated through reactive oxygen species [27]. A single dose of dexamethasone may significantly alter carbohydrate and lipid metabolism and induce glucose intolerance [28]. Pharmacological doses of glucocorticoids induce ob gene expression in rat adipocyte tissues within 24 h which is followed by complex metabolic changes like hyperleptinemia, resulting in decrease in food consumption, reduction in body weight with enhanced blood glucose, and triglyceride levels[4]. Disturbed utilization of glucose leads to increase in mobility of lipids in diabetes which leads to increase in serum lipid level [26]. In the present study Dexa control shows increase in total cholesterol, LDL,VLDL, serum triglycerides, atherogenic index and decrease in HDL was observed while NBF treated mice shows significant decrease in total cholesterol, LDL,VLDL, serum triglycerides, atherogenic index and increase in HDL. Possible mechanism for total cholesterol and triglyceride lowering activity of *NBF* may be either due to increase in uptake and utilization of glucose leading to subsequent inhibition of lipolysis. The hypolipidemic action of fraction might be due to inhibition of lipid peroxidation [1]. Also the steroids and phenolic contents present in NBF have potent antioxidant activity [25], this antioxidant effect may contributes to hypolipidemic action [29]. The same effects were seen in glibenclamide treated mice also.

Change in body weight is major consequence of experimentally induced diabetes in rats. This loss of body weight could be due to, dehydration and catabolism of fats and protein. In this study untreated diabetic rats shows significant loss in body weight where as significant increase in body weight was observed in NBF and glibenclamide treated group. This prevention of loss in body weight by NBF may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control.

Reactive oxygen species are major contributor in diabetes complications. Dexamethasone have ability to produce toxicity in insulin producing cell might be by an increase in oxidative stress, because beta cells are

particularly vulnerable and susceptible to ROS toxicity [27]. Further insulin resistance induced by Dexamethasone causes release of cytokines like TNF-alpha, IL-8 which leads to development of oxidative stress in liver by reducing the mitochondrial enzymes and producing H_2O_2 radicals and leads to increase in lipid peroxidation [4]. Diabetes results in increased oxidative stress. In lipid peroxidation malonaldehyde is generated as end product and as lipid peroxidation increases in diabetes malonaldehyde also increases. This malonaldehyde reflects the degree of oxidation in the body. The level of antioxidant enzymes was decreased and the concentration of malonaldehyde was increased results in increased value for lipid peroxidation [20,21,30]. In the present investigation there was significantly decrease in lipid peroxidation value was observed in NBF as compared to diabetic control group, indicating the significant efficacy of NBF in providing antioxidant defence. It may be due to effective antioxidant and concentration dependent radical scavenging activity [15]. NBF is reach in steroids and phenolic contents, which may be responsible for this protective effect of against lipid peroxidation.

Thus outcomes obtained from above study reveals that NBF fraction obtained from the flowers of *B.monosperma* may prove to be useful in hyperglycemic conditions owing to its strong antioxidant potential which may contributes in reducing serum glucose and lipid profile. The observed activity may be due to the steroids and phenolic content presents in the fraction. It is conceivable that antioxidant/free radical scavenging activity of NBF is unlikely to be the possible mechanism associated with antidiabetic effects. Further investigation on the mechanism of action and identification of lead molecule responsible for antidiabetic effects need to be explored.

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