

Original Research article

Evaluation of protein thiols and Liver glycogen content on Streptozotocin induced diabetic rats treated with aqueous extract of *Bixa orellana* leaves

Abstract

Aim: This study was conducted to determine the protein thiols and glycogen content to evaluate the hypoglycemic activity of the aqueous leaf extract of *Bixa orellana* using diabetes induced rats.

Methods: The leaves of *B.orellana* were cleaned and shade dried to make the extract. Wistar male rats weighing about 200-300g were used and all the animals were provided with wheat gluten feed pellets and water. Diabetes was induced using Streptozotocin and the experimental group (n=6) animals were treated with aqueous extract of 60 mg/kg body weight. The diabetic and normal control group animals were treated with 0.9% of saline.

Results: There was decrease in protein thiol and increase in liver glycogen content in diabetic control group when compared to normal control group. P value between normal control and diabetic control groups for protein thiols and glycogen was found to be 0.004 and 0.005 which were highly significant. When normal group was compared with test group there was statistical significance difference in which aqueous extract treated group had an increase in serum protein thiol (P<0.002) and a decrease in glycogen content (P<0.001). When Diabetic control group was compared with test group there was no statistical significance difference in which aqueous extract treated group and had an increase in serum protein thiol (P > 0.05) and decrease in glycogen content (P >0.05).

Conclusions: The study showed a significant increase in the protein thiol and decrease in liver glycogen in the test group of diabetic rats treated with aqueous extract of *B.orellana* leaves.

Keywords: Diabetes Mellitus; Protein Thiol; Glycogen; *Bixa orellana*

Introduction

Diabetes mellitus (DM) is a common metabolic disorder causing hyperglycemia affecting the world wide population of about 170 million people (1). It was found to be ineluctably connected with increased oxidative stress both in diabetic humans and hyperglycemic animals (2). Most of the plant extracts contain fatty acids, sterols, flavonoids, alkaloids, and vitamins which add to the potential antioxidant property and are used in the treatment of diabetes mellitus like *Ocimum sanctum* L. (OS), *Murraya koenigii* L. (MK), *Catharanthus roseus* L. (CR) and *Azadirachta indica* A.Juss. (AI) (3,4). Literature surveys show that the various parts of the plant *Bixa orellana* (annatto) are known to have hypoglycemic effects (5).

Thiols bound to albumin in its sulfhydryl group at cysteine 34 position contribute majorly to the protein bound thiols. They play significant role in detoxification reactions, apoptosis of the cell along with free radical defense mechanisms. Various renal, cardiovascular, neurological

disorders, metabolic disorders like diabetes mellitus, Liver cirrhosis due to alcohol all contribute to decrease in the serum thiols (6). Uncontrolled diabetes mellitus leads to raise in oxygen free radicals from autoxidation of glucose⁷ and glycosylation of scavenging enzymes and depletion of low molecular antioxidant causing oxidative stress which has been implicated in the pathogenesis of DM and its complications (8).

The pancreas will respond to hyperglycemia, by releasing insulin that promotes the liver and muscles to take up glucose from the blood, lowering blood glucose levels and stimulating glycogenesis. In case of diabetes, the glycogen metabolism is altered due to the lack of insulin.

More than 80% of the diabetic subjects are known to have glycogen accumulation in the liver and the reason behind this is said to be long standing insulin deficiency facilitating the synthase activity along with increased gluconeogenesis (9,10).

In this study, effect of aqueous extract of the plant *Bixa orellana* is used to study the oxidative stress and glycogen metabolism in diabetes mellitus.

B. orellana is a bushy shrub ranging from 4-10cm in height. They possess glossy ovate evergreen leaves with reddish veins. Pink flowers and brownish to maroon two valved fruit is a characteristic feature of the plant (8).

Several bioactive compounds in *Bixa* such as sesquiterpenes are known to have antifungal activity against *Candida albicans* and also it is shown to have an antibacterial activity against *Escherichia coli* (11). Studies done in dogs reveal that seeds of *Bixa orellana* are known to be effective against skin diseases (12).

Materials and Methods:

Preparation of the different extracts

The leaves of *B.orellana* were obtained, cleaned and shade dried. The dried leaves were powdered and the final yield of 53 g was stored in an air tight container.

Aqueous extract: Finely powdered leaves were soaked in water for 1-2 hour. Refluxed for 2-3 hour by reflux condensation method and decanted when hot. Concentrate the contents under reduced pressure (evaporate water). Keep in water bath and heat till the syrupy consistency was obtained. Preserve it in an air tight container and store it in a desiccator at room temperature.

The application for the research proposal was submitted to the Institutional Animal Ethics Committee. The Committee has scrutinized the project and approved it on the letter dated May 22, 2015.

Experimental animals:

Male wistar rats used in the study are bred in house weighing about 200-300g. They were housed in Central Animal House, Manipal, India. Solid bottom shoe box type cages constructed of durable plastic and contact bedding material of wheat husk were used. All the animals were

provided with wheat gluten feed pellets and water was provided in separate compartments of the cage.

Induction of diabetes:

Rats were deprived of food for about 8 hours. Diabetes inducing drug Streptozotocin of 60mg/kg body weight and the volume (dissolved in 0.1M citrate buffer of p^H 4.5) is 1ml/kg body weight was used to induce the diabetes. Normal control group was injected with 0.9% of saline.

After 7 days of stabilization period following the induction of the diabetes on the 8th day about 1-1.5ml of blood was drawn into a grey vacutainer containing sodium fluoride and potassium oxalate as anticoagulant. Blood was collected intraorbitally using mucap capillary and tested for serum glucose to confirm the diabetes. The diabetes induced groups were randomly assigned into different groups with 6 animals in each group. Day of confirmation of diabetes was considered as day 1 for further course of treatment as per the group. Estimation of protein thiols was done on day 30. After assaying all the parameters the animal was sacrificed and liver. Glycogen was estimated. Treatment schedule is given below.

Treatment

After the confirmation of diabetes, 8th day was considered as day 1 of treatment.

Group 1 (Normal Control (NC), n=6): treated with vehicle

Group 2 (Diabetic control (DC), n=6: treated with vehicle

Group 3 (Insulin treated (IN), n=6: treated with insulin intraperitoneally at a dose of 6 units/kg body weight

Group 4 (Standard drug (SD), n=6: treated with pioglitazone orally at a dose of 200 mg/kg body weight

Group 5 (Test (T), n=14: treated with aqueous extract orally at a dose of 200 m/ kg body weight

Estimation of protein thiols: (mg/dl)

Protein thiols were estimated by Spectrophotometric method using Dinitrobenzene (DTNB)-Ellman's method.

Estimation of Glycogen

The rats are sacrificed and the liver is isolated to estimate glycogen. Liver glycogen was estimated by the direct method of Morris et al. The organic anthrone reagent is used for the qualitative estimation of carbohydrates, hence glycogen. The mechanism of reaction between the carbohydrates and the anthrone reagent is that the sulfuric acid in the reagent causes dehydration of the sugar to furfural derivatives which then condenses with anthrone to form a blue colored compound which is estimated spectrophotometrically (13).

Statistical analysis:

All data obtained were analyzed using SPSS (commonly used Software version 17.0). The significance of difference among the groups was assessed using Kruskal Wallis test followed by Man Whitney 'U' test. Significant value was set to $p < 0.05$. Comparisons of data within the group at different intervals were assessed by Wilcoxon Signed Rank Sum test.

Results

The results of the present study confirms that the administration of aqueous extract of *B. orellana* possess antidiabetic activity against streptozotocin induced diabetic rats. Table 1 depicts P value for protein thiol and glycogen between NC, SD and Test group and is found to be 0.003 which is highly significant for protein thiol. Increase in protein thiol concentration was seen in the test group treated with aqueous extract (Figure 1). P value for glycogen was found to be 0.002 which is highly significant. Liver glycogen in the test group was less compared to that of NC (Figure 2).

There was decrease in protein thiol in diabetic control group when compared to normal control and increase in liver glycogen in the diabetic control group. The P value between normal control and diabetic control for protein thiols and liver glycogen were found to be 0.004 and 0.005 respectively and which are highly significant.

When Normal group was compared with Test group there was statistical significance difference in which aqueous extract treated group had an increase in serum protein thiol ($P < 0.002$) and decrease in glycogen content ($P < 0.001$).

When Diabetic control group was compared with Test group there was no statistical significance difference in which aqueous extract treated group and had an increase in serum protein thiol ($P > 0.05$) and decrease in glycogen content ($P > 0.05$).

There is statistical significant decrease in liver glycogen and increase in protein thiol in the group treated with aqueous extract compared to that of the normal control.

Discussion

In the present study we demonstrated that streptozotocin induced diabetes mellitus in rats causes hyperglycemia, renal function deficits and oxidative damage in liver and pancreas.

Measurement of protein thiol is a good reflection of free radical generation since the conformation of albumin is altered showing SH groups to be oxidized. Decrease in blood glucose due to aqueous extracts of *B. orellana* might have caused the decrease in free radical formations and hence the protein thiols. The major source of thiols in plasma is albumin and glycation modifies thiol groups to form disulfide bonds and intermolecular aggregates. The antioxidants in the extract decreased the glycemic excursion and hence increase in the protein thiol concentration as a result of decreased oxidative protein damage during the course of treatment

(14). A major modification of protein structure due to glycation is the loss of protein thiol groups which is the direct reflection of excess free radical generation.

Estimation of Liver glycogen level can be considered as the best marker for assessing the hypoglycemic activity of any drug. *B.orelance* might enhance glucose utilization by peripheral tissues and increase the glycogen stores in the liver. Treatment with methanolic extract of *Rhinacanthus nasutus* also showed an increase in the glycogen content in liver tissue of diabetic rats. The alcoholic extract of *Inula racemose* decreased blood glucose and enhanced liver glycogen in rats. Administration of streptozotocin causes increase in liver glycogen content due to increased glycogenolysis which is due to insulin deficiency so the normal capacity of the liver to synthesize glycogen is impaired. A significant decrease in liver glycogen by administration of *B.orellana* extract may be due to an increase in level of insulin by it. In patients with noninsulin dependent diabetes mellitus increased gluconeogenesis is given as an explanation for the preservation of liver glycogen (15). Liver glycogen changes may require higher doses of the extract and are not observed at lower doses.

Conclusion

The study showed a significant increase in the protein thiol and decrease in liver glycogen in the test group of streptozotocin induced diabetic rats treated with aqueous extracts of *B.orellana* leaves. The parallel effects of the aqueous extract with antioxidant properties along with the hypoglycemic effects had added up to the increase in protein thiol. The decrease in liver glycogen is due to decreased rate of gluconeogenesis and increase in insulin caused by the hypoglycemic property of the above studied plant.

References:

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Table 1: Descriptive – Protein Thiol and Glycogen

	N	Mean	Standard deviation	H	P
Protein Thiol (nmol/L)					
NC	6	.326	.059	11.827	0.003 hs
SD	6	.638	.098		
TEST	9	.667	.184		
Glycogen					
NC	6	73.317	5.075	12.453	0.002 hs
SD	6	42.567	10.865		
TEST	9	42.193	10.467		

NC-Normal control, SD-Standard Drug, Test-Aqueous extract of *B orellana*
H=Kruskal Wallis test

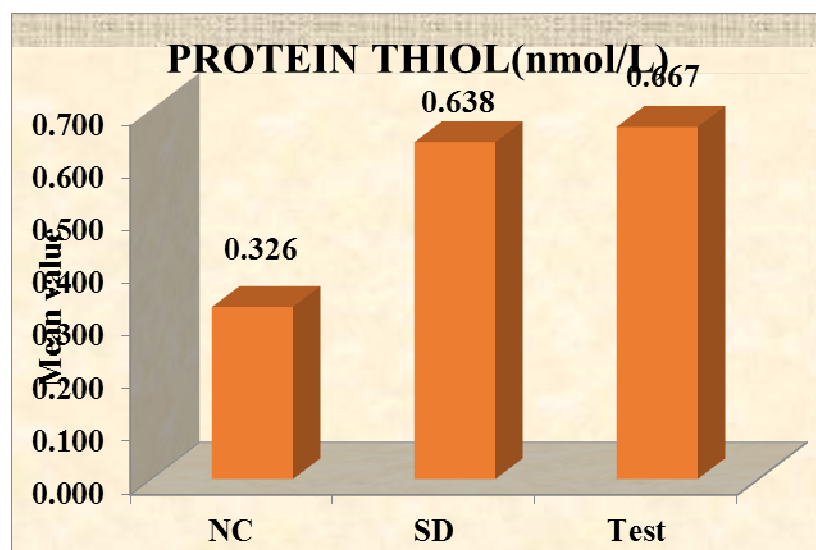


Figure 1: Protein thiol levels in animal group treated with aqueous extract.

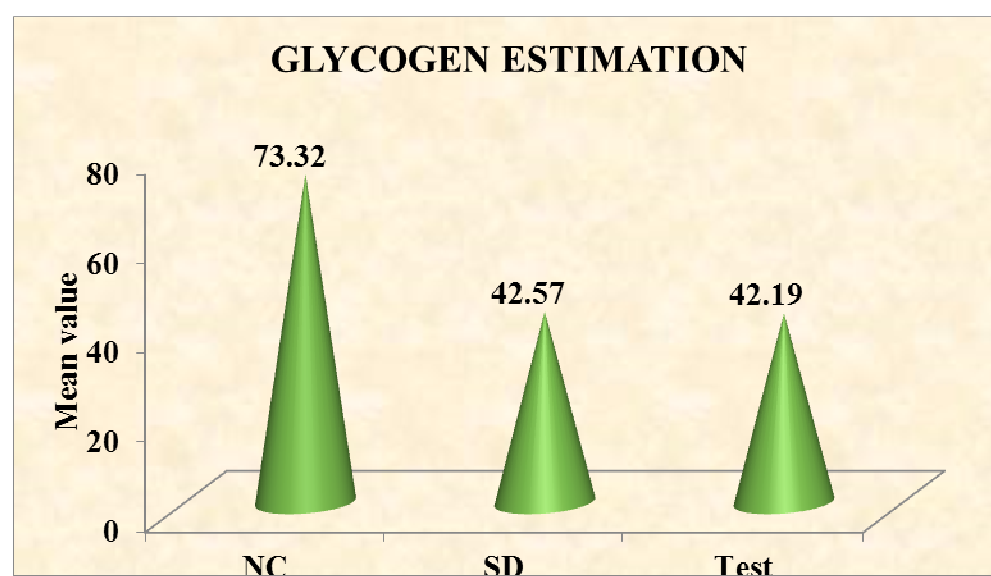


Figure 2: liver glycogen in Test group.