

Hepatoprotective and Renoprotective Potentials of the Aqueous Extract of *Bixa orellana* on Streptozotocin Induced Diabetic Rats

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Abstract

Objective: The present study was undertaken to evaluate the effects of the aqueous extract of *Bixa orellana* on the liver enzymes such as AST, ALT and ALP and also to evaluate the efficacy of the extract for the protection of the renal function in streptozotocin induced diabetic rats.

Methods: Diabetes was induced by administering streptozotocin dissolved in saline while the normal control group was given propylene glycol. Diabetes induced animals were randomly assigned into different groups. Blood samples were collected from all the control and experimental group. Estimation of urea, uric acid and creatinine as well as the liver enzymes such as AST, ALT and ALP were assayed. These estimations were carried out on day 30 only.

Results: The 30 days treatment with the aqueous extract (200 mg/kg body weight) showed no statistical significance with respect to urea and uric acid but there was statistical significance in the levels of creatinine which is an indicator of glomerular filtration rate. The alteration in the levels of ALT and ALP were highly significant statistically but no changes observed in the level of AST.

Conclusion: The employed dose of the extract is nontoxic to the kidney and it even caused a significant decrease in level of liver enzymes when diabetic induced rat subjected to treatment hence this may indicate the hepatoprotective properties of this extract.

Keywords: Hepatoprotective; Antidiabetic; *Bixa orellana*; Standard drug (SD)

Introduction

The oldest form of health care available to mankind is the use of medicinal plants and the World Health Organization (WHO) defines medicinal plant as any plant which in one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1,2]. The plant Bixa orellana used in our study is historically suggested to have the property of reducing blood glucose levels [3]. Diabetes Mellitus is a clinical condition characterized by hyperglycemia with the classical symptoms of polyuria, polydipsia and polyphagia is a third leading cause of death in the world and India is considered to be the "Diabetic Capital of the World [4]. Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 [5]. Liver and renal function tests assessment is of great significance in diabetes since it is used to diagnose the impaired organ functions and the progressive loss of both liver and kidney functions over time [6]. Devastating late complications in kidney and the liver may lead to diabetic nephropathy and liver damage in patients with diabetes mellitus [7]. Diabetic nephropathy is the major microvascular complication which leads to the over production of reactive oxygen species (ROS) [8]. The increased ROS damages the liver tissue which causes tremendous metabolic derangementas liver is the largest internal organ involved in various metabolic function [9]. Our study aimed to find out the effect of aqueous extract of Bixa orellana leaves on liver function and kidney function markers in the streptozotocin induced diabetic rats.

Material and Methods

Ethical Clearance is obtained from Institutional Animal Ethics Committee to carry out this study.

Experimental animals

Male wistar rats weighing about 200-300 g were housed in Central Animal House, Manipal in a solid bottom shoe box type cages constructed of durable plastic and contact bedding material of wheat husk and were provided with wheat gluten feed pellates and water. Rats were deprived from food for about 8 hours and are induced with Streptozotocin with the dosage of about 60 mg/kg body weight and the volume is 1 ml/kg body weight, dissolved in 0.1 M citrate buffer (pH 4.5), stored in brown bottle and kept in ice chest and the normal control group was injected with 0.9% saline intraperitoneally.

Plant material and extract preparations

B. orellana is a bushy shrub ranging from 4-10 cm 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 [10]. The leaves of *Bixa orellana* were obtained, cleaned and sun dried. The dried leaves were powdered and stored in an air tight container. The plant was identified by a botanist. It is locally available and its medicinal uses are given in the literature. The plant is used to treat gonorrhea, dysentery and hepatitis, reduce cholesterol, increase the volume of urine in cases of renal insufficiency or cystitis and also

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Received: July 28, 2018; Accepted: August 14, 2018; Published August 21, 2018

Citation: Bhat VR, Chianeh YR, Udupa P, Anushree U, Sheikh S (2018) Hepatoprotective and Renoprotective Potentials of the Aqueous Extract of *Bixa orellana* on Streptozotocin Induced Diabetic Rats. Biochem Pharmacol (Los Angel) 7: 255. doi: 10.4172/2167-0501.1000255

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the Bixin and Norbixin, the carotenoids found in the plant are used to lower blood sugar levels [11].

Aqueous extract

Finely powdered leaves were soaked in water for 1-2 hours. Refluxed for 2-3 hours by reflux condensation method and decanted when hot. The contents concentrated under reduced pressure (evaporate water). It has beenkept in water bath and heated till the syrupy consistency was obtained, then it is Preserved in an air tight container and stored in a desiccator at room temperature.

Experimental design

Confirmation of the diabetes was done after 7 days of stabilization by intraorbitally withdrawing the blood using mucap capillary. About 1-1.5 ml of blood was collected in grey vacutainer containing sodium fluoride and potassium oxalate. The 8th day was considered to be the 1st day of treatment and it was followed for 30 days. The sample was centrifuged after 30 minutes and the plasma was used for the assay (Table 1).

Biochemical estimation

The Urea was estimated using Urease or Glutamate Dehydrogenase (GLDH) method. Uric acid was estimated byuricase or Phenol and 4-aminoantipyrine (PAP) method, Creatinine by Modified Jaffe's method.

Alkaline phosphatase: Modified 2-amino-2-methyl-1-propanol (AMP) method.

Alanine Transferase (SGPT) and Aspartate Transferase (SGOT): Modified IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) method.

Group	Number of animals	Drug	Dose	Route
Normal control	6	Vehicle	-	Orally
Diabetic control	6	Vehicle	-	Orally
Insulin treated	6	Insulin	6 units/kg body weight	Intraperitoneally
Test	14	Aqueous extract Vehicle (propylene qlycol)	200 mg/kg body weight	Orally

 Table 1: Represents the treatment schedule.

Principle: Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory MEDICINE) Modified.

Statistical analysis: All data obtained were analyzed by using SPSS (commonly usedsoftware version 17.0). The significance ofdifference among the groups was assessed using Kruskal Wallis test followed by MannWhitney 'U' test. Significant value was set top <0.05. Comparison of data within the group atdifferent intervals was analyzed by Wilcoxoll Signed Rank Sum test.

Results

The administration of Aqueous extract of *B. orellana* showed no statistical significance in relation with Urea and Uric acid but showed statistically high significance p value of 0.01 in relation with creatinine (Table 2 and Figures 1-3). The liver enzyme AST was not statistically significant as its level may rise due to the damage to other organs particularly heart, muscle etc. The enzyme ALT and ALP shows high statistical significance with p value of 0.004 and 0.002 respectively on administration of the plant extract (Table 3 and Figure 4).

Discussion

Diabetes plays a central role in the initiation and progression of liver injury and this progressive disease is an independent risk factor for the development of chronic liver disease [12]. Significant increase in AST and ALT mainly due to the leakage of these enzymes from liver cytosol into the blood stream which is an indication of hepatotoxic effect of Streptozotocin and consistent with previous studies which described increase in liver oxidative stress. However in our study decrease in these enzymes could be attributed to their ability to protect /repair liver tissue damage. There are no clear distinct studies has been progressed with this plant extract and as such an attempt has been made to study their activities which gives us a more promising results so as to minimize the extent of liver damage, diabetes mellitus is also associated with complications in renal system. Our results reconfirmed that there was statistical significance in creatinine level as a significant biomarker of renal dysfunction, so however treatment of diabetic rats with the aqueous extract of *B. orellana* reduced serum creatinine levels which implies that the extract could normalize the function of kidney in diabetic rats. The characteristic loss of glucose homeostasis due to the defect in the secretion and function of insulin refers to the worldwide metabolic disorder of diabetes mellitus [13]. The deficiency of

Variable	NC (n=6)	DC (n=6)	Test (n=9)	P value			
Urea (mg/dl) Mean ± SD	84.42 ± 13.89	93.45 ± 7.9	110.82 ± 37.74	<0.067			
Uric acid (mg/dl) Median IQ	1.92(1.77,2.11)	2.38(1.90,3.88)	2.12(1.52,3.46)	<0.444			
Creatinine (mg/dl) Median IQ	0.47(0.405,0.525)	0.83(0.57,1.33)	0.93(0.53,1.33)	<0.01			

NC-Normal control DC – Diabetic control Test-Aqueous extract of *B. orellana*

Table 2: The concentration of urea, uric acid and creatinine in different groups.

Variable	NC (n=6)	DC (n=6)	Test (n=9)	P value
AST(U/L) Mean ± SD	142.15 ± 27.56	101.94 ± 26.04	110.49 ± 26.86	<0.096
ALT(U/L) Mean ± SD	134.11 ± 17.69	69.96 ± 25.32	71.3 ± 31.93	<0.004
ALP (U/L) Mean ± SD	88.22 ± 7.38	154.73 ± 41.91	138.3 ± 39.69	<0.002

 Table 3: Concentration of AST, ALT and ALP in different groups.

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Figure 1: Urea levels in different groups. (NC-Normal control DC – Diabetic control Test-Aqueous extract of *B. orellana*).



insulin impairs the metabolism of glucose, lipids and proteins. In our study, Diabetes mellitus was induced to the experimental animals by intraperitoneally injecting the drug Streptozotocin which disrupts the ß cells of Islets of Langerhans of pancreas and consequently lead to wide range of metabolic disorder such as glucose, lipid as well as protein metabolism [14]. The long lasting hyperglycemia if left untreated can lead to diabetic nephropathy with concomitant effects to the largest internal metabolic organ the liver. The major cause of diabetic nephropathy is the generation of free radicals by glycosylation reaction which leads to the formation of oxidative stress and is toxic to the tissues [15,16]. This toxicity in turn damages the hepatocytes of the liver and effects the various metabolism in the body. The dysfunction of kidney and liver is assessed by the organ function tests. Kidney function tests include Urea, Uric acid and Creatinine whereas the damage to liver releases cellular enzymes such as AST, ALT and ALP. In our study the oral administration of the aqueous extract of Bixa orellana showed no statistical significance with respect to Urea and Uric acid but there was statistical significance related to the level of creatinine. The level of creatinine is used as an indicator of glomerular filtration rate and







the increased level can cause osmotic diuresis with depletion of extracellular fluid. The plant extract was observed to decrease the creatinine level. The liver enzymes are used as a diagnostic tool to monitor the treatment for liver damage and it includes AST, ALT and ALP. AST is not a specific marker as the level can rise in damage to other organs of the body. In our study the level of ALT and ALP was in diabetic rats which was monitored by the oral administration of the plant extract and was observed to possess high statistical significance with the p value of 0.004 and 0.002 for ALT and ALP respectively. The result of this study is consistent with previous studies on the use of medicinal plants to treat diabetes is gaining importance as the researchers are focusing on the therapeutic applications of the plants.

Conclusion

The present results indicate that the leaves of the plant possess hepatoprotective property and could be exploited for the development of antidiabetic-hepatoprotective agents. Citation: Bhat VR, Chianeh YR, Udupa P, Anushree U, Sheikh S (2018) Hepatoprotective and Renoprotective Potentials of the Aqueous Extract of *Bixa orellana* on Streptozotocin Induced Diabetic Rats. Biochem Pharmacol (Los Angel) 7: 255. doi: 10.4172/2167-0501.1000255

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