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Original Research Paper

Biochemistry

VALIDATION OF ANTIOXIDANT PROPERTY OF ACANTHOSPERMUM HISPIDUM DC IN CONTROL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT Today diabetes became a global public health issue. More than 1200 species of plants are identified as herbal remedy for diabetes. In present study, Ethyl acetate and aqueous extract of *Acanthospermum hispidum* DC. was tested on blood glucose levels (mg/dl) at different time intervals of diabetic induced rats and found that the blood glucose levels are decreased and also able to alter the levels of antioxidants including TBARS, SOD, Catalase, GPX, GSH in diabetic rats after 9 hours in all the three groups of rats with body weights of 150mg/kg, 300mg/kg and 450mg/kg that are taken for testing.

KEYWORDS : Diabetes, Ethyl acetate extract, blood glucose, antioxidants, *Acanthospermum hispidum.*

Introduction:

India holds credibility of diverse social, cultural and medical heritage with an unbroken tradition coming down across millennia. Though medical heritage is centuries old, million people in rural area still depend on traditional medicine to congregate their healthcare needs (Ved and Goraya, 2008). Today diabetic mellitus is in alarming rise and became globally an important public health issue. According to world health organization projection, the diabetes population is likely to increase to 300 million or more by the year 2025. The International diabetes federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025. (Zimmet, P. 2000).

Ethno botanical studies of traditional herbal remedies used for diabetes around the world have identified more than 1200 species of plants. There are apparent evidences that bioactive phyto chemicals will reduce the risk of diseases. Thus for the present study ethyl acetate and aqueous extract of *Acanthospermum hispidum* DC. was taken to test on fasting blood glucose levels (mg/dl) at different time intervals of diabetic induced rats.

Materials and Methods:

Leaves of *A.hispidum* were collected from Rangampet and S.V.University, Tirupati campus and surrounding areas of Chittoor district. The plant is identified with the help of floras and voucher specimen was deposited in the herbarium of Department of Botany, S.V.University, Tirupati.

Leaves were shade dried in the laboratory and made into powder. The powered plant material was extracted in a Soxhlet's apparatus with ethyl acetate. The extract was distilled and used for the experiment.

Healthy albino wistar rats aged 3 1/2 months with a body weight 150 – 450 mg were procured from Sanzyme Pvt.Ltd. Hyderabad, Telangana were used in this study. Diabetes was induced into these rats by intraperitonial administration of STZ. All the animals were allowed to free access to tap water and pellet diet and maintained at room temperature.

Ethyl acetate and aqueous plant extract of *A.hispidum* is dissolved in distilled water and administered orally for 15 days. After an overnight fast the plant extract suspended in distilled water is fed by using a force feeding needle. Group 1 and Group 2 rats are fed distilled water alone. Blood samples are collected for the measurement of blood glucose from the tail vein before plant extract treatment and after plant extract treatment. Blood glucose is measured and the results are compared with those of normal rats.

The rats were divided into 5 groups and each group consists of 6 rats.

Group I – Untreated normal rats
Group II – Untreated diabetic rats
Group III – Diabetic rats with 150mg/kg body weight treated with plant extract.
Group IV – Diabetic rats with 300mg/kg body weight treated with plant extract.
Group V - Diabetic rats with 450mg/kg body weight treated with plant extract.

The blood glucose level was estimated by using dextrostics with Ames Glucometer at different time intervals as 0hr., 1hr., 3hr., 5hr., 7hr., and 9th hr.

3.15. ESTIMATION OF REDUCED GLUTATHIONE

The estimation of total Glutathione (reduced) was carried by the method of Moron, et al., (1979).

REAGENTS

1. Standard glutathione
2. 5.5-dithiobis 2-nitrobenzoic acid (DTNB), 0.6 mM in 0.2 m phosphate buffer, pH 8.0.
3. Trichloroacetic acid 5%

PROCEDURE

To 0.1 ml of tissue homogenate added 1.9 ml of TCA and the protein were precipitated. The contents were centrifuged at 3000 rpm for 15 min and the precipitated was discarded. To 1 ml of the supernatant 2 ml of DTNB reagent was added and the color developed was read at 412 nm against a blank containing TCA instead of sample. The amount of glutathione was expressed as µ moles of GSH/mg protein

ASSAY OF GLUTATHIONE PEROXIDISE

Assay of GP_x was carried by the procedure of Wendell, et al., (1981).

REAGENTS

1. 0.25 M Potassium phosphate buffer, pH 7.0 containing 2.5 mM Na_2 EDTA and 2.5 mM sodium azide
2. 2. Glutathione reductase (GR from yeast) in the phosphate buffer activity 6 units /ml
3. 10 mM GSH in water
4. 2.5 mM NADH in 0.1 % $NaHCo_3$
5. 12 mM t- butyl hydro peroxide
6. Drabkin's solution
7. Transformation solution: 4.5 mm KCN and 0.45 mM K_3 $(Fe(CN)_6)$ prepared in phosphate buffer just before use.

PROCEDURE

Heparinised venous blood was centrifuged and the plasma and leucocytes were removed. The erythrocyte sediment was washed three times with isotonic NaCl. A homolysate containing 3 mg Hb/ml was prepared by diluting the homolysate. To 0.5 ml of

transformation solution was added and left it for 10 min at room temperature, 100 ml of buffer, GR, GSH, and NADPH were transferred in to a 1 ml cuvette containing 500 ml of sample and incubated at 37°C for 10 min then the reaction was started by the addition of 100 ml of t- butyl hydro peroxide. The linear decrease in NADPH absorption was recorded at 340nm. The simultaneous reaction without NADPH and substrate was kept as blank. The activity of GPX was expressed as units/g Hb/min at 25°C.

ASSAY OF SUPEROXIDE DISMUTASE

The SOD activity was according to the method of Mishra and Fridovich (1975). After the removal of haemoglobin in the hemolysate by the method of Concetti, et al., (1976). This method is based on the ability of superoxide dismutase to inhibit the oxidation of epinephrine.

REAGENTS

1. Chloroform
2. Ethanol
3. 0.05 M carbonate buffer pH 10.2
4. Epinephrine, 0.3 mM

PROCEDURE

To the 2ml of hemolysate containing 3 mg Hb/ml, 0.5 ml of Chilled ethanol and 0.25 ml of chilled chloroform were added, centrifuged at 4000 rpm for 10 min and the supernatant was used for the assay of superoxide dismutase. The assay medium containing 50 mM sodium carbonate bicarbonate buffer, pH 10.2 containing 0.1 mM EDTA< 0.6 mM adrenaline, an sample in a total volume of 2.4 ml. Adrenaline was the last component to be added and the absorbance of adrenochrome formed was recorded at 470 nm in a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of adrenaline auto oxidation.

MEASURING OF CATALASE ACTIVITY

The activity of catalase was determined by the method of Aebi, et al., (1984). After removing of the plasma from the blood, the sediment was washed thrice with isotonic sodium chloride. The RBC were lyses with cold distilled water and a stock homolysate was prepared containing 5 g of haemoglobin per 100 ml this homolysate was diluted 500 times with phosphate buffer immediately before the assay and the amount of haemoglobin present in the hemolysate used for the assay was determined by the method of Drobkin and Austin, et al., (1932).

REAGENTS

1. Phosphate buffer (50mM)

a. KH_2HPO_4(50 mM)

b. Na_2HPO_4 (50mM)

Sodium A and B were mixed in 1:15 proportion to get the pH 7.0

2. Hydrogen peroxide, 30 mM (0.34 ml of 3% H_2O_2 was dissolved 100 ml of phosphate buffer

In to the sample cuvette and reference cuvette 2 ml each of homolysate was pipette and 1ml of 30 mM H_2O_2 was added to the sample cuvette and 1 ml of phosphate buffer was added to reference cuvette. The decrease in the absorbance was recorded spectrophotometrically for a minutes at 240 nm. The catalase activity was expressed as KU/g HB.

ESTIMATION OF THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) (Niehuas and Samuelson, 1968)

Malondialdehyde and other thiobarbituric acid reactive substances (TBARS) were measured by their reactivity with thiobarbituric acid (TBA) in acidic condition. The reaction generates a pink colored chromophore which was read at 535nm.

Reagents

1. 5 % TCA
2. HCL – 0.025N.
3. TBA – 0.375 % in hot distilled water.
4. TCA – TBA – HCL reagents. Solution 1, 2 and 3 were mixed freshly in the ratio of 1:1:1.
5. Stock standard – 4.4 mol/l solution of stock was prepared from 1, 1-3, 3 - tetra methoxy propane in distilled water.
6. Working standard – Stock solution was diluted to get concentration of 50 m.mol / ml.

PROCEDURE

Liver and kidney tissue homogenate was prepared in Tris – HCL buffer (0.025 M, pH 7.8). To 1.0ml of the plasma/tissue homogenate, 2.0ml of TCA – TBA – Hcl reagent was added and mixed thoroughly. The mixture was kept in a boiling water bath for 15 minutes. After cooling the tubes were centrifuged at 1000 rpm for 10 minutes and the pink colour develop in the supernatant was measured in a systronic Spectrometer at 535 nm against a reagent blank. series of standard solution in the concentration of 2.5-10 moles were treated in a similar manner and values were expressed as nMol/mg protein in tissue and nMol/dl in plasma.

Results and Discussion: The TBARS, antioxidant enzymes and GSH levels of liver and kidney are mentioned in Table 1 and 2 respectively. In untreated diabetic rats there is an enhancement in catalase activities of erythrocytes, liver and kidney compare those normal ones. But the activities of SOD and GPX decreased in all tissues in diabetic rats compared to those in normal rats. Similarly the levels of reduced glutathione in kidney and liver were decreased significantly than those of normal rats.

The treatment of *A. Hispidum DC* Ethyl acetate extract and aqueous extract resulted the activities of SOD and GPX increased and decreased the activity of catalyse in treated diabetic rats. GSH levels also increased more than normal after the treatment of plant extract in diabetic treated rats.

The present study shows that catalase activity was increased and SOD and GPX activity were decreased in diabetic rats. This is due to accumulation enzymes in tissues, where as others reported the increased levels of both SOD and catalase in the erythrocytes of diabetic rats (Terakhina et al., 1998) the possible researches for the enhanced activity of catalase is comparatively mechanism of H_2O_2 saving to avoid tissue damage.

The treatment of diabetic rats with *A. hispidumDC* resulted in the improvement of kidney function by normalize the alterations. In diabetic conditions the diabetic liver showed structural alteration the major alteration are depletion of hepatic glycogen granules and the treatment with plant extracts normalised these structural changes and improved hepatic glycogen granules. Under diabetes in pancreas, there is a destruction of islet and degranulation of beta –cells. Diabetes is associated with several morphological changes of kidney. Pathological changes in kidney due to hyper glycaemia lead to nephropathy (Horie et al 1997). The degenerative changes in diabetes may be due to increased oxidative stress also which could be auto oxidation and glycosylation processes. In this study the changes observed in diabetic rat kidneys were normalized with treatment of plant extract.

Table.1. TBARS, SOD, Catalase, GPx, GSH, level in the liver of normal and experimental animals.

Groups	Mean ±SD %change	TBARS	SOD	Catalase	Glu peroxidase	Glutathione
Group 1	Mean ±SD	0.76 ±0.06[a]	41.52±1.54[d]	29.65±3.22[a]	0.39±0.06[c]	138.68±3.23
Group2	Mean ±SD	1.69±0.08[d]	14.42 ±1.12[a]	58.25±3.46[b]	0.12±0.02[a]	72.53±3.12[a]
Group3	Mean ±SD	1.36[c]±0.14[c]	26.45 ±1.72[b]	41.15±3.21[a]	0.28±0.02 12[b]	99.13±4.15[b]
Group4	Mean ±SD	1.12±0.14[b]	36.53 ±0.98[c]	32.46±1.16[a]	0.33±0.08[c]	126.11±3.64[c]
F-Value		96.409	299.152	51.325	92.456	152.632
Significance	0.000	0.000	0.000	0.000	0.000	0.000

Table. 2. TBARS, SOD, Catalase, GPx, GSH, level in the Kidney of normal and experimental animals.

Groups	Mean ±SD %change	TBARS	SOD	Catalase	Glu peroxidase	Glutathione
Group 1	Mean ±SD	64.26 ±2.55 [a]	36.55 ±1.99 [c]	91.12 ± 5.26 [a]	0.26 ±0.04 [d]	82.24±6.22 [d]
Group2	Mean ±SD	92.52±2.45 [d]	13.23 ±1.24 [a]	382.39 ± 5.86 [d]	0.14 ±0.02 [a]	34.53 ±1.96 [a]
Group3	Mean ±SD	76.15±3.89 [b]	24.26± 1.32 [b]	162.11 ± 7.23 [c]	0.16 ±0.006 [b]	54.62±3.02 [b]
Group4	Mean ±SD	82.18 ±3.96 [c]	33.16 ±1.22 [c]	116.15± 5.13 [b]	0.21 ±0.008 [c]	72.46 ±1.55 [c]
F-Value		59.582	128.346	1346.48	52.152	84.125
Significance	0.000	0.000	0.000	0.000	0.000	0.000

Table.3. SOD, Catalase, GPx, level in the Plasma of normal and experimental animals.

Groups	Mean ±SD %change	SOD	Catalase	Glu peroxidase
Group 1	Mean ±SD	13.36 [d] ±1.51 [d]	15.92±1.56 [a]	54.42±2.16 [d]
Group2	Mean ±SD	3.42±0.31 [a]	45.78±3.48 [c]	25.49±2.12 [a]
Group3	Mean ±SD	7.22±0.61 [b]	32.19±1.82 [b]	38.12±1.51 [b]
Group4	Mean ±SD	10.42 [c] ±1.26 [c]	20.83±1.26 [a]	49.78 ±2.56 [c]
F-Value		94.12	159.648	132.62
Significance	0.000	0.000	0.000	0.000

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