



EVALUATION OF ETHANOLIC EXTRACT OF ORYZA SATIVA (VAR. JOHA RICE) FOR ANTI DIABETIC ACTIVITY IN STREPTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study evaluated the anti-diabetic activity of the ethanolic extracts of *Oryza sativa* (var. Joha rice) (Ethanolic Extract of Joha Rice) on blood glucose of albino rats. Ethanolic Extract of Joha Rice was administered at doses of 200 and 400 mg/kg body weight respectively on Streptozocin induced diabetic rats for 17 days. Diabetic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 200 & 400 mg/kg body wt. /day resulted in a marked decrease in the levels of fasting blood glucose, with a concomitant increase in body weight. Streptozocin induced diabetic rats treated with Ethanolic Extract of Joha Rice (200 & 400 mg/kg) significantly reversed all these changes to near normal. Quantification of antioxidants of the Ethanolic Extract of Joha Rice revealed that Joha rice had high antioxidant property. These results suggest that Ethanolic Extract of Joha Rice possess anti diabetic in Streptozocin induced diabetic rats.

KEY WORDS

Ethanolic extract, streptozocin, joha rice, fasting blood glucose, antioxidant property

INTRODUCTION

Diabetes mellitus type 1 (also known as **type 1 diabetes**) is a form of diabetes mellitus in which not enough insulin is produced. This results in high blood sugar levels in the body. The classical symptoms are frequent urination, increased thirst, increased hunger, and weight loss.

Diabetes mellitus type 2 (also known as **type 2 diabetes**) is a long-term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Common symptoms include increased thirst, frequent urination, and unexplained loss. Symptoms may also include increased hunger, feeling tired, and sores that do not heal.

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable degree with onset or first recognition during pregnancy. A study found this

condition to be associated with persistent metabolic dysfunction in women at 3 years after delivery, separate from other clinical risk factors.

Prescribing pattern: Rational use of the drugs is a defined as follows: "That patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time and at the lowest cost to them and their community". Rational use of the drugs in populations can be effectively evaluated with drug utilization studies.

Drug utilization studies seek to monitor, evaluate and implement remedies in the prescribing practice with the aim of making the medical care rational and effective.

The goal of management in people with diabetes is to provide:

- Relief from diabetic symptoms and improve quality of life.
- Prevention of acute complications, Prevention of infections. The compliance of people with diabetes management covers treatment as well educating programs include.
- Appropriate self-care skills.
- In depth information about diabetes its complications and treatment.
- Appropriate resources of self-care.
- Self-monitoring skills.

Utilization pattern of anti-diabetic drugs:

Drugs: Monotherapy:

- Biguanides: Metformin
- Sulfonylurea: Glimepiride
- Alpha-glucosidase inhibitors: Voglibose
- Insulin

Combination therapy:

- Insulin + (Glimepiride + Metformin)
- Insulin + Glipizide
- Metformin + Insulin
- Metformin + (Glimepiride + Metformin)
- Insulin + Glimepiride
- (Glimepiride + Metformin) + Voglibose
- Insulin + (Pioglitazone + Glimepiride)
- (Glimepiride + Metformin) + (Voglibose+ Metformin)
- Insulin + (Metformin+Chromium+Pioglitazone)
- Insulin+(Glimepiride+Metformin+pioglitazone)

CO-MORBIDITIES IN TYPE 2 DM: Co-morbidity is defined as the occurrence of one or more chronic conditions in the same person with an index-disease, occurs frequently among patients with diabetes. Common co-morbidities are:

- Hypertension
- Micro vascular diseases
- Cholelithiasis
- Arthritis
- Kidney disorders
- Hyperlipidaemia
- Macro vascular diseases

MATERIAL AND METHODS

Apparatus and Equipments:

General laboratory glass ware includes volumetric flasks, measuring cylinders, beakers, pestle and mortar, filter papers, test tubes, micro pipette, weighing balances and water bath. Specific equipment used for extraction and study include soxhlet apparatus, centrifuge.

Plant Material

Collection and Authentication of plant material

Oryza sativa (var.Joha Rice) was collected from local areas of Assam state and was authenticated by Prof. Dr.K.Madhava chetty, Taxonomist, SVU University, Chittoor, Andhra Pradesh (India).

Preparation of Extracts

In general plant material after drying is then grounded into a fine powder. Grinding of plant materials into smaller particles facilitates subsequent extraction procedures by rendering the sample more homogeneous, increasing the surface area, and facilitating the penetration of solvent.

Oryza sativa (var.Joha Rice) was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and extracted successively with ethanol. Finally, extract was concentrated by distilling off the solvent and then evaporated to dryness on the water-bath, until to get the extract. The extract obtained with each solvent was weighed and its percentage in terms of the air-dried weight of the plant material was then calculated and also the consistency of the extract was noted. The extracts were then subjected to preliminary phytochemical screening for the detection of various plant constituents and used for the pharmacological investigations.

Percentage Yield Calculation

Oryza sativa (var.Joha Rice) powder was weighed and subjected to hot extraction. Total 1300 grams Joha rice and 750 ml ethanol were used for extraction. Percentage yield was calculated using following formula:

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{Weight of total rice taken}} * 100$$

Evaluation of Pharmacological Activities:

Experimental Animals:

Wistar Albino Rats (150–200g) were obtained from the Animal House, CRC group, Hyderabad. Rats were maintained on standard pellet diet and tap water *ad*

libitum. They were kept in clean cages under a 12-hour light/dark cycle, at room temperature 22–24°C and were acclimatized to the environment for 2 weeks prior to experimental use. The fresh diet and water for the animals has to be supplied daily to the animals. The condition of the animals has to be supervised daily till the completion of the experiment. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee.

Vehicles and Preparation of Doses:

To prepare the dosage forms **Ethanollic Extract of *oryza sativa* (var. Joha Rice)** is made a suspension with 1% tween 80. The dose in required concentration was administered at 1ml/100g body weight of the animal.

Evaluation for Anti-diabetic activity

Experimental Design:

Animal: Rats, **Sex:** Either, **Weight:** 150-200 gms, **Age:** 2-3 months

Induction of experimental diabetes:

- Rats, overnight fasted, were injected with single intraperitoneal injection of freshly prepared streptozotocin solution (60 mg/kg, i.p; dissolved in 0.1 M cold citrate buffer; pH 4.5) to induce experimental type 1 diabetes.
- For the i.p. injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone- iodine solution and the designated amount of STZ was injected in the caudal abdominal cavity using sterile 25g needle.
- STZ induce fatal hypoglycaemia as a result of massive pancreatic insulin release, the rats were provided with 5% dextrose (glucose) solution after 6 h of STZ administration for next 24 h to prevent drug induced hypoglycaemia.
- Streptozotocin induces diabetes within 3 days by destroying the beta cells
- Diabetes was confirmed at 72 h, after induction of diabetes by polydipsia and polyuria along with measuring the non-fasting plasma glucose levels by measurement of tail vein blood glucose levels with the glucose meter (Eidi, *et al.*, 2006).
- Animals, which did not develop more than 200 mg/dl glucose levels, were rejected.
- Group the diabetic animals.
- Then animals were treated with extract and standard drug according to the recommended dose on 3rd day of diabetes induction (1st day of the treatment).

- Take the fasting blood glucose levels on 1st day of the treatment after 90 mins of treatment.
- After 7 days of treatment, blood glucose levels and body weights were measured.
- In the same way BGLs and body weights were measured on 14th, 21st days of treatment.
- Then stop the treatment on 14th day and measure BGLs for withdrawal effect on 21st day.
- Control rats were injected with citrate buffer alone.

TREATMENT OF ANIMALS - MULTIPLE DOSE STUDIES

The diabetic rats were divided into 4 groups. One normal control group was included

Group 1: Control rats received distilled water, Oral

Group 2: Diabetic rats received distilled water, Oral

Group 3: Diabetic rats received plant extract

(ETHANOLIC EXTRACT OF JOHA RICE) 200 mg/kg, Oral

Group 4: Diabetic rats received plant extract

(ETHANOLIC EXTRACT OF JOHA RICE) 400 mg/kg, Oral

Group 5: Diabetic rats received standard drug (Glipizide) 5mg/kg, Oral

Care of diabetic animals:

Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day and, in some circumstances, more than once per day. Diabetic rats should have sufficient food and water; therefore, only three diabetic rats have been housed per cage to avoid competition for feed and water.

Collection of serum samples:

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anaesthesia on different occasions i.e., day 0, day 1, day 3, day 7, day 14 and day 21. The blood samples were allowed to clot for 30 min at room temperature and then they were centrifuged at 5000 rpm for 20 min. The resulting upper serum layer was collected in properly labelled, clean and dry micro-centrifuge tubes. The blood samples were stored at 2-8 °C and analyzed within one week. This serum specimen was used for the estimation of different biochemical parameters.

Biochemical parameters analyzed:

Blood glucose levels were estimated from the serum by using standard kits. (Oliver-Bever, Rai MK, Sarti C,)

a) Body Weight

The body weight of each animal was recorded on the day 0, 1, 3, 7, 14 and 21st day using weighing balance i.e. the days corresponding to serum glucose analyzed.

b) Fasting Serum Glucose Estimation

Serum glucose was estimated by GOD/POD method with help of clinical chemistry analyzer (Metro Lab, 1600 DK-R) GLUCOSE LIQUID STABLE REAGENT (Swemed Diagnostics, Bangalore).

Principle

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose peroxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidases to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

Glucose oxidase

Glucose + O₂ + H₂O₂ Gluconate + H₂O₂ → H₂O₂ + 4-aminoantipyrine + Peroxidase + Phenol Red Quinoneimine dye + H₂O

Procedure

To 1000 µl of the reagent, 10 µl of standard glucose (100 mg/dl) was added and incubated for 5 min at 37 °C. This incubated mixture was aspirated, and concentration of standard was calibrated to show a value of 100 mg/dl. The fasting serum glucose was estimated by adding 10

µl of the serum sample to 1000 µl of the reagent, mixed well and incubated at 37 °C for 5 min. This incubated mixture was aspirated, and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

Calculation

Glucose (mg/dl) = $\frac{\text{Absorbance of test} \times \text{conc. Of STD}}{\text{Absorbance of STD}}$

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one-way analysis of variance (ANOVA). The test was followed by Dennett's 't'-test, p values less than 0.05 were considered as significance.

RESULTS

Results of Extraction

The successive solvent extraction was done using ethanol using standard procedure. The behavior of various extracts like texture and colour and extractive yield were calculated. The result is given in **Table—1**

Table-1: % Yield of ethanolic extract of *oryza sativa* (var. Joha Rice)

Solvent	Texture of Extract	Colour	% Yield
Ethanol	Oily semisolid	Brownish black	3.6

Result of Evaluation of Anti-diabetic activity

Blood glucose levels:

Effect of ethanolic extract of Joha Rice on blood glucose levels in diabetic rats depicted in Table- 2. In animals treated with streptozotocin (60 mg/kg i.p) (Group II), a significant increase in serum glucose level was observed on 3rd, 10th, 17th and 25th day when compared with normal rats (Group I). Group V received Glipizide (5 mg/kg p.o.) showed decrease in blood glucose level

when compared with diabetic control rats (Group II). After the oral administration of ethanolic extract of *Joha Rice* in diabetic control rats, a significant reduction in blood glucose level was observed on the 3rd, 10th, 17th and 25th day compared with diabetic control rats (Group II). On 25th day, observed withdrawal effect in standard group but no such effect in test groups. The results were shown in **Table- 2** and values were plotted in **Figure- 1**.

Table 2. Effect of *Oryza sativa* (Var. Joha Rice) on Fasting Blood Glucose level in Streptozocin induced diabetic rats

S.NO	GROUPS	TREATMENT	Fasting Blood Glucose Levels(mg/dl) MEAN±SEM				
			0 Day	3 rd Day	10 th Day	17 th Day	25 th Day
1	I	Normal	89.83±1.30	87.5±1.56	86.5±1.33	88.5±1.05	90.0±0.57
2	II	Control	88.33±1.11 ^{ans}	538±26.10 ^{a***}	533.7±26.94 ^{a***}	445±16.63 ^{a***}	416.3±20.68 ^{a***}
3	III	EEJR(200mg/kg)	85.67±1.11 ^{bns}	467.5±17.88 ^{b*}	241.2±35.39 ^{b***}	91.83±1.57 ^{b***}	93.0±1.39 ^{b***}
4	IV	EEJR(400mg/kg)	89.83±0.60 ^{bns}	385.8±7.98 ^{b***}	205.3±21.61 ^{b***}	80.17±2.89 ^{b***}	85.17±1.81 ^{b***}
5	V	Glipizide (5mg/kg)	98.33±1.11 ^{bns}	589.7±8.50 ^{bns}	323.0±18.67 ^{b***}	95.67±1.62 ^{b***}	124.8±5.09 ^{b***}

Effect of EEJR on Fasting Blood Glucose level on Streptozocin induced diabetic rats

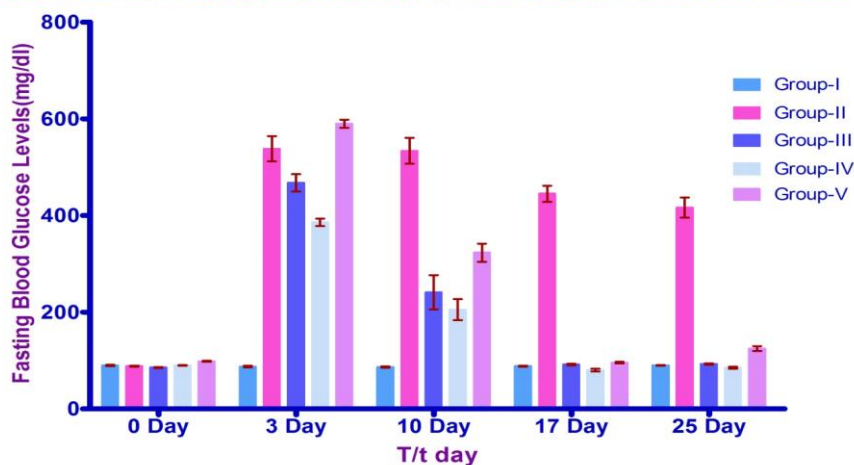


Fig 1. Effect of ETHANOLIC EXTRACT OF JOHA RICE on BGLs in Streptozocin induced diabetic rats

Body weight:

Effect of ethanolic extract of Joha Rice on body weight in diabetic rats depicted in Table-3. In animals treated with streptozotocin (60 mg/kg i.p) (Group II), a significant increase in body weight was observed on 3rd, 10th, 17th and 25th day when compared with normal rats (Group I). Group V received Glipizide (5 mg/kg p.o.) showed decrease in body weight when compared with

diabetic control rats (Group II). After the oral administration of ethanolic extract of *Joha Rice* in diabetic control rats, a significant reduction in body weight was observed on the 3rd, 10th, 17th and 25th day compared with diabetic control rats (Group II). On 25th day, observed withdrawal effect in standard group but no such effect in test groups. The results were shown in Table- 3 and values were plotted in Figure- 2.

Table 3. Effect of *Oryza sativa* (Var. Joha Rice) on Body weight on Streptozocin induced diabetic rats

S.NO	GROUPS	TREATMENT	BW (mg/dl) MEAN±SEM				
			0 Day	3 rd Day	10 th Day	17 th Day	25 th Day
1	I	Normal	155.8±2.00	156.3±1.56	157.5±1.58	159.7±2.60	160.5±1.85
2	II	Diabetic Control	156.7±2.108 ^{ans}	104.7±2.91 ^{***}	98.5±0.42 ^{a***}	97.83±0.47 ^{a***}	97.83±0.60 ^{a***}
3	III	Extract(200mg/kg)	152.5±3.09 ^{bns}	115.0±4.83 ^{bns}	130.0±2.88 ^{b***}	154.2±2.38 ^{b***}	156.0±1.91 ^{b***}
4	IV	Extract(400mg/kg)	158.3±3.07 ^{bns}	121.8±2.83 ^{b***}	138.3±1.66 ^{b***}	151.5±9.20 ^{b***}	160.2±2.31 ^{b***}
5	V	Standard	157.5±3.59 ^{bns}	110.2±3.76 ^{bns}	132.5±2.14 ^{b***}	152.3±1.76 ^{b***}	142.3±1.76 ^{b***}

Effect of EEJR on body weight of streptozocin induced diabetic rats

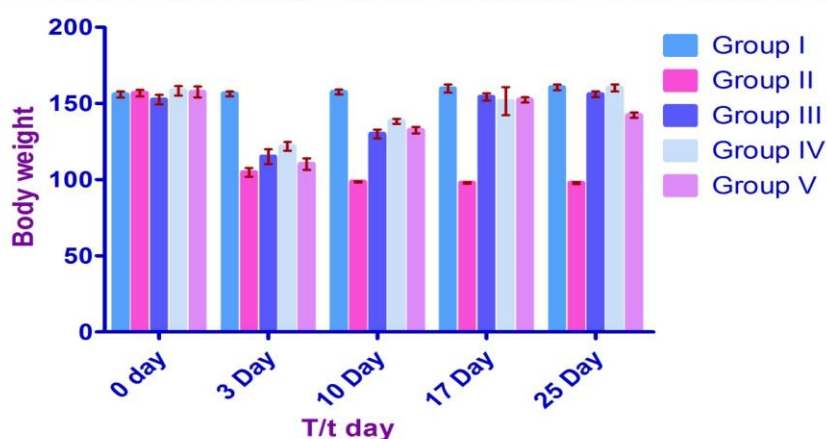


Fig 2. Effect of EEJR on Body Weight in Streptozocin induced diabetic rats

CONCLUSION

The present study suggests ethanolic extract of *Oryza sativa* (var. Joha Rice) possess good Antidiabetic activity on experimental animals.

This ultimately, proves the traditional uses of this rice for protecting diabetics by native tribal peoples of Assam.

Finally, it can be concluded that by further extensive research, it can be used as drug in the treatment/management of these diseases and can explore globally this indigenous variety of rice (i.e. *Oryza sativa* (var. Joha Rice) from Assam for its medicinal value.

DISCUSSION

Aromatic rice are found to have anti diabetic activity. ETHANOLIC EXTRACT OF JOHA RICE also possess anti-oxidant potential. So, it was evaluated for anti – diabetic activity. Diabetes mellitus was induced by Streptozocin (60mg/kg b.w.) the treatment group received standard drug Glipizide (5mg/kg b.w.) and ETHANOLIC EXTRACT OF JOHA RICE was given to the test groups- low dose (200mg/kg b.w.) and high dose (400mg/kg b.w.) for a period of 25 days. The blood serum was collected frequently on 0, 3rd, 10th, 17th, 25th days and measured the biochemical parameters like blood glucose levels, total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, very low-density lipoproteins, serum creatinine, and blood urea. Along with these parameters body weight was also taken.

Streptozocin decreases the insulin secretion by destroying the β -cells of islets of langerhans in pancreas. Due to this insulin deficiency, diabetes mellitus occurred. Glipizide, the standard drug and ETHANOLIC EXTRACT OF JOHA RICE, the extract showed the anti-diabetic activity by preventing the destruction of β -cells and enhancing the secretion of insulin.

The estimation of these parameters in the standard group (Glipizide) and test groups (ETHANOLIC EXTRACT OF JOHA RICE) revealed that the level of Blood Glucose levels was increased than the negative control group in a significant manner when evaluated statistically by using the software Graph Pad Prism 5.0 ANOVA study was done for the results of the level of parameters obtained by using Dunnet t-test.

The standard drug decreased the level of blood glucose than the negative control. The Test drug- high dose (ETHANOLIC EXTRACT OF JOHA RICE) decreased the

level of blood glucose than the negative control. The differences were found to be significant. The Test drug- low dose (ETHANOLIC EXTRACT OF JOHA RICE) decreased the level of blood glucose than the negative control. The differences were found to be significant. Hepatic enzyme levels were also significantly increased in standard and test groups. After 7th day of the treatment standard group showed the withdrawal effect, but test groups not shown such effects.

Thus, it can be reported that the ethanolic extract of *Oryza sativa* (var. Joha rice) possess significant anti-diabetic activity.

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