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ROLE OF AQUEOUS EXTRACT OF *JUSSIAEA REPENS* ON CELLULAR OXIDATIVE STRESS AND METABOLIC STATUS IN DIABETIC RATS INDUCED BY STZ.

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ABSTRACT: This work aimed to explore how crude aqueous extract of *Jussiaea repens* affected oxidative stress and metabolic status in streptozotocin (STZ) induced diabetic male rats. The diabetic rats were gavaged with aqueous extract of *Jussiaea repens* (50 mg/100 g body weight/day for 32 daysconsicutively) after an induction of STZ (8 mg/100g body weight, i.p.). Fasting blood glucose, insulin, serum LDH, total GSH, MDA, G-6-PDH, amylase, lipase, catalase and superoxide dismutase were measured. The hepatic levels of hexokinase, PFK, G-6-Pase, F-1,6-BPase and glycogen were also measured. The aqueous extract of *Jussiaea repens* decreased blood glucose level in diabetic control group. The extract supplemented group also demonstrated a notable improvement in blood insulin levels, SOD, GSH and G-6-PDH level in comparison to diabetic group. In diabetic group, the LDH and MDA levels were significantly higher than non-diabetic control rats and were significantly recovered in extract supplemented group. But catalase level showed no significant change. The liver glycogen, hexokinase and PFK were lower in the diabetic group, whereas G-6-Pase and F-1,6-BPase were higher, showing statistical recovery in the extract-treated group. Serum amylase and lipase followed the pattern of recovery like G-6-Pase and F-1,6-BPase. These findings suggested that aqueous extract of *Jussiaea repens* could regulate diabetes mellitus and oxidative stress in diabetics. This work will aid in the development of cheap herbal medicine for the amelioration of diabetes mellitus in future.

INTRODUCTION: Diabetes, usually referred as hyperglycemia, which is a disorder marked by a lack of or resistance to the insulin hormone, which is produced by pancreatic β cells [1]. Nearly 382 million peoples are affected worldwide by diabetes, with the figure expected to climb towards 592 million by 2035, as such India was called the "Diabetes Capital of the World" as diabetes cases soared [2].

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Generally, diabetes is linked with physiological stress as well as oxidative stress causing cell damage, which leads to a rise in the free radical generation *viz.* superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), along with the decreased antioxidant defense system [3].

WHO recommends ancient and complementary herbal medicinal therapy for diabetes because they are fruitful, harmless, have less or no adverse effects and are good candidates for oral therapy [4]. However, many wetland plants have been scientifically confirmed as anti-diabetic herbs. Of these, *Jussiaea repens* (*J. repens*), belonging to the Onagraceae family, is a potent herb and is mostly found in wetlands all over India and most of the

countries of the world [5]. So, the primary goal of this study is to find out whether the aqueous extract of *J. repens* could help to alleviate diabetes mellitus by lowering oxidative stress in diabetic male rats.

MATERIALS AND METHOD:

Crude Aqueous Extract Preparation: During summer, the plant *J. repens* was collected from the marshes of Katwa, West Bengal, India. The plant, which includes stem and leaves, was collected and dried before being crushed in a mixer grinder.

For extraction, 50 g dry powder of *J. repens* was poured into 0.5L of distilled water. Then the solution was left for cooling at normal temperature overnight after being boiled for 30 min. After filtering the mixture, it was thickened by incubation at 40°C, followed by drying at 40°C until a thick paste was prepared [6].

Animal Selection and Maintenance: Eighteen male albino rats weighing about 100–120 g were chosen for this study. They were kept in standard laboratory settings (25°C, 12 h dark/light cycles, relative humidity of 40-60%) and fed a standard food and water recommended by the ICMR, NIN, Hyderabad, India [7]. The Institutional Animal Ethics Committee (IAEC No: IEAC/BST/2016/003) authorized the research dated 21.12.2016.

Induction of Diabetes: Animals were administered streptozotocin (STZ) (8mg/100g body weight, i.p. dissolved in 0.1M cold citrate buffer, pH 4.5) after a 12h fast.

The fasting blood glucose was measured at the day of STZ administration and after 7 days and they were marked as induced diabetic animals to be used in future experiments [6].

Experimental Model: The animals were split into three groups of eight animals each (n=8) at random as -

Group I (Vehicle Control): Given 0.1ml distilled water/100g body weight/day through gavage feeding.

Group II (Diabetic Control): Given a single dose of STZ.

Group III (Supplemented Group): Given 0.5 ml (50mg of aqueous extract of *J. repens*/100g body weight/day) for 32 days consecutively through gavage in STZ induced diabetic rats.

Measurement of Blood Glucose: Accu-check active glucometer was used to measure fasting blood glucose level. Blood was drawn from the rat's tail vein with light anesthesia. The blood glucose level was tested on the 0, 7^{th}, 18^{th}, 29^{th} and 39^{th} day of treatment.

Estimation of Insulin: Insulin was measured by Electrochemiluminescence immunoassay (ECLIA), with standard protocol [8].

Estimation of Antioxidant Enzymes: Lipid peroxidation was assessed by measuring malondialdehyde (MDA) by the Niehaus and Samuelson method (1968) [9], serum GSH level was estimated by standard kit [10], Superoxide dismutase (SOD) activity was determined by the process of Beauchamp and Fridovich (1971) [11], Catalase enzyme level was estimated by the method of Claiborne (1985) [12], G-6-PDH and LDH level were measured by Standard kits [13, 14].

Estimation of Metabolic Enzymes: Hexokinase, PFK, F-1,6-BPaseand G-6-Pase status in hepatic tissue were measured by the protocol of Brandstrup *et al.* [15],Castano *et al.* [16], Hikaru and Toshitsugu [17] and J. M. Gancedo and C. Gancedo [18] respectively.

The hepatic Glycogen level was measured following the method of Ong and Khoo[19]. The serum Amylase and Lipase levels were measured by standard kits [20, 21].

Statistical Analysis: The observed result was denoted as a Mean±SEM. One-way ANOVA with post hoc Turkey's multiple comparison testing was used to analyze the experimental groups.

GraphPad in Stat version 3 was used to conduct the tests. The significance level of $p<0.05$ is considered to be statistically significant.

RESULTS:

Effects on Glycemic Level: In contrast to the group I, the glucose level in group III remained considerably increased ($p<0.01$), whereas in group III, a significant ($p<0.05$) decline was observed after the intervention **Fig. 1.**

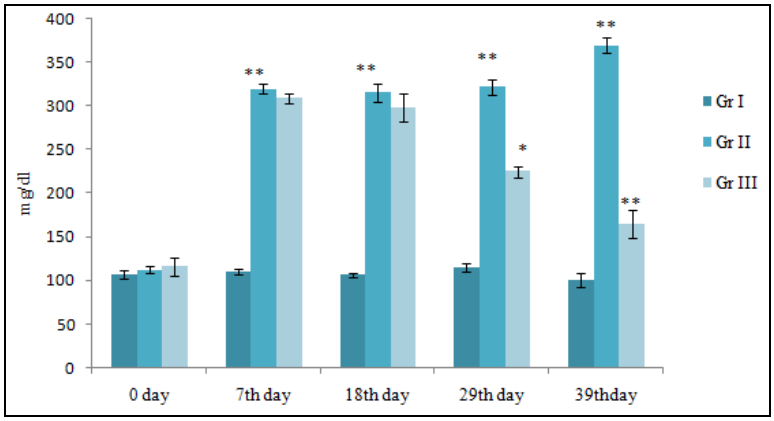

FIG. 1: GRAPHICAL REPRESENTATION OF RELATIVE CHANGES IN THE BLOOD GLUCOSE LEVEL OF DIFFERENT GROUPS. *P<0.05, ** P<0.01.

Alteration in Insulin Level: The serum insulin level in the diabetic group was remarkably (p<0.001) lower than group I. Insulin level in group III was considerably (p<0.05) increased after 32 days of treatment with aqueous extract of *J. repens* **Fig. 2.**

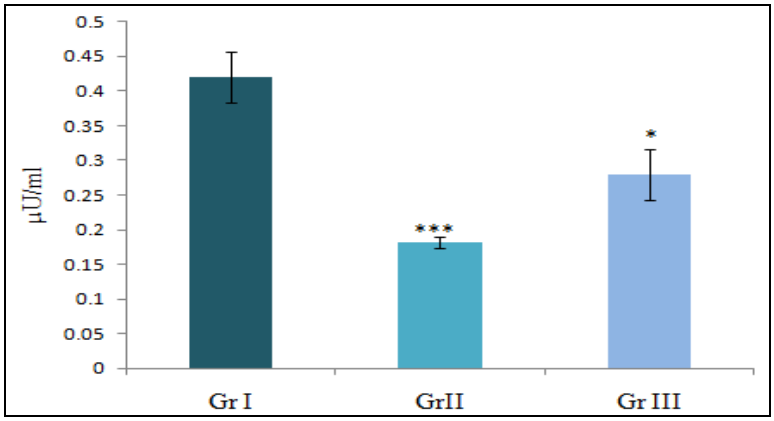

FIG. 2: GRAPHICAL REPRESENTATION OF RELATIVE CHANGES IN SERUM INSULIN LEVEL OF DIFFERENT GROUPS. *P<0.05, ***P<0.001.

Changes in Antioxidant Enzymes and Oxidative Stress Markers: The activities of serum LDH and MDA were dramatically enhanced in group II compared to group I in this study, but the aqueous extract of *J. repens* effectively lowered the serum LDH and MDA levels in group II animals. In contrast to group II, serum GSH, G-6-PDH and liver SOD levels were considerably higher in group III. However, no significant change was observed in Catalase level between the groups **Table 1.**

TABLE 1: EFFECT OF AQUEOUS EXTRACT OF *J. REPENS* TREATMENT ON ANTIOXIDANT ENZYME AND OXIDATIVE STRESS MARKERS

Groups	LDH (IU/L)	MDA (nmol/ml)	GSH (µmol/ml)	G-6-PDH (µ/gHb)	SOD (U/mg of protein)	Catalase (µmols of H_2O_2 decomposed/min/mg protein)
Group I	136.899±9.235	2.305 ±0.386	0.635 ±0.038	2.95±0.23	119.52 ±6.95	5.81 ±1.31
Group II	277.333±20.498***	7.198±0.452***	0.281±0.011***	0.91 ±0.14***	60.075±7.875*	3.09 ± 0.97
Group III	165.666 ±11.882**	3.948±0.372***	0.470±0.033***	1.72 ±0.15*	102.22 ±3.1*	4.75 ±0.88

The data are expressed as mean ± SEM, n=8. *p<0.05, **p<0.01, ***p<0.001. Comparison between Gr-I vs Gr-II, and Gr-II vs Gr-III.

Effect on Hepatic Glycogen Content: Diabetic control rats had considerably lower hepatic glycogen content (p<0.05) in comparison to vehicle control rats, which was increased significantly (p<0.05)in extract-treated rats when compared with diabetic control group **Table 2.**

Effect on Metabolizing Enzymes: The impact of administering the aqueous extract of *J. repens* on metabolizing enzymesin diabetic control rats was shown in **Table 2.** In case of hexokinase level, there was a considerable ($p<0.05$) recovery in group III but PFK level showed no such remarkable recovery in respect to STZ induced diabetic group. The group II animals expressed a higher level of F-1,6-BPase and G-6-Pase than group I. After 32 days of extract treatment, the recovery of activities of such enzymes showed no satisfactory changes in group III. The amylase and lipase levels were elevated in group II but statistically ($p<0.05$) declined in group III.

TABLE 2: EFFECT OF AQUEOUS EXTRACT OF *J. REPENS* TREATMENT ON ENZYMES

Parameters	Group I	Group Ii	Group Iii
Liver glycogen (mg/g)	21.89±3.02	8.45±0.65*	17.25 ±1.85*
Hexokinase (nmols of g-6-phosphate formed/min/mg/protein)	300.68±25.65	95.79±3.81*	177.15 ±12.32*
Phosphofructokinase (U/mg protein)	3.34 ±0.09	2.19 ±0.23*	2.72 ±0.17
Glucose-6-phosphatase (nmols of Pi liberated/min/mg protein)	45.46 ± 1.84	110.45 ±5.89**	75.34 ±4.05*
Fructose 1,6-bisphosphatase (nmols of phosphate liberated/min/mg protein)	24.39 ±4.88	150.12 ±19.52*	90.78 ±11.95
Amylase (U/L)	1848±90.87	2313.07±72.77	1541.8±116.7*
Lipase (U/L)	12.66±0.95	29.15±0.94**	17.5±1.6*

The data are expressed as mean ± SEM, n=8. * $p<0.05$, ** $p<0.01$.Comparison between Gr-I vs Gr-II, Gr-II vs Gr-III

DISCUSSION: Streptozotocin causes oxidative cell damage in the pancreas, liver, kidneys, and among other organs. Oxidative stress indicators are high in primary phases in many diseases where the liver is considered the major organ for free radical generation [22]. In the present study, after 32 days of chronic treatment, STZ-induced diabetic rats treated with crude aqueous extract of *J. repens* significantly dropped blood glucose.

The anti-hyperglycemic effect of *J. repens* is linked to an increase in plasma insulin levels, implying that the plant extract has insulin-like activity. This suggests that the aqueous extract of *J. repens* promotes pancreatic β-cell insulin production. There have been numerous studies on medicinal plants that have an anti-diabetic effect *via* insulin secretion in this setting [23]. In other studies, Li-Hsiang Chen and Li-Wei Wu found a comparable increase in LDH levels in diabetic patients. They explained that lactate is converted to glucose from pyruvate in the glucogenic flux with the help of the functioning enzyme lactate dehydrogenase (LDH) [24]. It also strengthens our findings of recovery in LDH activity in diabetic rats treated with *J. repens* extract, possibly following the stimulation of NADH oxidation. Earlier it was reported that hydroxyl radicals and singlet oxygen produced conjugated dienes, lipid peroxyl radicals and hydroperoxides, while oxygen-free radicals acted on membrane lipids to make MDA. The chemical alteration of MDA and 4-hydroxynonenal by lipid peroxidation may cause protein degradation [25]. In this study, treatment of aqueous extract of *J. repens* at the dose of 50mg/100 g body wt. exhibited a considerable decrease in MDA level, indicating the amelioration of protein damage.

Despite the fact that catalase is a primary oxidative stress marker, any remarkable difference has not been found between the diabetic and *J. repens* treated groups in this investigation. This experimental studies found a substantial reduction in SOD activity in group II, which may be associated with the rise of the generation of superoxide anions. SOD deficiency in the hepatic tissues could be caused by H_2O_2 inactivation or glycation of an enzyme [26]. Such findings in this study could reveal that aqueous extract of *J. repens* may enhance SOD activity. Diabetes indirectly causes GSH depletion, which leads to oxidative stress. GSH detoxifies reactive oxygen species (ROS) produced by STZ exposure through the glutathione redox cycle. The aqueous extract of *J. repens* treated rats exhibited a considerable recovery in GSH levels in this experiment. NADPH

is also one of the physiological chemicals that work in tandem with the antioxidant defense system. This NADPH is produced by G-6-PDH as a part of Glucose-6-phosphate (G-6-P) metabolism. Because of its involvement in free radical scavenging, G-6-PDH deficiency may cause oxidative stress in diabetes mellitus [27], which showed partial recovery by supplementing the aqueous extract of *J. repens* in diabetic control rats. Hexokinase is an enzyme that helps in the formation of G-6-P from glucose. It's reduced activity slows the glucose oxidation *via* glycolysis, resulting in a diabetic condition and reduced adenosine triphosphate (ATP) production. Here, the diabetic rats revealed lower hexokinase levels, even *J. repens* stimulated the enzyme activity. The results obtained may be related to elevation in insulin level [28].

In both gluconeogenesis and glycogenolysis, G-6-Pase facilitates the breakdown of G-6-Pto glucose. Insulin slows glucose production by diminishing the level of G-6-Pase and F-1,6-BPase in hepatocytes [29]. In diabetic rats, both hepatocellular enzymes were found to be considerably increased. The level of these enzymes recovered after extract administration. But the PFK level expressed no recovery in the supplemented group.

We know insulin suppresses glycogenolysis and gluconeogenesis in the liver while promoting glycogen synthesis. Insulin enhances glycogen production and glycolysis in muscle by stimulating the facilitated transfer of glucose *via* the GLUT4 glucose transporter [30]. The oral administration of aqueous extract increased hepatic glycogen content in diabetic rats. This could be related to higher insulin secretion, causing the glycogen synthase system to reactivate.

The exocrine and endocrine dysfunction may result from pancreatic impairment in diabetes mellitus, leading to the rise of amylase and lipase levels in diabetics [31]. In the present work, both these enzyme activities were recovered partially by plant extract treatment. The antioxidant characteristics of *J. repens* aqueous extract are positively correlated with phenolic components such as gallic acid, cholinergic acid, p-caumaric acid, sinapic acid and flavonoids. Possibly Myricetin, rutin and quercetin, the flavonoids which are actively present in this aqueous extract, may have both antioxidant and anti-diabetic activities. It may help to prevent oxidative stress by scavenging free radicals [32, 33].

CONCLUSION: We may conclude that *J. repens* can alter the activities of various oxidative stress markers in diabetic conditions, which supports the ethnobiological use of *J. repens* in diabetes treatment. In the near future, this plant extract could be patented as an anti-diabetic herb, making it more accessible to the common people.

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