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Aqueous extract of *Jussiaea repens* L. ameliorates diabetes associated complications in STZ induced diabetic male rats

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Abstract: Nowadays, diabetes is a 'World Threat' in human health. For prevention and control of diabetes mellitus, different synthetic drugs were consumed deliberately by the patients inspite of knowing the harmful effect. In the modern scenario, people are becoming conscious about the uses of herbal antidiabetic drugs. Among so many herbs, *Jussiaea repens* L. (Keshardam) has ethnobiological reports on antidiabetic activity. In this study, the aqueous extract of *J. repens* (50mg/100gm/body wt/day for 32 days consecutively) was fed (oral gavage) in STZ induced (8mg/100gm/body wt, a single i.p. dose) male diabetic rats, which caused marked recovery in different physio-chemical disturbances in diabetic rats. The Blood glucose level was significantly reduced ($p<0.05$) in *J. repens* extract-treated group at 39th day (day of sacrifice). The SGOT, SGPT, Total Serum Protein, Total Cholesterol, Triglycerides, Serum Alkaline Phosphatase, Urea, and Creatinine level were showed a significant change in the supplemented group compared to the diabetic group. In the liver, necrosis of hepatocytes caused vacuolation in between hepatic chords in diabetic group, which was partially recovered by *J. repens* extract showing compactness of liver tissues. Similarly, hypertrophy was also observed in renal glomeruli and renal tubules due to STZ treatment. So, the present study highlights that *J. repens* extract bears the potentiality of antidiabetic activity. As in the diabetic rats, it may be due to poor level of antioxidants, causes rise of blood glucose, cellular degeneration, hepatotoxicity, nephrotoxicity etc., which can be prevented by *J. repens* extract possibly due to presence of antioxidant-polyphenol compounds. In broader future, the *J. repens* extract should be an herbal patent against diabetes mellitus, which will be of low cost-effective and easily available for the common people.

Keywords: *Jussiaea repens*; Diabetes mellitus; Blood glucose; Cholesterol; Urea; Triglycerides.

Introduction

Diabetes mellitus is found in almost all nations of the world, so it is called a global disease. Moreover, the morbidity and mortality rate is increased continuously worldwide, it has an estimated 135 million people with diabetes, and it would rise to 380 million by the year 2025.[1] Diabetes mellitus is a group of metabolic disorders characterized by abnormal carbohydrate metabolism resulting in chronic hyperglycemia caused by defective insulin production or appropriate and efficient insulin utilization by cells. It is thought to contribute to various biochemical changes in cellular metabolisms, vascular complications, and hematological changes.[2] Diabetes exists with a number of causes, such as chronic pancreatitis, pancreatectomy, hyperthyroidsm, pheochromocytoma, and genetic or acquired defects. To prevent diabetes mellitus, man consumes a variety of synthetic drugs with dreaded side effects[3], where herbal drugs are cheaper and safer than synthetic drugs and may be used without or minimum side effects.

Nowadays, researchers are trying to develop different drugs with therapeutic uses from plant extracts. They found that medicinal plants and their constituents act similarly as modern drugs and sometimes better without the dreaded side

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effects. Such a medicinal plant, *Ludwigia adscendens* L. (Synonym – *Jussiaea repens* L. family–onagra-ceae) is a herb, commonly known as creeping water prime rose. *Jussiaea repens* L, locally known as 'Kesardam' in different parts of India. It has been found in the shallow waters of streams and lakes and freshwater swamps, canal etc. throughout the different districts of West Bengal, Jharkhand, Orissa, and Manipur in India as well as in China, Africa, Thailand, Malaysia, Australia, New Guinea, and the Philippines at low and medium altitudes. By chromatographic, chemical, and spectroscopic studies, different scientists reported that aerial parts of this plant is composed of different metabolites like rutin, kaempferol, quercetin, terpenes, triterpenes, trifolin 2″-O-gallate, hyperin 2″-O-gallate, guaijaverin, reynoutrin, juglanin, avicularin, hyperin, trifolin etc. [4-5]A new acylated avicularin, namely avicularin 2″-(4‴-O-n-pentanoyl)-gallate along with these metabolites have also been isolated from the ethyl acetate extract of the aerial parts of *Jussiaea repens* L.[6] Pharmacologists reported this plant's clinical uses as hepatoprotective, antihelmintic, anti-dysenteric, anti-inflammatory, anti-bacterial, fibrinolytic anti-gonadal, and anti- fertile property.[6-8] It also has some therapeutic uses i.e., in ulcer, fever, cough, diuretic, urinary tract infection etc.[9] Despite the different therapeutic uses of *Jussiaea repens*, it may be used to treat diabetes mellitus like other medicinal herbs, but it does not appear in any scientific research and publication in support of the claim. Therefore, this study was carried out to investigate the anti-diabetic potential of *Jussiaea repens* L., in a dose and duration-dependent manner.

Materials and Method

Plant Material

The plant *Jussiaea repens* L. was collected from wetlands of 24 Parganas (N), West Bengal, India, during March - April. The material was identified and authenticated by taxonomist of Central National Herbarium (Kolkata), Botanical Survey of India (BSI), Shibpur, Howrah, West Bengal, India, having voucher specimen no. JRL/SG/NKP/IC/2011 NP-01. Fresh plants were carefully washed under running tap water and then with distilled water, air-dried at 35-40°C for 4-5 days, then homogenized to a coarse powder by mixer grinder and stored for extraction.

Extract Preparation [10]

100 gm of dry powder of *J. repens L.* was taken for extraction in 1 liter of hot distilled water for 30 minutes. Then the solution was cooled and kept overnight at room temperature. The extract was filtered, and the resulting solution was then concentrated by incubation at 40 °C till thick paste, and further dried at 40 °C.

Animal Selection and Maintenance

Eighteen adult male albino rats (*R. norvegicus* L. of Wistar strain) weighing 120±10gm were selected and used for the experiment. The rats were maintained under standard laboratory conditions (temperature 25 ± 2°C, 12/12hr dark, and light, relative humidity 40-60%) with free access to a standard normal diet, prescribed by ICMR, NIN, Hyderabad, India [11] and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IEAC/BST/2016/003).

Induction of diabetes

After overnight fasting, diabetes was induced by intraperitoneal injection of Streptozotocin (STZ) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 80 mg/kg. After a 1-week time for development, the rats with moderate diabetes having glycosuria and hyperglycemia were considered diabetic rats and used for the experiment. [12]

Experimental Design and Extract Administration

The animals were randomly divided into three groups of six animals each (n=6), as –

a) Group I - given a 0.1ml distilled water/ 100gm body weight/day, for the whole experiment, through the gavage method.
b) Group II - given Streptozotocin (STZ) single dose (8mg/100gm body weight, dissolved in 0.1ml citrate buffer) through i.p injection.
c) Group III - given 0.5 ml (50mg of aqueous extract of *Jussiaea repens* /100gm body weight/day) extract for 32days through the gavage method in STZ treated diabetic rats.

Preparation of serum

Blood samples collected in vials were kept for at least 30 minutes in undisturbed condition at room temperature. After that, it was centrifuged for 15 minutes at 3000 rpm by a Spin win. The transparent fluid was collected from the upper layer of the tube. The collected serum was then preserved at – 20˚C for further use.

Measurement of blood glucose:

Fasting blood glucose levels were measured by standard strip method using glucometer. Blood was collected from tail vein with recurring light anesthesia. The glucose level was measured on 0, 7th, 18th, 29th, and 39th day of treatment.

Biochemical analysis

SGOT and SGPT were determined by Reitman's and Frankel methods.[13] Serum alkaline phosphatase was measured by Mod. King and King's method[14].Total protein estimated by Biuret method[15]. The DAM method measured serum urea and creatinine [16] and the Alkaline picrate method[17]. Triglycerides were determined using the glyceryl phosphate oxidase method and total cholesterol measured by the CHOD-PAP method.[18] All assays were done by standard kit.

Histopathological Studies:

A portion of liver and kidney tissue was dissected out and fixed in formal saline, and processed. After fixation, tissues were embedded in paraffin. Fixed tissues were cut at 5 µm and stained with hematoxylin and eosin. The sections were examined under light microscope, and photomicrographs were taken.[19]

Statistical Analysis

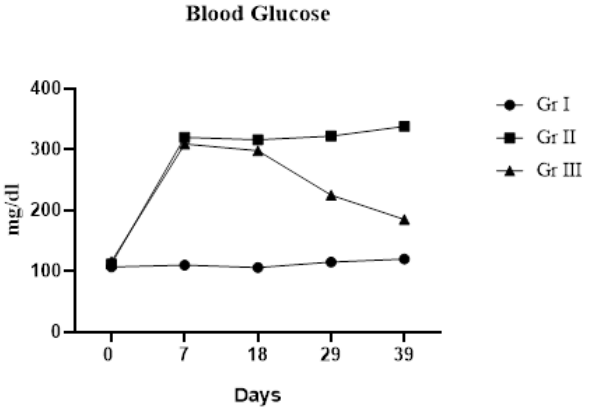
The recorded values are expressed in mean ± SEM. The control group and experimental groups were compared to each other by using one-way ANOVA with post hoc Turkey's multiple comparison test. The tests were performed using Graph pad InStat version 3. The value of $p<0.05$ is considered to be statistically significant.

Result

Effect on blood glucose level

The effect of *J. repens* extract on blood glucose is given in Figure 1. The blood glucose level was increased significantly in STZ induced diabetic rats, but in the extract-treated diabetic group, a significant ($p<0.05$) reduction has been found at the end of the treatment.

Figure 1. Graphical representation of relative changes of blood glucose levels at different groups of rats after 0, 7, 18, 29, 39 days of treatment with *J. repens* extract.



Significance level $p<0.05$.

Effect on SGOT, SGPT and ALP level

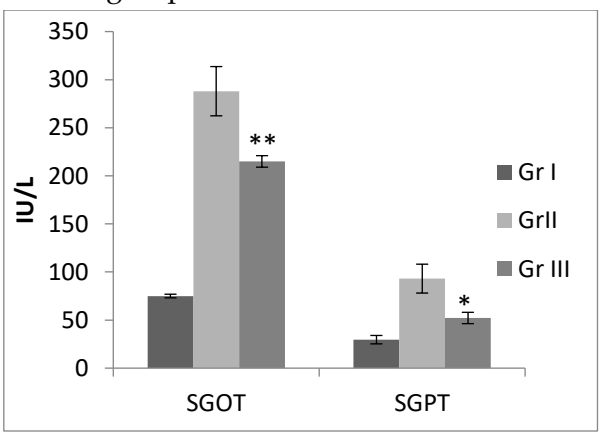
In the present study, SGOT, SGPT, and ALP levels were determined at the end of the experimental study. SGOT, SGPT, ALP was increased in the diabetic group than the extract-treated group and control group. The *J. repens* at the dose of 50 mg/100 gm body wt., significantly reduced the serum SGOT, SGPT

and ALP level compared to the diabetic control group. (Figure 2 & 3)

Effect on Total Protein, Urea and Creatinine level

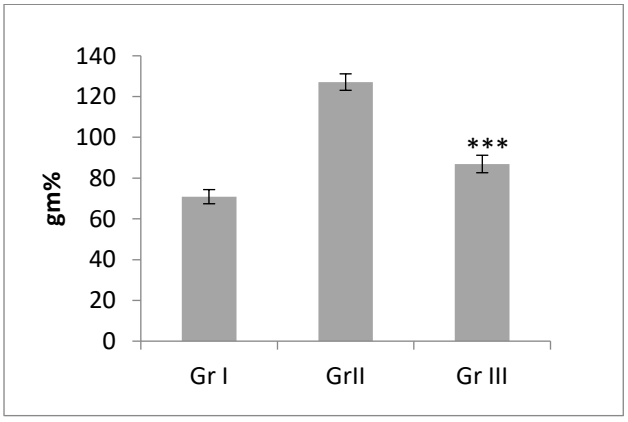
In our experiments, urea and creatinine level was increased, whereas total protein level was decreased in STZ induced diabetic group. But in the extract-treated group, the serum urea and creatinine levels were significantly reduced, and the total protein level was elevated. (Figure 4 & 5)

Figure 2. Graphical representation of relative changes of Serum SGOT and SGPT level of different groups.



*=p<0.05, **= p<0.01, ***=p<0.001.

Figure 3. Graphical representation of relative changes of Serum ALP level of different groups.

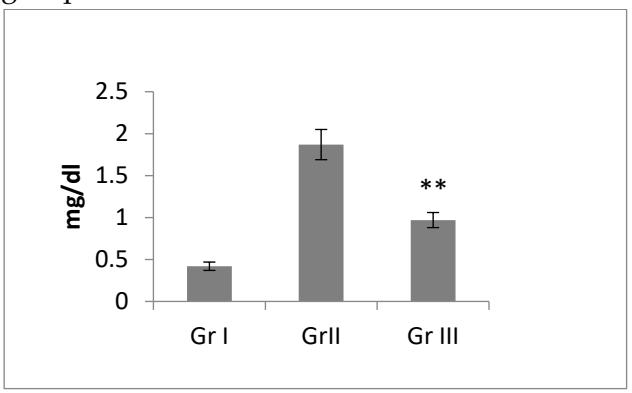


*=p<0.05, **= p<0.01, ***=p<0.001.

Effect on lipid content level

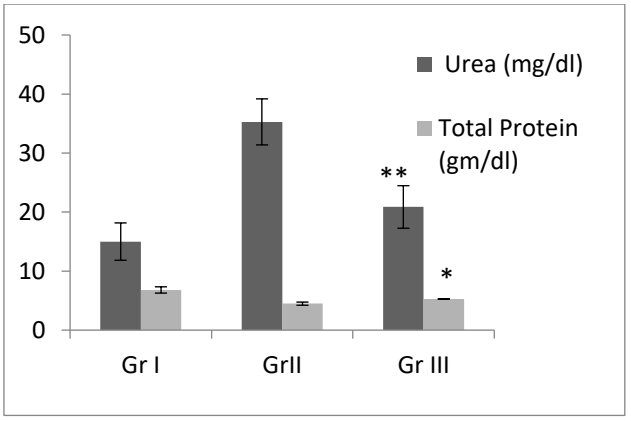
In the STZ induced diabetic rats, the increase in blood glucose level is accompanied by an increase in serum cholesterol and triglycerides level. But the extract-treated group showed a marked significant reduction in both the parameter. (Fig 6)

Figure 4. Graphical representation of relative changes of Serum Creatinine level of different groups.



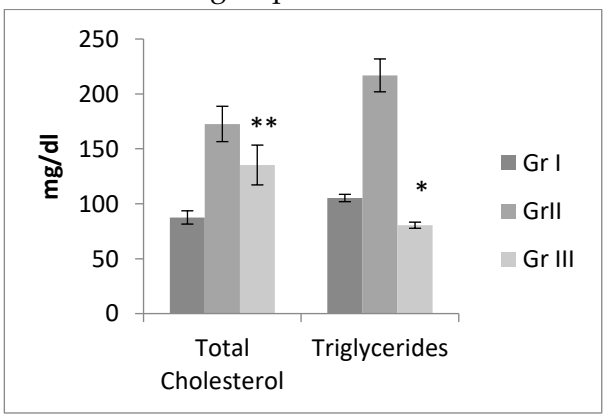
*=p<0.05, **= p<0.01, ***=p<0.001.

Figure 5. Graphical representation of relative changes of Serum Urea and Total Protein level of different groups.



*=p<0.05, **= p<0.01, ***=p<0.001.

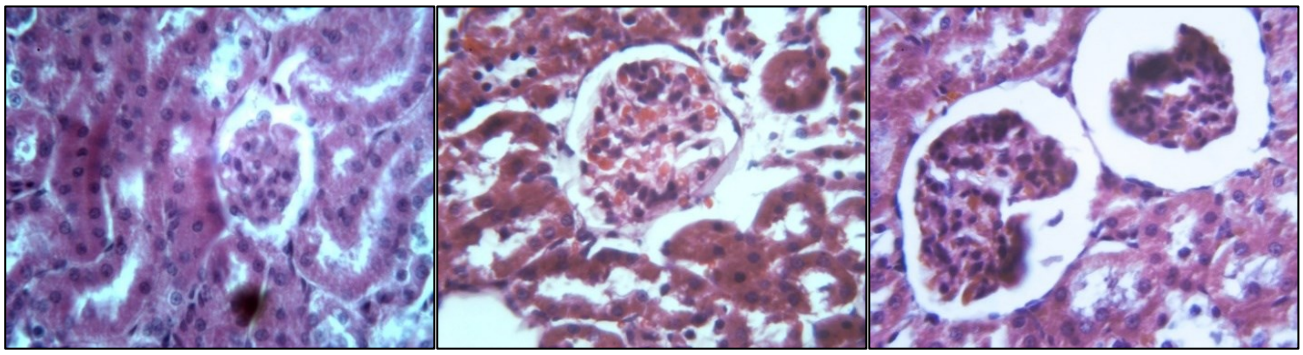
Figure 6. Graphical representation of relative changes of total cholesterol and triglycerides level of different groups.



*=p<0.05, **= p<0.01, ***=p<0.001.

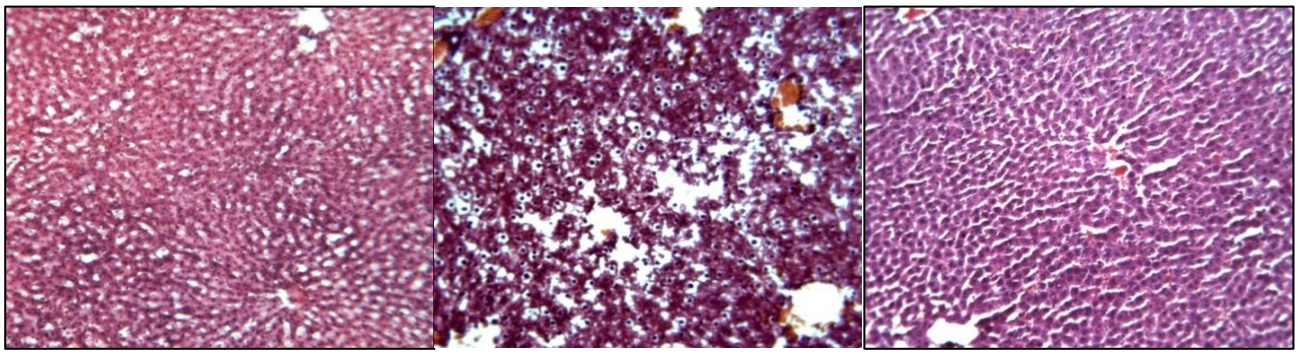
Effect on Histopathological study

Histological changes of liver and kidney tissues in different groups are described in fig. 7 and fig. 8.



A B C

Figure 7. Paraffin sections stained by haematoxylin and eosin for histopathological examination of the kidney of rats. A. kidney tissue of control showing normal structure of glomerulus and Compact renal tubules. B. kidney tissue of diabetic rats showing glomerular hypertrophy, tubular necrosis. C. kidney tissue of diabetic + aqueous extract of *J. repens* showing enlarged Bowmans capsule, prominent glomerulus. H&E, 40X.



A B C

Figure 8. Paraffin sections stained by haematoxylin and eosin for histopathological examination of hepatocytes of rats. A. liver tissue of control showing normal structure, central vein (C.V), normal arrangement of hepatic cords and hepatocytes; B. liver tissue of diabetic rats showing degenerative hepatocytes, necrosis; C. liver tissue of diabetic + aqueous extract of *J. repens* showing normal structure, central vein, normal hepatic cords, few necroses, less degenerative changes. H&E, 10X.

Discussion

From ancient times, diabetic patients have used medicinal plants to maintain blood glucose level.[20] In this regard, the present study is extended to show the influence of *J. repens* extract on blood glucose level, lipid profile, hepatotoxicity, nephrotoxicity in STZ induced diabetic rats.

It is known that hyperglycemia in both animals and humans with type 1 diabetes results from the increase in hepatic glucose output and the decrease in peripheral glucose utilization.[21]

This study shows that *J. repens* produced a marked decrease in blood glucose level in diabetic rats after treatment. The antidiabetic effect may be due to the increased release of insulin from the existing β cells of pancreas.[22]

SGPT and SGOT are the intracellular enzymes released into the bloodstream, and they serve as a marker of tissue injury, chiefly hepatocyte as well as renal injury. In addition, an increased level of liver function enzymes SGPT and SGOT in serum are not only used for the identification

of liver damage but also for metabolic syndrome diabetes mellitus. Our result obtained suggests that oral administration of *J. repens* extract showed a hepatoprotective effect.[23]

Kondeti *et al*.[24] reported that STZ induced diabetic rats account for the observed decrease in the total protein content. Increased urea production in diabetes might result from enhanced catabolism of liver and plasma proteins [25]. *J. repens* extract treatment has appreciably normalized the content of protein and urea. In response to STZ treatment, creatinine was increased in the serum, suggesting an impairment of kidney functions[25]. *J. repens* showed a clear improvement in kidney functions, perhaps due to the antioxidant properties.

In STZ induced diabetic rats, altered lipid metabolism was observed, indicated by increases in total cholesterol and triglycerides levels. The hypertriglyceridemia observed in diabetic rats may be due to increased absorption and formation of triglycerides and decreased triglycerides uptake in peripheral tissues. Hypercholesterolemia may be attributed to increased altered enzymatic pathways for the metabolism or increased dietary cholesterol absorption. Our study results showed that there was a decrease in both parameters in the supplemented group. It has been proposed that *J. repens* may be increasing insulin production, which lowers the cholesterol and triglycerides levels by increasing the activity of enzyme lipoprotein lipase and peripheral tissue utilization of cholesterol.[26]

Histological observation in renal tissue showed enlargement of glomerulus structure in diabetic group caused by diabetogenic metabolic stress and was partially corrected by *J. repens L.* extract. The renal tubular necrosis caused by diabetes and also caused partially rectified. In diabetes, the glomeruli showed degenerated infiltration by inflammatory cells and thickening of the basement membrane. The proximal convoluted tubule (PCT) showed edematous changes with the deposition of mucopolysaccharides and hyaline substances.[27] This was partially corrected by herbal supplementation due to antioxidants, which cause the prevention of inflammatory changes.

In liver tissue, the disintegration of hepatic cords, distorted, sinusoids tissue necrosis, vacuolations and degenerations were observed in diabetic rats. This was markedly corrected by *J. repens L.* extract supplementation, causing tightness, integration of hepatic tissues with prominent central vein and sinusoids, but karyolysis by diabetes was not markedly notified. Lipid peroxidation is supposed to cause destruction and leads to changes in membrane permeability, fluidity, and enhanced protein degradation by STZ in the diabetic group.[28] In the present study, STZ increases free radicals in diabetic cells, which *J. repens* (L) extract was ameliorated. This study also coincides with Reham and Md Salem, 2015 in paracetamol-induced hepatotoxicity prevention by *Nigella sativa* extract.[29-30]

Conclusion

The study showed that aqueous *J. repens L.* had antidiabetic property, which causes rectification of anomalies in different physiological parameters i.e., blood glucose, lipid profile, etc., STZ induced diabetic male rats. In conclusion, the present study provides evidence of *J. repens L.* as a potential medicinal herb for integrative management of diabetes and associated complications.

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