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Original article

Hepato-Renal Protective Actions of Aqueous *Lactuca scariola Linn* (Prickly Lettuce) leaves extract in Diabetic Rats

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Abstract:

Background: Diabetes mellitus results in a high blood sugar level over a prolonged period. Chronic hyperglycemia causes tissue and organ damage. The present study was carried out to investigate the Antidiabetic activities and possible Hepato-Renal protective action of aqueous *Lactuca scariola Linn* leaves extract on alloxan induced diabetic rats.

Materials and Methods: The alloxan induced diabetic rats were used in the experimental groups; control, diabetic, diabetic + aqueous *Lactuca scariola Linn* extract. Rats of all the groups were studied for Oral Glucose Tolerance Test (OGTT) followed by sub chronic supplementation of leaves extract on rats as per the experimental design. Histopathology studies of liver and kidney were also performed.

Results: The study showed a significant decrease in the blood glucose level of the experimental rats treated with aqueous *Lactuca scariola Linn* extract. It was also revealed from the Histopathology studies that, the sub chronic supplementation of *Lactuca scariola Linn* leaves extract showed improvement in the liver and kidney tissues of diabetic rats.

Conclusion: As per the results obtained from the experiments it can be revealed that in addition to Antidiabetic properties, *Lactuca scariola Linn* leaves extract has a protective effect on liver and kidney tissue damage in diabetic rats.

Key words: Histopathology, Diabetes Mellitus, Lactuca scariola Linn, Hepato-Renal protection, Prickly Lettuce

Introduction:

Diabetes mellitus (DM) is a group of metabolic diseases, in a course of time leads to high blood sugar level [1]. Chronic hyperglycemia during diabetes leads to oxidative stress, which causes redox imbalance of the body due to surplus production of reactive oxygen species [2]. Accordingly, it causes tissue and organ damage [3]. Diabetes causes many complications like heart disease, stroke, chronic kidney failure etc. if left untreated [4, 5]. Diabetes tends to increase the oxidative stress in several organs and liver in particular [6]. Liver plays an important role in the regulation of carbohydrate metabolism [7]. It is also important to note that oxidative stress due to hyperglycemia is effective in development of kidney related diseases [8].

Lactuca scariola Linn is an herb commonly known as Prickly lettuce. It is being used in the folkloric medicine for managing respiratory, gastrointestinal and other ailments. It is also found to possess anti-diabetic and cardio protective properties. Hence, the present study aimed to examine the anti-diabetic property of *Lactuca scariola Linn*

leaves extract on alloxan induced diabetic male rats and also its protective action on liver and kidney damage due to hyperglycemia.

Materials and methods

Collection of Plant sample:

The fresh green leafy plants were collected directly from the agriculture field or from the local market. The plant sample was authenticated as *Lactuca scariola Linn* at the Department of Botany, BLDEA's S.B. Arts and K.C.P. Science College, Vijayapur, Karnataka, India.

Preparation of plant extract:

Freshly collected green leaves on washing thoroughly with distilled water, soaked for few minutes. 5 g of soaked leaves were added to 0.1 L distilled water in a glass beaker. The beaker was placed inside the waterbath. The mixture was heated at a temperature of 50^0 – 60^0 C for about one and half hour to get the plant extract. The extract was filtered through Whatman filter paper 1.

Phytochemical analysis:

The plant extract was analyzed using standard procedures to find out phytochemical components such as alkaloids, saponins, triterpenes, flavonoids, tannins, steroids etc.

Animals:

The male albino rats of Wister strain were selected for carrying out antidiabetic studies. The weight of each rat was around 200 g. Three rats were housed for 12h light and 12h dark cycles in a metabolic wire cage having the dimension 0.6m X 0.3m X 0.2m. Hindustan Unilever rat feed (Mumbai, India) was given as diet for rats during the experiments.

Ethics:

The ethical clearance was obtained from "Institutional Animal Ethical Committee" of BLDE (Deemed to be University), Vijayapur, Karnataka, India. This ethical clearance was approved as per the ethical norms of "CPCSEA, Ministry of Social Justice and Empowerment, Government of India".

Oral toxicity study of the extract:

OECD guidelines received from "CPCSEA, Ministry of Social Justice and Empowerment, Government of India" was followed to study oral toxicity of the extract [9]. Different doses of aqueous leaves extract from 5 mg / 100 g.b.wt to 125 mg / 100 g.b.wt were fed to the rats. The stepwise doses did not show any significant signs of toxicity. Hence, one tenth of this maximum dose was used to study antidiabetic and Hepato-Renal protective action of the plant extract.

Induction of diabetes:

Diabetes was induced to overnight fasted rats by injecting (i.p.) freshly prepared 15% alloxan monohydrate solution (15 mg / 100g.b.wt.) in normal saline. After 72 h fasting blood glucose levels were measured in the blood samples collected from the rat's tail vein. Only the rats which showed the blood glucose level above 250 mg / dL were considered diabetic and were used for the study.

Experimental design:

The experimental rats were divided into three groups of six rats each as shown in Table **1**.

Table1: Experimental groups

Group	Supplementation	Dosage
Group I	Control	Distilled water
Group II	Diabetic	Alloxan monohydrate, 15mg/100g bwt, *i.p* one dose and subsequently distilled water.
Group III	Diabetic + *Lactuca scariola Linn*	12.5 mg/100 g, orally

Anti-diabetic activity of aqueous study:

Oral glucose tolerance test (OGTT):

In the first step of OGTT, fasting blood sugar (FBS) was measured for all three groups of rats. Subsequently, rats of all the groups were supplemented with aqueous leaves extract half an hour prior to the administration of glucose (0.35 mg/100g.b.wt.). An Accu check glucometer was used to check Blood glucose level before glucose administration and at every half an hour for 2.0 hours [10].

Sub chronic (13 days treatment) studies on leaves extract in diabetic rats:

From the day of OGTT, oral supplementation of aqueous leaves extract (12.5mg/100g.b.wt) was continued in the designed experimental groups (group II and group III) for 13 days [11]. Blood collected from rat tail is measured for blood glucose level in the same manner after half an hour of feeding aqueous leaves extract on every alternate day starting from Day 1 to Day 13 in rats of all groups [11].

Histopathology examination

Tissue collection and fixation:

All the animals were sacrificed on day 14. After proper dissection of animals, the tissue samples from liver and kidney were isolated and washed thoroughly in normal saline. Tissue samples were then fixed in 10% buffered formalin for 24 h.

Section cutting and staining:

The tissue fixation was carried out routinely followed by embedding in paraffin and 5µm thick sections were taken out using a soft brush. Tissues were then stained with H&E and examined under a light microscope. Lastly, sections were made to mount in Dibutylphthalate Polystyrene Xylene (D. P. X.).

Photomicrographs were captured; morphological changes were observed and evaluated to know the extent of diabetes induced necrosis in all the tissue samples stained with H&E.

Results

Phytochemical analysis:

On carrying out the preliminary phytochemical analysis, the phytochemical components present in experimental aqueous leaves extract is listed in **Table 2**

Table 2: Phytochemical constituents of *Lactuca scariola Linn*

Sl. No.	Phyto constituent	Status of Constituent
1	Test for alkaloids	Negative
2	Test for carbohydrates	Negative
3	Test for Flavanoids	Positive
4	Test for Saponins	Positive
5	Test for Tannins	Negative
6	Test for Triterpenes	Negative
7	Test for Steroids	Positive
8	Test for Carotenoids	Negative

Anti-diabetic effect of aqueous *Lactuca scariola Linn* leaves:

Influence of aqueous *Lactuca scariola Linn* leaves extract on acute exposure (OGTT) on diabetic male rats:

The OGTT results of group I control rats showed the normal blood glucose level. The blood glucose level in Group II diabetic rats was increased significantly. The diabetic group III rats on feeding with the extract a significant decrease in blood glucose level was seen **(Figure 1).**

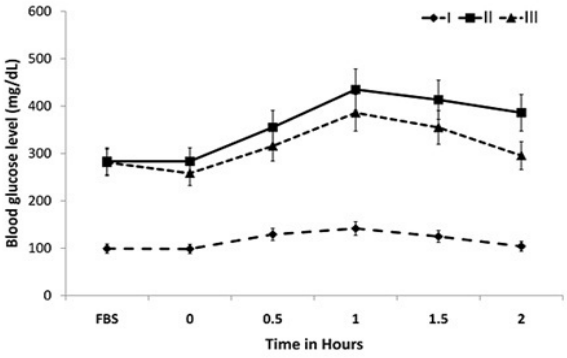

Figure 1. Treatment groups: Group I, control; Group II, diabetic control; Group III, diabetic + *L. scariola Linn* leaves extract (12.5 mg/100g.b.wt)

Effect of aqueous *Lactuca scariola Linn* leaves extract on the Sub chronic (13 days) exposure on diabetic male rats:

Sub chronic exposure of group I rats showed normal blood glucose level whereas Group II diabetic rats showed a significant increase in blood glucose level over a period of 13 days. However, the diabetic rats of group III showed considerable decrease in blood glucose level on supplementation with aqueous *Lactuca*

scariola Linn leaves extract. The blood glucose level decreased to near normal **(Figure 2)**.

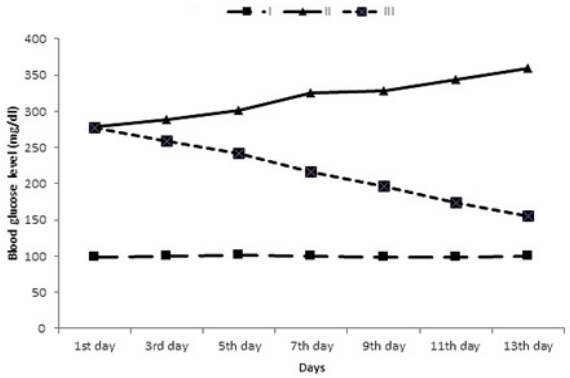

Figure 2. Treatment groups: Group I, control; Group II, diabetic control; Group III, diabetic + *L. scariola Linn* leaves extract (12.5 mg/100g.b.wt)

Histopathology findings

Histopathology of liver:

In group I untreated rats, the histology structure of the liver showed normal architecture, i.e., cords of hepatocytes and sinusoids which contain blood radiate from central vein to the peripheral portal triads. The central veins lined by endothelial cells with a ring of collagen fibers.

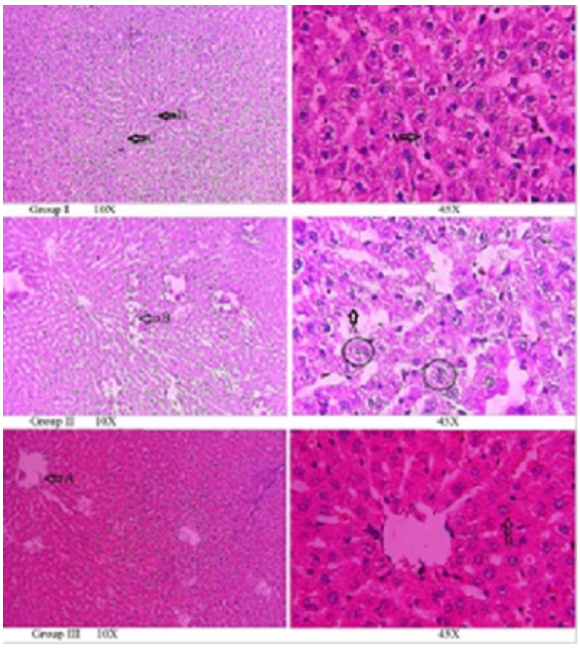

Figure 3: H.E stain (10 x and 45x) of normal hepatic parenchymal tissue of pre diabetic (group I): normal prediabetic rat liver showing normal architecture; diabetic (group II): showing distorted lobular architecture of liver parenchyma; diabetic + *Lactuca scariola Linn* (group III): showing mild enlargement of hepatocytes, Periportal mid zone and Centrilobular appear to be normal.

The sinusoids have flattened nuclei with unclear cytoplasmic margin and are lined by both endothelial cells and Kupffer cells. The polygonal shapes hepatocytes are with distinct borders. The single round nucleus has clear chromatin pattern with 1 to 2 amphophilic noticeable nucleoli. Finely granular eosinophilic cytoplasm is seen in Figure 3. In case of group II diabetic rats, the majority of distorted "lobular" architecture of liver parenchyma was observed. Swollen hepatocytes with vacuolated microvesicular and eosinophilic cytoplasm and increased number of mitotic figures were also observed. Portal area along with fibrous tissue found to be mildly proliferated and mixture of acute and chronic inflammatory cells were found to be infiltrated as seen in Figure 3. However, in group III diabetic rats upon treating with *Lactuca scariola Linn* extract showed mild enlargement of hepatocytes although periportal, mid zone and centrilobular appear to be normal. The central veins lined by endothelial cells appeared to be slightly dilated (lesser than group II) as shown in **Figure 3.**

Histopathology of Kidney:

In group I, section studied under H&E stain showed normal renal parenchymal tissue which is composed of glomeruli and tubules separated by small amount of interstitial connective tissue containing peritubular capillaries. Each glomerulus is made up of interconnected capillaries inside Bowman's space lined by flat parietal cells. The visceral epithelial cells cover outer portion of glomerular capillaries. It also shows normal appearance of capillary tufts and the mesangium. Tubules appear to be normal too. There is no evidence of tubular atrophy. Glomeruli seem to be normal in morphology and in cellularity (Figure 4). In group II diabetic rats, it can be seen that glomeruli are hypercellular with thickening of glomerular basement membrane and mesangial proliferation. Tubules show focal tubular

basement membrane mild thickening with cloudy swelling (coagulation necrosis). The lumen shows eosinophilic protienacious (pink body), suggestive of acute tubular necrosis (ATN). Interstitium is edematous with infiltration of inflammatory cells. Vessels are congested and sclerotic (Figure 4). In group III diabetic rats which were treated with *Lactuca scariola Linn* extract, glomeruli are hypocellular with thickening of glomerular basement membrane and show sclerotic changes. Tubules show focal tubular necrosis suggestive of acute tubular necrosis (ATN) with regenerative epithelium. Interstitium is edematous with infiltration of inflammatory cells. Vessels are congested and sclerotic **(Figure 4).**

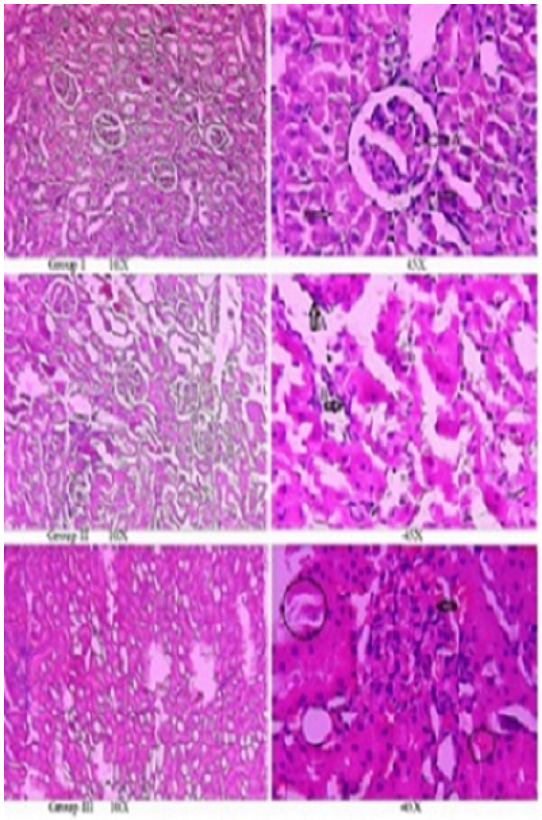

Figure 4: H.E stain (10 x and 45x) of renal parenchymal tissue of pre diabetic (group I): Glomeruli seem to be normal in morphology and in Cellularity.; diabetic (group II): Glomeruli are hypercellular with thickening of glomerular basement membrane and mesangial proliferation; diabetic + *Lactuca scariola Linn* (group III): Tubules show focal tubular necrosis suggestive of Acute Tubular Necrosis (ATN) with regenerative epithtelium.

Discussion:

Phytochemical constituents:

The phytochemical constituents present in the plant extracts are flavonoids, saponins and steroids.

Anti-diabetic effect of plant extract *in vivo*:

The aqueous plant extract has exhibited a remarkable improvement in the blood glucose level. The elevated blood glucose level due to diabetes is significantly reduced almost to the normal level during OGTT and Sub chronic exposure of *Lactuca scariola Linn* leaves extract. The anti-diabetic capabilities of plant extract may be due to the phytochemical constituents such as, flavonoids and saponins [12, 13]. The decrease in blood glucose level may be because of enhanced secretion of pancreatic insulin by the β cells of islets of Langerhans [11]. Flavonoids and saponins are known bioactive anti diabetic components [11]. Flavonoids are found abundantly in plants and are known for their action on different cell organelles to regulate various metabolic functions [14, 15]. Flavonoids are also able to terminate the free radicals and prevent oxidative stress [16]. Saponins on the other hand influence structural changes of glucose molecule through glycone and glucuronic acid [17]. Saponin shows a protective action on pancreatic β cells and increase insulin secretion and hence it is beneficial to treat diabetes mellitus [17]. Hypoglycemic effects of medicinal plants may be due to the presence of antioxidants which are responsible for enhanced hepatic glucose regulatory system and reduce intestinal glucose uptake [18]. Hypoglycemic effects of medicinal plants may also be due to increased insulin secretion or increase in peripheral glucose uptake mechanisms by inducing insulin mediated cell signaling pathways [19-21]. The bioactive compounds might protect and heal the pancreatic β cells of islets of Langerhans [22].

Histopathology of Liver:

It is observed that control group of animals is found to be stable (Figure 3) whereas, diabetic control group is found to possess cellular

abnormalities comprising of necrosis, cellular and vascular degeneration. In diabetic rats majority of the 'lobular' architecture of liver parenchyma was found to be distorted, little swollen hepatocytes was observed, vacuolated micro vesicular and eosinophilic cytoplasm besides increase in number of mitotic figures. It is observed that disruption and cytotoxicity of pancreatic β-cell membrane occurs due to administration of alloxan to experimental rats [23]. These histopathology changes in diabetic rats may be due to hyperglycemia and accumulation of mucopolysaccharide deposits [24]. Antioxidants have significant effects on most probable impairments in diabetes such as, oxidative stress, protein glycation and glucose metabolism [25]. The liver, a central metabolic organ, gets exposed to reactive oxygen species caused by oxidative damage due to diabetes [26]. However, on supplementation with *Lactuca scariola Linn* leaves extract; hepatocytes have reappeared and also periportal, mid zone and centrilobular appeared to be normal. The central veins lined by endothelial cells were appeared to be slightly dilated. Our findings indicate possible hepato-protective role played by aqueous *Lactuca scariola Linn* leaves extract.

Histopathology of Kidney:

Figure 4, shows normal renal parenchyma tissue composed of glomeruli and tubules separated by small amount of interstitial connective tissue containing peritubular capillaries. Tubules appear to be normal with no evidence of tubular atrophy. Glomeruli seem to be normal in morphology and in cellularity. In diabetic rats, glomeruli appeared to be hypercellular with thickening of glomerular basement membrane. Tubules show focal tubular basement membrane mild thickening with cloudy swelling (coagulation necrosis). The lumen shows eosinophilic protienacious suggestive of acute tubular necrosis (ATN), collectively may be due to deposition of mucopolysaccarides [26].

The study carried on lipid metabolism and glomerulo sclerosis in diabetes mellitus shows that the SREBP-1 expression increases in diabetes which plays significant role in lipid synthesis leading to Triglyceride accumulation, mesangial expansion [27]. However, upon treating with *Lactuca scariola* Linn leaves extract, glomeruli were hypocellular with thickening of glomerular basement membrane showing sclerotic changes. Tubules show focal tubular necrosis suggestive of acute tubular necrosis (ATN) with regenerative epithtelium.

Conclusion:

Lactuca scariola Linn extract showed remarkable improvement in the blood glucose level clearly suggesting that this plant extract possess potential anti diabetic properties possibly through modulating insulin signaling mechanisms. Hence, *Lactuca scriola Linn* extract may be useful to treat complications associated to diabetes in liver and kidney.

Conflict of interest: Nil

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