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Research Article

Effect of *Cucumis sativus Linn*. in Liver Pathogenicity of Alloxan Induced Diabetic Rat

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Abstract

Background: Diabetes mellitus (DM) is the most prevalent chronic disease which can be related with liver pathogenicity and its effect with induction of *Cucumis sativus* (CS) Linn. since its constituents play role in bioactive in glycogen, fat and as antioxidant lowering agent. Aim of the study is to observe effect of CS Linn. in liver of alloxan induced diabetic rat.

Materials and Methods: Total twenty four albino wistar rats were randomly assigned in to four groups, each group consist of six rats Group I (control), Group II (diabetic control treated 120 mg/kg body weight single dose intraperitoneally), group III (diabetic treated with low dose of 200 mg/kg body weight/day) and Group IV (diabetic treated with high dose of 400 mg/kg body weight/day) for 21 days .

Results: Diabetic rat were found with reduced significantly in body weight (111.20 ± 9.48 gm), blood glucose level (364.0 ± 11. mg/dl), aspartate transaminase (AST) (112.8 ± 2.56 IU/L), Albumin (2.00 ± 0.13 mg%), and Alanine transaminase (ALT) (110.5 ± 2.67 IU/L). Liver pathogenicity of diabetic rat was revealed by lymphatic infiltration, intracellular vacuolation and apoptotic hepatocyte whereas low and high doses of CS treated diabetic rat reported significantly improved positive parameters in contrast to diabetic rat.

Conclusions: Oral Administration of CS Linn. shows an ameliorating effect to liver function and its pathogenicity of alloxan induced albino wistar rat.diabetic rat.

Keywords: Body Mass Index; Blood Glucose Level; Liver Function; Hepatopathogenicity

Abbreviations

DM: Diabetes Mellitus; ALT: Alanine Transaminase; AST: Aspartate Transaminase; CS: *Cucumis sativus*; NAFL: Non-alcoholic fatty *liver*

Introduction

Diabetes mellitus (DM) at present is one of the most prevalent chronic disease world-wide which cause morbidity of 8.5% of adult in 2014 and mortality of 1.5 million directly [1,2] and 2.2 million indirectly [3]. This can be correlate with as reported previously that chronic hyperglycemia, carbohydrate, lipid and protein metabolism disorders which lead into hepatogenous diabetes approximate 96% of patient suffers from liver cirrhosis from glucose intolerance and this leads into accumulation of glycogen in liver may cause the hepatomegaly and elevated enzyme due to DM [4,5]. Non-alcoholic fatty liver is associated in DM with excess accumulation of triglycerides within the hepatocyte [6]. NAFL in DM may progress to liver disease from steatosis to steatohepatitis with evidence of inflammation and cell injury, hepatic fibrosis, and ultimately liver failure was reported about 88% [7,8].

Cucumis sativus(CS) Linn. belongs to the cucurbitaceae constitutes saponins, steroids, carotenes, flavones, amino acids, resin, tannins, proteins and proteolytic enzymes which can be a good agent for bioactivities of glycogen, lipid lowering and anticytotoxicity [9,10].

Present study is aimed to observe the leaves of CS Linn. activity in liver of alloxan induced diabetic rat as experimental model.

Materials and Methods

Collection and Preparation of CS Linn. leaves extract

The fresh leaves of CS Linn. were collected from village area of Kanbargi Belagavi, Karnataka and stored in air tight container. The plant sample was sent for authentication at Regional Medical Research Centre (RMRC), Belagavi. The leaves of CS Linn. were first washed, dried at room temperature for 2 days and then dried at 45°C for 36 hr in an electric oven, crushed in an electric grinder, passing through sieve #40. The dried powdered material was taken in a clean, flat bottomed glass container and soaked in ethanol for seven days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material and normal filter pa-

per respectively. Filtrate was concentrated using rotary evaporator to obtain a crude ethanol extract of leaves and extract obtained was stored under refrigeration conditions. The residue was suspended in a mixture of tween 80 and distilled water (1:3) in a fixed dose and used for treatment.

Selection of animals and animal care

Male Albino Wistar rats weighing 150 - 250 gm were used for this experiment. They were procured from Shri. Venkateshwara Enterprises, Bangalore and housed in a clean polypropylene cage in group of six and were maintained under 12 hr light-dark cycle at 25 ± 2˚C ambient temperature, 45 - 55% relative humidity. They were allowed to acclimatize two week before the experiment. The rats were allowed free access to water and standard laboratory diet. After 14 days of acclimatization period, they were randomly selected for different experimental groups. All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (Resolution No.-KLECOP/IAEC/Res.22-10/10/2015) KLEU's College of Pharmacy, Belagavi before conducting the experiment.

Selection of animals and animal care

Alloxan: Freshly dissolved in the normal saline and administered with a single dose of 120 mg/kg body weight, intraperitoneally and observed for 4 days subsequently.

CS Linn.: The suspension of CS Linn. was prepared by using Tween 80. The CS Linn. leaves ethanolic extract dose selection is done based on acute toxicity study. For the present study 200 mg/kg (low dose) and 400 mg/kg (high dose) were taken as effective doses and suspended in distilled water and administered orally for 21 days.

Administration of doses

The test substances are administered in a single dose per orally. The animals were fasted prior to dosing, the animals were weighed and test substance was administered. After the dose was administered, food was withheld for a further 3 - 4 hours in rats.

Induction of experimental diabetes mellitus in rats

The overnight fasted rats were made diabetic with single dose of alloxan (120 mg/kg) prepared in normal saline. Glucose (4% w/v) was given in drinking water for 48 hours to prevent hypoglycaemic phase. All animals were allowed free access to food and water and maintained at room temperature. Diabetes was confirmed on 4th day after alloxan injection by determining the blood glucose concentration. The same day is considered as 'day 0th'. Only animals with BGL more than 180 mg/dl were considered for the experiment.

Experimental study design

In the experiment, rats were divided into four groups (n = 6) after induction of alloxan as diabetes and received the following treatment. The experimental period was 21 days.

- **Group I:** Normal Control **-** Normal saline (5 ml/kg body weight per oral)
- **Group II:** Diabetic Control **-** Alloxan induced intraperitoneally at single dose of 120 mg/kg body weight.
- **Group III:** Diabetic treated with *Cucumis sativus* low dose**-** CS Linn. leaves extract low dose (200 mg/kg) daily for 21 days.
- **Group IV:** Diabetic treated with CS Linn. high dose **–** CS Linn. leaves extract high dose (400 mg/kg) daily using intragastric tube for 21 days.

The body weight (gm), blood glucose of all the rats were recorded at weekly interval on 0th, 7th, 14th, 21st day and water intake (ml) were observed on daily basis during the experimental period (21 days) from blood collected from the tip of the tail by using On Call Plus glucometer. Body weight was recorded weekly parameters and water intake (ml). At the end of experimental period, the animals were overnight fasted and blood was taken from retro-orbital plexus under mild anesthesia for Alanine transaminase (ALT) and Aspartate transaminase (AST) (ERBA Diagnostics Manheim GmbH, Germany), and Albumin (YUCCA Diagnostics, Kolhapur, India) estimation was done by kit method purchased. The serum is separated from the blood by centrifugation at 4500 rpm for 10 minutes. Then animals were sacrificed by cervical dislocation then after immediately liver was autopsied for the histopathology studies.

A liver was fixed in formalin solution (10%) and subjected to histopathology studies. The section of liver in about 4 - 6 µm was processed and embedded in paraffin wax stained with hematoxylin and eosin staining and microphotograph was taken at 40X.

Statistical analysis

All Values are expressed as Mean ± SD in excel sheet of MS 2007. The results were analyzed for statistical significance by one-way ANOVA post hoc analysis followed by Bonferroni Multiple Comparison (BMC) Test using Graph Pad version 6.0, $P < 0.05$ was considered as significant.

Results

DM association with physiological changes

Physiological changes in body weight and food intake changes were observed in DM and CS Linn. treated during experiment by ANOVA, post hoc analysis with Bonferroni multiple comparison test with $p < 0.0001$ significant value. It was observed that the body weight of diabetic rat at 21 day of experiment was found to be reduced (111.20 ± 9.48 gm) with highest amount of food intake (24.19 ± 4.01 gm) in comparison to control and CS Linn. treated group. Body weight of CS treated in DM was found to be increased (206.5 ± 0.72 gm) with lesser amount of food intake (19.14 ± 1.63 gm) comparison to diabetes group rat (Figure 1 A, B and C).

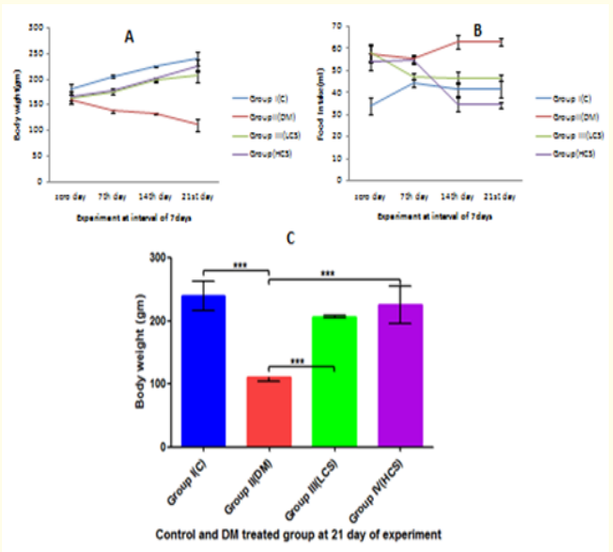

Figure 1: Body weight of experimental rat showing A: weekly parameter of body weight in gm at 7 day interval in relation with DM, B: weekly parameter of food intake at 7 days interval in relation with DM and CS Linn. treated with $p < 0.0001$.

Blood glucose level in control and DM treated

Alloxan was induced in single dose to the rat to make diabetic. For 21 days along with CS Linn. was induced against diabetic rat daily. Time dependent line graph showed alloxan induced diabetic animal showed a significant ($p < 0.001$) increase in blood glucose level (364.0 ± 11.1 mg/dl) from day zero onwards with respect to normal control group (80.17 ± 0.6540 mg/dl). Treatment with low dose i.e. 200 mg/kg of CS showed significant ($p < 0.001$) decrease in blood glucose level from 14th day (242.7 ± 15.36 mg/dl) to 21st day (161 ± 15.76 mg/dl) (Figure 2).

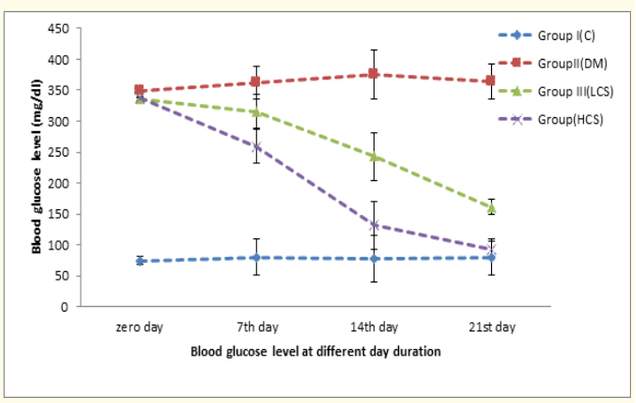

Figure 2: Time dependent line graph of blood glucose level (mg/dl) at interval of 7day in control and treated group against CS Linn.

Biochemical activity of liver function

Liver function was essayed from estimation of AST, serum albumin and ALT from blood serum at 21 day of treatment. It was founded that the impairment of liver function was assessed, DM rat was found to be significantly increased in AST, serum albumin and ALT in Group II (DM) and Group IV with $p < 0.0001$ in compared to Group I Control, whereas Group III was found to be significantly increased in AST, ALT with $p < 0.001$, $p < 0.0001$, where serum albumin was found to be non-significant in compared to control group (Table 1).

	Group I (C)	Group II	Group III	Group IV
AST (IU/L)	65.50 ± 1.61	112.8 ± 2.56***	97.67 ± 3.04**	71.67 ± 3.40***
Albumin (gm%)	3.70 ± 0.20	2.00 ± 0.13***	2.55 ± 0.19	3.35 ± 0.13***
ALT (IU/L)	45.83 ± 0.87	110.5 ± 2.67***	78.67 ± 0.80***	57.00 ± 1.93***

Table 1: Liver function showing by parameters as AST, ALT and Albumin among different group ***$p < 0.0001$ and **$p < 0.001$ in comparison Group II vs I, Group II vs III and II vs IV. Group II (DM) and Group IV (HCS) AST, ALT, Albumin were significantly increased in compared to Group I (Control), Group III (LCS) ALT, AST were significantly increased with ***$p < 0.0001$, **$p < 0.001$ and albumin is non-significant in compared to Group I (Control).

Histopathological study of liver

Diabetic liver induced by alloxan

Diabetic rat liver tissue was observed under microscope Haematoxylin and Eosin (H&E) stain shows in low dose CS Linn. against diabetic lymphatic infiltration in hepatocytes, hepatocytes undergoes mitosed, vaculation around hepatocytes, acute hepatic necrosis whereas in high dose CS Linn. lymphatic infiltration in wall of the central vein, apoptosis, and enlarged sinusoids were observed (Figure 3 and 4).

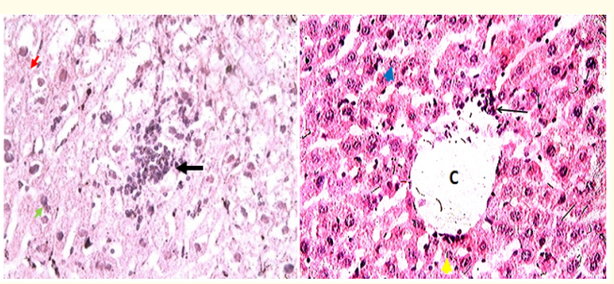

Figure 3: Histological features of diabetic rat liver tissue (Haematoxylin and Eosin (H&E) stain 40X) treated by CS Linn. showing lymphatic infiltration in hepatocytes (black arrow), hepatocytes undergoes mitosed (green arrow), intracellular vacuolization in hepatocytes (red arrow), acute hepatic necrosis (yellow arrow), apoptosis (blue arrow) (A); lymphatic infiltration in wall of the central vein (black arrow), enlarged sinusoids (red arrow) (B).

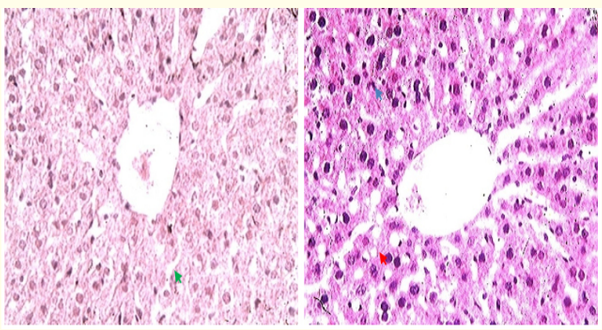

Figure 4: Histological features of diabetic rat liver (HE stain 40X) treated by CS Linn. showing mild sinusoidal congestion (green arrow), accumulation of lipid droplets in hepatocytes (red arrow), fragmented nuclei in hepatocytes (blue arrow).

Discussion

Present study was undertaken to correlate the CS Linn. activity and liver function in DM rat. In the present study, albino wistar rat was induced with alloxan to make rat the diabetes and CS Linn. was induced in diabetes rat. Since CS Linn. was reported that CS Linn. was found to exhibits as wide spectrum of activity of antioxidant, amylolytic, antibacterial and anticytotoxic [11-13]. The present study was undertaken in liver activity as physiological phenomenal, blood glucose level in each day during experiment, LFT by AST, serum albumin and ALT, and histopathological.

Physiological behavioral phenomena of food intake increased with body weight decreased significantly during treatment in diabetic group. The relatively low weight of diabetic rat can be caused by several factors such as vascular changes limit delivery of nutrients to liver [14]. Similarly, catecholamine release from the adrenal glands and nerve cells due to the exposure to diabetic can cause vasoconstriction [15], insulin signaling disorder to inhibit glucose synthesis in the liver [16]. In diabetic, food intake can be associated with DM type II [17], which shows similar with the present study that diabetic rat increased the food intake amount. In administration of CS Linn. in low dose and high dose against, body weight increased significantly with constant average food intake amount in comparison to control.

Present study reported that blood glucose level was significantly raised from day zero in group diabetic control and other treated group of CS Linn. Since alloxan is diabetogenic chemical, pathological effects and toxic glucose analogues that preferentially accumulates in pancreatic beta cells via the glucose transporter 2 [18]. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species in a cyclic redox reaction with its reduction product, dialuric acid. Auto oxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low anti-oxidative defence capacity, and the ensuing state of insulin-dependent alloxan diabetes which may transport the glucose in extrahepatic tissues and carbohydrate metabolism. Low and high dose of CS Linn. in alloxan induced the diabetic rat decreased the blood glucose level significantly due to antidiabetic activity. Studies of antidiabetic activity in streptozotocin and alloxan induced rat by CS Linn. seed and leave extract in BGL was reported [10,19,20].

To correlate the pathogenicity of liver in diabetic and CS Linn. induced against diabetes, diabetic rat showed AST, serum albumin and ALT level increased significantly in comparison to control. This study showed liver pathogenicity undergone that ALT and AST intra hepatic concentration leaked in the circulation and indicates as hepatocyte injury where serum albumin represents the liver synthetic function. In administration of CS Linn. in diabetic rat the concentration of ALT, AST and serum albumin significantly decreased in comparison to diabetic control and normal control. This markers to correlate with pathogenicity of liver satisfied in diabetic rat showed that hepatocyte undergone apoptosis, necrosis, fatty liver and abundantly presence of infiltration. The liver pathogenicity is significantly decreased by presence of only few apoptotic and necrosed hepatocyte.

Conclusion

Present study concluded that CS Linn. was administered at two different doses i.e. low dose (200 mg/kg) and high dose (400 mg/kg) against diabetic rat. This study has shown the potential antidiabetic effects liver of CS Linn. on alloxan-induced diabetes in albino wistar rat, which is confirmed by improvement of physiological food intake, body weight, blood glucose level, AST, ALT, serum albumin and liver pathogenicity.

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