

Effect of Alcohol Extract of *Cucurbita maxima* (Pumpkin) Seeds on Alanine Aminotransferase (ALT) Levels in Streptozotocin Induced Diabetic Male Long Evans Rats

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Abstract

An experimental study was done in the Department of Pharmacology & Therapeutics, Dhaka Medical College, Bangladesh, between January and December of 2023, to evaluate the hepatoprotective effect of alcohol extract of *Cucurbita maxima* (pumpkin) seeds on alanine aminotransferase (ALT) in streptozotocin induced diabetic male long Evans rats. A total of 25 rats were divided into 5 groups (i.e., 5 rats in each group): group A (non-diabetic control), group B (untreated diabetic control as diabetes induced by streptozotocin 50mg/kg body weight given intraperitoneally), group C (low dose experimental group treated with pumpkin seeds 100mg/kg body weight orally), group D (high dose experimental group treated with pumpkin seeds 200mg/kg body weight orally), and group E (treated with metformin 100 mg/kg body weight orally along with laboratory diet). Group A had normal fasting blood glucose and serum ALT level, while group B had hyperglycemia with significant increase in ALT level ($p < 0.001$). Post-treatment serum ALT levels of group C as well as group D were found lower than that of group B ($p < 0.001$), while group E also showed significant reduction of serum ALT level compared to group B ($p < 0.001$). This in vitro study suggests that alcohol extract of *Cucurbita maxima* (pumpkin) seeds has hepatoprotective effect against streptozotocin induced diabetes in rats.

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Introduction

The liver is the largest organ of the body, which is involved in the metabolism and excretion of unwanted compounds, which may be exogenous (e.g., drugs and poisons) or of endogenous origin (e.g., steroid or catecholamine hormones). It performs a wider range of biochemical functions than any other organ.¹ It is connected with most of the physiological processes, which include growth, immunity, nutrition, energy metabolism and reproduction.² Liver plays an important role in filtering and clearing blood received from digestive tract prior to passing it to other body tissues and organs. Besides, it is transforming, detoxifying, and accumulating metabolites and in mediating drug transformations and metabolism.² Hence, the liver is highly susceptible to damage from different toxins, viruses, and reactive oxygen and nitrogen species.^{2,3} This damage of the liver is often associated with liver metabolic and synthetic dysfunctions which can result in many disorders, e.g., hepatic fibrosis, cirrhosis of the liver, etc.⁴ Elevated

levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) (considered as hepatic functioning markers) signify hepatic

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derangement. Hepatic dysfunctions are more common in diabetes mellitus and metabolic syndrome.⁵

Antidiabetic phytomedicines as found in *Cucurbita maxima* (pumpkin) seeds can counteract the harmful toxic side effects of synthesized drugs by providing by enhancing hepatoprotective efficacy at low cost, which is also more helpful in diabetic conditions. Indeed, a number of in vivo studies have shown that plants can be used as chemopreventive agents against hepatic damage. The current modern approach to treat diabetes, including insulin and various oral antidiabetic synthetic drugs, has demonstrated its limits, with unsatisfactory results and many side effects.^{6,7} The pumpkin (*Cucurbita maxima*) belongs to the Cucurbitaceae family. It is a trailing annual herb with somewhat prickly or hairy stem and axillary tendrils, leaves simple, alternate; flowers large, yellow, unisexual, solitary; fruits fleshy, round or oval, brown; seeds ovoid or oblong, compressed.⁷ Its seeds have also been explored by various research group for their antidiabetic, antihyperlipidemic and antioxidant potential.⁸ Therefore, the present study was done to evaluate the hepatoprotective effect of alcohol extract of *Cucurbita maxima* (pumpkin) seeds on alanine aminotransferase (ALT) levels in streptozotocin induced diabetic male long Evans rats.

Methods

This experimental study was done in the Department of Pharmacology & Therapeutics, Dhaka Medical College, Bangladesh, between January and December of 2023. At first, fresh pumpkins (*Cucurbita maxima*) were collected from Sadullapur, Savar, Dhaka and authenticated by Bangladesh National Herbarium (BNH), Mirpur, Dhaka. A voucher specimen (DACB Accession Number-90624) was

kept in the herbarium department for future reference. The fresh *Cucurbita maxima* (pumpkin) was cut and seeds were collected. The seeds were cleaned well with water and dry in a shadow place. After complete drying, the seeds were made into fine powder with auto-mix blender. Then the powder was dissolved into 1.8 litre of 99% alcohol in a round bottom flask with intermittent shaking for 5 days in room temperature to allow alcohol extraction. This alcoholic mixture then filtered with cotton filter. Then the filtrates were evaporated by using a vacuum rotary evaporator at 65-75 rpm in the Drug Research Laboratory, University of Dhaka, to convert into oily form. Then this oily extract was collected in an air tight plastic jar with proper labeling. Finally, this extract was stored at 4°C refrigerator until further use. Before each use, required amount of extract were diluted in adequate amount of distilled water to obtain fresh preparation with desired concentration.

The experiment was carried out on a total number of 25 healthy, 10-12 weeks aged, male long Evans rats weighing between 150-200gm, which were purchased from animal house from Bangladesh Medical University (BMU), Dhaka. The rats were acclimatized in the lab in a metallic cage in animal house in Dhaka Medical College under standard condition and fed with standard pellet diet and water ad libitum except during the day of sacrifice and collecting blood samples. They were allowed free access of water during only fasting.⁶ The total duration of experiment was 22 days. It was comprised of 25 rats divided into 5 groups (5 rats in each group). Group A (non-diabetic control group) rats were given lab diet and distilled water for 21 days; Group B (untreated diabetic control group) rats were given streptozotocin 50mg/kg body weight intraperitoneally only for day 1 along with laboratory diet and distilled water for next 21 days;⁶ Group C (low dose pumpkin seeds treated

group) rats were given streptozotocin 50mg/kg body weight intraperitoneally only for day 1 and treated with pumpkin seeds 100mg/kg body weight along with laboratory diet and distilled water from day 4 to day 21;⁷ Group D (high dose pumpkin seeds treated group) rats were given streptozotocin 50mg/kg body weight intraperitoneally only for day 1 and treated with pumpkin seeds 200mg/kg body weight along with laboratory diet and distilled water from day 4 to day 21;⁷ and Group E (metformin treated group) rats were given streptozotocin 50mg/kg body weight intraperitoneally only for day 1 and then metformin 100 mg/kg body weight orally along with laboratory diet and distilled water from day 4 to day 21.⁷

Streptozotocin (STZ) was purchased from Jonaki Scientific Store, Dhaka. It was injected intraperitoneally after dissolving in ice cold 0.1M citrate buffer solution (pH 4.5) at a dose of 50mg/kg body weight at day 1.^{5,6} After 3 days of STZ administration, all rats developed marked hyperglycemia. Alcohol extract of pumpkin seeds was suspended in distilled water and administered orally through Ryles tubes (either 100 mg/kg body weight or 200 mg/kg body weight as applicable). The active ingredient of metformin as a standard drug was obtained from Ad-din Pharmaceuticals Ltd., Dhaka, (Batch No. MF/12031196). It was given orally through Ryles tube at a dose of 100 mg/kg body weight mixed with normal saline from day 4 to day 21.

Blood (2-3 ml) was collected through cardiac puncture from all rats and allowed to clot for 30 minutes. Serum was separated by centrifugation at 2500 rpm for 30 minutes and was taken to Department of Clinical Pathology, Dhaka Medical College Hospital, Dhaka, for analysis of serum alanine aminotransferase (ALT).

Data was presented as mean \pm SD (standard deviation). Statistical significance of different groups

were determined by one-way ANOVA, followed by Bonferroni tests. Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 26.0 for Windows. Results were considered as statistically significant, when p-value was found <0.05.

This experimental study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka, Bangladesh (ERC-DMC/ECC/2023/158).

Results

The mean serum alanine aminotransferase (ALT) levels were found 45.00 \pm 6.20 mg/dl, 110.40 \pm 7.4 mg/dl, 85.80 \pm 12.3 mg/dl, 56.60 \pm 7.77 mg/dl, 48.20 \pm 3.42 mg/dl in group A, B, C, D, and E respectively. The difference was found statistically significant (p<0.001). A significant increase of serum ALT level of group B was seen in comparing to group A, while significant reductions of serum ALT levels of group C, D, and E were observed in comparing to group B. (Fig. 1). Table-I shows the comparison of serum alanine aminotransferase (ALT) levels between groups. The difference was found statistically significant (p<0.001), except for group D vs. group E (p>0.05).

Fig. 1: Bar diagram showing serum alanine aminotransferase (ALT) levels of all groups

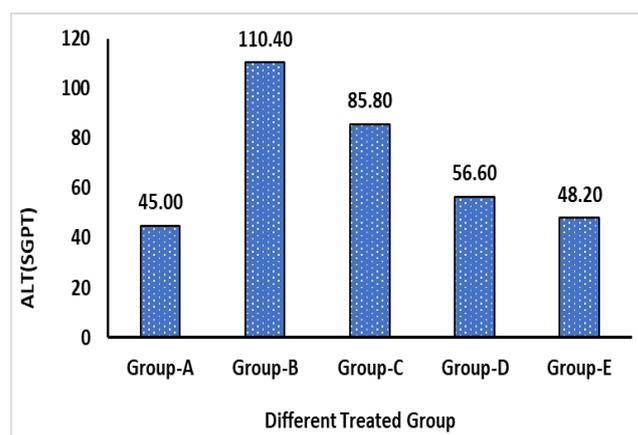


Table-I: Comparison of serum alanine aminotransferase (ALT) levels between groups

Serum ALT	Mean Difference	95% CI		
		Lower	Upper	p-value
Group A vs. Group B	-65.4	-75.388	-55.4121	<0.001***
Group B vs. Group C	24.6	9.76252	39.43748	<0.001***
Group B vs. Group D	53.8	42.71198	64.88802	<0.001***
Group B vs. Group E	62.2	53.75864	70.64136	<0.001***
Group D vs. Group E	8.4	-0.35067	17.15067	>0.073 ^{NS}

p-value reached from one-way ANOVA, followed by Bonferroni tests; ***=highly significant, NS=not significant

Discussion

In the present study, 5 rats were kept as non-diabetic control, while for the rests, diabetes mellitus was induced through administration of streptozotocin (STZ) at day 1. After completion of treatment on day 22, all rats were sacrificed and blood was collected to estimate the serum alanine aminotransferase (ALT) levels. The mean serum alanine aminotransferase (ALT) levels were found 45.00±6.20 mg/dl, 110.40±7.4 mg/dl, 85.80±12.3 mg/dl, 56.60±7.77 mg/dl, 48.20±3.42 mg/dl in group A, B, C, D, and E respectively. Comparison between group A (non-diabetic control group) and group B (untreated diabetic control group) showed significant difference ($p<0.001$) as increased serum ALT was found in untreated diabetic control group because of hepatocellular damage due to STZ induced DM. Similarly, comparison between group B (untreated diabetic control group) and group C (low dose extract of pumpkin seeds 100mg/kg body weight experimental group) as well as group B and group D (high dose extract of pumpkin seeds 200mg/kg body weight experimental group) showed significant

difference ($p<0.001$) as decreased serum ALT levels were found in group C and group D; the results indicate pumpkin seeds extracts can reduce serum ALT level in diabetic rats. Comparison between group B (untreated diabetic control group) with group E (metformin 100 mg/kg body weight treated group) also showed significant reduction of serum ALT level ($p<0.001$). In several previous studies, significant reduction of serum ALT levels were observed after treating with pumpkin seeds extract and metformin. Those findings are consistent with our study results.⁶⁻⁸

The functions of liver may also be affected by the changes in the levels of insulin, which provides rapid uptake, storage as glycogen and usage of glucose in liver. Structural and biochemical changes occur in the liver as a result of the absence of insulin in diabetes. Estimating the activities of serum marker enzymes, like serum ALT, AST and ALP can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol, are released in to the blood stream. The aminotransferase is abundant in the liver and are released into the blood stream following

hepatocellular damage, their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.^{4,5}

In this study, diabetes mellitus was induced in rats by administration of streptozotocin (50 mg/kg body weight) and then treated with alcoholic extract of pumpkin seeds (either 100 or 200 mg/kg body weight) – as similar procedure was followed in several previous studies.⁶⁻⁹ However, Kushawaha *et al.*⁸ studied with 5 separate doses (50, 100, 150, 200, 250 mg/kg body weight respectively) of pumpkin seeds extract. In that point of view, in the present experiment, 2 different doses were administered to observe the exertion of the effect in STZ induced diabetic rats. In a similar study done in the same institution, diabetes was induced in rats by using alloxan to see the hypoglycemic effect of the ginger juice.¹⁰

Conclusion

Our data suggests that alcohol extract of *Cucurbita maxima* (pumpkin) seeds have significant hepatoprotective effect in experimentally induced diabetic rats. The seed extracts of *Cucurbita maxima* can be considered as a potent source of antidiabetic agents, which may be due to presence of flavonoids, phenols or saponins in the extract. This study also provides a new therapeutic avenue against diabetes and diabetes related complications in humans.

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