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HYPOGLYCEMIC AND HEPATOPROTECTIVE ACTIVITY OF *ELETTARIA CARDAMOMUM* EXTRACT IN HFD/STZ INDUCED DIABETIC MICE.

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Abstract:

Chronic hyperglycemia affects metabolism of carbohydrates, proteins, lipids and it can lead to various organ dysfunction. The present study examined the effect of ethanolic extract of *Elettaria cardamomum* (ECE) on hepatocytes of HFD/STZ induced diabetic mice. In this study male mice were allocated into three groups (n=7 per group): Control, Diabetic and Recovery group. Diabetes was induced by feeding high fat diet (HFD) for two weeks followed by an injection of streptozotocin (STZ) (40 mg/kg body weight, intraperitoneally). In recovery group, diabetic mice were fed ECE at dose of 200mg/kg for 28 days. Blood glucose and histology of liver in all experimental groups were examined. The blood glucose level significantly increased in diabetic mice as compared to control. In recovery group, administration of ECE for 28 days significantly decreased the elevated blood glucose near to normal. Histopathological studies in liver tissue showed that ECE restored altered histopathological changes of hepatocytes in diabetes and were near to normal. This study revealed ECE exerted a hypoglycemic and hepatoprotective effect that proves its potential as an antidiabetic agent.

Keywords: Hyperglycemia, *Elettaria cardamomum* extract (ECE), Hepatoprotective, Streptozotocin

Introduction:

Diabetes mellitus is a group of heterogeneous disorder that is characterized by hyperglycemia and in the more severe case, it is associated with increased risk of liver dysfunction. It is well known that the liver is insulin dependent organ and plays a vital role in carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose level and of a continuous supply of glucose to organs that require it (Levinthal, 1999). Diabetes is associated with several structural and functional liver abnormalities that affect glycogen and lipid metabolism (Sanchez, 2000; Bolkent, 2004; Koyuturk, 2005). Excess glycogen deposition, fibrosis, cirrhosis, steatohepatitis and biliary disease in the liver has been reported in 55 – 80% of diabetic patients (Stone, 1985).

Plants used in traditional medicine for the treatment of liver disorders are of great interest, as they may serve as potential sources for new therapeutic agents that could be applied in the management and prevention of hepatic injuries. Plants rich in different phytochemical derivatives such as triterpenes, flavonoids or polyphenols, have been reported to exhibit antihepatotoxic effects in experimental liver-injury models induced by different types of hepatotoxicants, such as carbon tetrachloride, cadmium, galactosamine, acetaminophen and thioacetamide (Iwu1990; Dwivedi1993).

Elettaria cardamomum is a well-documented spice used as flavoring agent in food preparation. Apart from its use as a flavoring agent in food preparations, cardamom is used both in ancient and modern medicine (Korikanthimathm, 2001). Cardamom has been reported as an antioxidant and has anti-inflammatory and anti-carcinogenic properties (Agaoglu, 2006; Suneetha, 2005). In a recent study, Ahmed *et al*. reported antidiabetic activity of cardamom and its role in glucose metabolism (Ahmed 2007). Hence this study investigates the potential antihepatotoxic properties of the *Elettaria cardamomum* extract (ECE) in type 2 diabetes.

MATERIALS AND METHODS:

Chemicals:

STZ was purchased from Sigma-Aldrich Company (India). The other histological chemicals and stains were purchased from Hi Media (Mumbai, India).

Preparation of *L. sativum* seed Extracts:

Elettaria cardamomum seeds were collected from local market of Kolhapur. They were (100 gm) cleaned and ground to fine powder using a grinding machine. Extraction was carried out by soxhlet method. Ethanol was used for extraction for six hrs. The extract was evaporated to dryness under reduced pressure at 60°C by rotary evaporator. Extract was placed in dark bottle and stored at -8°C.

Animals:

Three-month-old male Swiss albino mice (*Mus musculus*) weighing 30-40 g were used for the present study. Mice were housed in an approved departmental animal house (1825/PO/EReBi/S/15/ CPCSEA). They were kept under a 12:12 hr L: D cycle. Mice had free access to standard rodent pelleted diet (Nutrivet Life Sciences, Pune), high fat diet (VRK Nutritional Solutions, Sangali) and water *ad libitum*. Present study was initiated upon receiving the approval of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All these animals were maintained and treated as per the directions of the Institutional Animal Ethical Committee (IAEC).

Experimental design:

21 male mice were used in present investigation. Mice were divided into three groups and seven animals kept in each.

Control group: Mice were fed standard diet throughout the experiment and injected with 0.5 ml citrate buffer intraperitoneally (IP), pH 4.5.

Diabetic group (HFD/STZ group): Mice were fed HFD (40% fat as a percentage of total kcal) for two weeks and then injected with multi low dose of STZ (40mg/kg body weight) intraperitoneally (IP); in citrate buffer; pH 4.5 for five consecutive days (Mohapatra, 2016)

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Recovery group (HFD/STZ+ECE group): Diabetic mice supplemented with ECE (200mg/kg body wt., orally) for 28 days.

Blood glucose:

After 28 days of treatment, the mice were kept in fasting condition for overnight. Blood glucose was measured by collecting a drop of blood from the tail after incision with a sharp blade. Blood glucose was measured using glucometer (Accucheck). The results were expressed in terms of milligram per deciliter of blood (Bopanna, 1997).

Statistical Analysis:

All values were expressed as mean ±SD. Statistical analysis was carried out by one-way ANOVA, Turkey's HSD test.

Histological examination:

Liver was obtained on day 28 from all the groups of animals for the histological study. The tissues were fixed in 10% buffered formal saline and processed for routine histological evaluation. Tissue were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with Haematoxylin and Eosin (Harris, 1990).

RESULT:

Table No-1: Effect of *Elettaria cardamomum* seed extract (ECE) on blood glucose level (mg/dl) of STZ induced diabetic mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sr No	Treatment (n=5)	Blood glucose (mg/dl)	Statistical significance
1	Control	101.8±5.11	1:2 P<0.01
2	Diabetes	315.6±50.7	2:3 P<0.01
3	Recovery	189.4±12.7	1:3 P<0.01
P<0.01=Significant			

Plate No.1

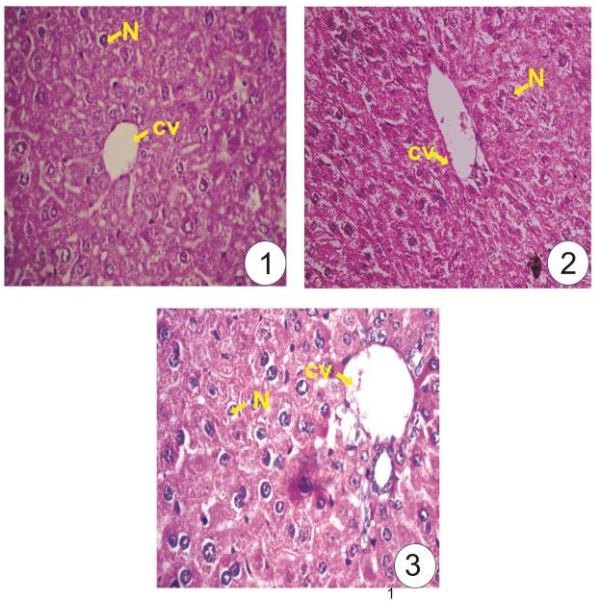

Plate1: Histopathological changes in Liver of experimental mice. All the sections stained by HE.

Fig. 1: Control mice liver shows normal lobular architecture, central vein and normal arrangement of hepatic cords X 400

Fig. 2: Diabetic mice liver shows destroyed architecture, dilation of hepatic sinusoids X 400

Fig. 3: Diabetic mice treated with ECE showing marked improvement in hepatocytes architecture X 400

Captions: CV- Central vein N-Nucleus

Effect of ECE on hyperglycemia in HFD/STZ induced diabetic mice:

The blood glucose level in control group was 101.8±5.11 and it was increased to 315.6±50.7 in diabetic mice and increase was significant as compared to control group (1:2, P< 0.01). In recovery group, after the treatment with ECE for 28 day significant decrease in blood glucose level 189±12.7 was observed as compared to HFD/STZ induced diabetic group (2:3, P< 0.01).

Effect of LSE on histological changes Liver of HFD/STZ induced diabetes:

Control mice liver had normal histology with normal hepatocellular architecture with central vein (Plate No. I Fig 1). Cytoplasm of hepatocytes stained with pink in color while prominent nuclei appear violet in color. The cells have well defined cell borders, are polygonal and are arranged in sheets. In HFD/STZ induced diabetic mice, damage to liver cells were observed. Along with hepatocytes, damaged central vein and portal triad also observed. Severe necrosis in hepatocytes with irregular size and shape were observed. Nucleus also showed displaced, enlarged and vacuolated appearance (Plate No. I Fig.2). In recovery group, moderate recovery in hepatocytes was observed after treatment with cardamom seed extract. In histopathological observations, hepatocytes of this group showed well-defined cell border definite polygonal shape but loss of lobular structure along with dilated central vein and blood vessels (Plate No. I Fig. 3)

Discussion:

In this study, we investigated the effect of ethanolic extract of *Elettaria cardamomum* seed (ECE) on liver's histopathology during the diabetes mellitus (DM). Hamden *et al.*, (Hamden, *et al.,* 2009) showed that the formation of free radicals in diabetes mellitus due to oxidative stress caused many complications including hepatopathy and nephropathy. Therefore, oxidative stress is a common destructive mechanism contributing the beginning and development of hepatic damage in many liver diseases (Medina and Moreno-Otero, 2005).

The administration of ethanol extract of *Elettaria cardamomum* seed (ECE) to the diabetic mice showed a significant (P<0.01) reduction in the blood glucose level of the diabetic mice. The result of this study correlates with Ahmed *et al* (2017) which reported α-amylase and α-glucosidase activity of cardamom in the regulation of diabetes mellitus. Medicinal plants contain several bioactive which may exert their hypoglycemic effects by reducing insulin resistance, increasing release and decreasing glucagon secretion, slowing the digestion and absorption of carbohydrates or by decreasing hepatic glucose production (Katzung, 1995). *Elettaria cardamomum* seed (ECE) also contains flavonoids, terpenoids, glycoside and alkaloids (Korikanthimathm,2001) as its bioactive compounds, which elicit their anti-diabetic effect by causing an increase in insulin output or by inhibition of the intestinal absorption glucose or to the facilitation of metabolites in insulin dependent processes.

In the present study, the increased oxidative stress in liver of diabetic mice was clearly demonstrated by histopathological studies. The hepatocytes of STZ induced diabetic mice showed loss of their normal architecture, cellular integrity, degenerated hepatocytes with polymorphic nuclei and dilated sinusoids. Increased free radical formation leads to increasing oxidative stress in liver tissue, due to which hepatocytes lost their structural integrity. STZ is a diabetogenic chemical and it is main source of oxidative stress in liver and increased diabetic complication (Yoon, *et al*., 2003). In recovery group after treatment of cardamom seed extract, hepatocytes showed restoration in their normal histological structural. Mohamed, 2016 reported Hepatoprotective effect of aqueous extract of cardamom against gentamicin induced hepatic damage in rats. On basis of present findings, it indicates that administration of cardamom seed extract markedly decreased the oxidative stress in liver by preventing formation of reactive oxygen species (ROS) and recovering the structural damages. Liver protective herbal drugs contain a variety of chemical constituents like coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids, xanthenes, terpenoids, reducing sugars, resins saponins and stilbeans (Gupta, 2006). These are present in the *Elettaria cardamomum* seed (ECE) and so may be responsible for hepatoprotective effect. It shows that the *Elettaria cardamomum* seed (ECE) have appreciable ability to prevent damage to the liver.

Conclusion:

The intraperitoneal injection of STZ in mice induces type 2 diabetes with hepatic damage. The data obtained from the study specified that oral administration of ethanol extract of *Elettaria cardamomum* seed extract (ECE) has antidiabetic and antihepatotoxic properties. It is therefore concluded from the result that *Elettaria cardamomum* seed extracts (ECE) possess antidiabetic as well as hepatoprotective activity and it may be useful in the management of diabetes.

References:

1. Levinthal, G.N. and Tavill, A. S. 1999. Liver disease and diabetes mellitus. Clin Diabetes, 17(2):73–93.
2. Sanchez, S.S., Abregu, A.V., Aybar, M. J. and Sanchez Riera, A.N. 2000. Changes in liver gangliosides in streptozotocin-induced diabetic rats. Cell Biol Int, 24:897–904.
3. Bolkent, S., Yanardag, R., Karabulut-Bulan, O. and Ozsoy-Sacan, O. 2004. The morphological and biochemical effects of glibornuride on rat liver in experimental diabetes. Hum Exp Toxicol, 23:257–264.
4. Koyuturk, M., Tunali, S., Bolkent, S. and Yanardag, R. 2005. Effects of vanadyl sulfate on liver of streptozotocin-induced diabetic rats. Biol Trace Elem Res, 104:233–247.
5. Stone, B.E. and VanThiel, D.H. 1985. Diabetes mellitus and the liver. Sem Liver Dis, 5:8–28
6. Iwu, M.M., Igboko, O.A., Elekwa, O.K. and Tempesta, M.S. 1990 Prevention of thioacetamide-induced hepatotoxicity by biflavanones of Garcinia kola. Phytother Res, 4:157–159.
7. Dwivedi, Y., Rastogi, R., Garg, N.K. and Dhawan, B.N. 1993. Perfusion with picroliv reverses biochemical changes induced in livers of rats toxicated with galactosamine or thioacetamide. Planta Med, 59:418–420
8. Korikanthimathm, V.S., Prasath, D. and Govardhana Rao 2001. Medicinal properties of cardamom Elettaria cardamomum Journal of medicinal & Aromatic Crops, 22/A 683-685.
9. Agaoglu, S., Dostbil, N. and Alemdar, S. 2006. Antimicrobial Effect of Seed Extract of Cardamom (Elettarıa cardamomum Maton). YÜ Vet Fak Derg, 16(2), 99– 101.
10. Suneetha, W.J. and Krishnakantha, T. P. 2005. Cardamom extract as inhibitor of human platelet aggregation. Phytother Res, 19(5): 437–440.
11. Ahmed, A.S., Ahmed, Q., Saxena, A.K. and Jamal, P. 2017. Evaluation of in vitro antidiabetic and antioxidant characterizations of Elettaria cardamomum (L.) Maton (Zingiberaceae), Piper cubeba L. f. (Piperaceae), and Plumeria rubra L. (Apocynaceae). Pak. J. Pharm. Sci, 30(1):113–126.
12. Mohapatra, L., Bhattamishra, S.K., Panigrahy, R., Parida, S. and Pati, P. 2016. Antidiabetic Effect of *Sargassum wightii* and *Ulva fasciata* in High fat diet and Multi Low Dose Streptozotocin Induced Type 2 Diabetic Mice. UK Journal of Pharmaceutical and Biosciences, 4(2): 13-23.
13. Bopanna, K.N., Kannan, J., Gadgil, S., Balaraman, E.R. and Rathore, S.P. 1997. Antidiabetic and antihyperglycemic effects of neem seeds kernel powder on alloxan diabetic rabbits. Indian Journal of pharmacology, 29: 62-67.
14. Harris, H.S. 1900. On the rapid conversion of hematoxylene in to haematin in staining reaction. J. Appl microsc, 3: 777.
15. Hamden, K., Allouche, N., Damak, M. and Elfeki, A. 2009. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. Chem Biol Interact, 180(3):421-32.
16. Medina, J., Fernández-Salazar, L.I., García-Buey, L. and Moreno-Otero, R. 2004. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. Diabetes Care, 27(8):2057-66.
17. Katzung, B.G. 1995. Basic and Clinical Pharmacology, 6 Edition London. Practice Hall International (UK) Limited.
18. Yoon, K.H., Ko, S.H., Cho, J.H., Lee, J.M., Ahn, Y.B., Song, K.H., Yoo. S.J., Kang, M.I., Cha, B.Y., Lee, K.W., Son, H.Y., Kang, S.K., Kim, H.S., Lee, I.K. and Bonner- Weir, S. 2003. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab, 88(5): 2300-2308.
19. Mohamed Aboubakr, Abdelazem Mohamed Abdelazem. 2016. Hepatoprotective effect of aqueous extract of cardamom against gentamicin induced hepatic damage in rats International Journal of Basic and Applied Sciences, 5 (1) :1-4

20. Gupta, A.K. and Misrag N.2006. Hepatoprotective Activity of aqueous ethanolic extract of Chamonile capitula in paracetamol intocicated albino rats. American Journal of Pharmacological Toxicology, 1: 17-20.