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EFFECT OF *TRIGONELLA FOENUM* GRAECUM ON LACTATE DEHYDROGENASE (LDH) ACTIVITY OF BLOOD, LIVER AND PANCREAS IN NORMAL AND ALLOXAN- INDUCED DIABETIC MICE

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ABSTRACT

The effect of aqueous seeds extract of *Trigonella foenum graecum* Linn was studied on Lactate dehydrogenase (LDH) activity of blood, liver and pancreas in normal and alloxan- induced diabetic mice. Our study showed that aqueous seeds extract, Oral administration of 50 mg/animal (0.5 ml of extract) in alternative days up to 7 days (1st, 3rd, 5th & 7th day). In alloxan induced diabetic mice, there was a significant increase in LDH activity of all the three tissues. The enzyme Lactate dehydrogenase showed significant decrease in the diabetic group treated with aqueous extract of tested plant when compared with the diabetic group. It is clear from the current data in this study that ginseng aqueous extract was the most efficient of the tested plant.

INTRODUCTION: Diabetes is a chronic disease that cannot be completely cured and may develop complications if not properly regulated. A serious metabolic disorder with micro- and macrovascular complications results in significant morbidity and mortality. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetics worldwide. In fact, despite the implementation of dietary intervention strategies and the presence of numerous educational programs, the prevalence of diabetes continues to increase [1].

Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system. The doubts about the efficacy and safety of the oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes [2]. Sheweita *et al*. reported that hypoglycemic herbs could alleviate the deleterious effects of carcinogenic compounds in the liver of diabetic rats since these herbs reduced the hepatic content of cytochrome P450 and other associated enzyme activities compared to the diabetic group. Such alterations in the activity of phase I and II drug metabolizing enzymes should be considered when therapeutic drugs are administered to diabetic patients since most of drugs are metabolized mainly by the cytochrome P450 system.

According to the previous investigations there are some medicinal plants and herbs which exert hypoglycemic effect such as *Balanites aegyptiaca* fruits [4], *Trigonella foenum* (Hulba seeds) [5] and two *Acacia* species (*Acacia nilotica* and *Acacia farnesiana* L. Wild) [6]. Additionally, there are reports indicating that worldwide, over 1200 species of plants have been recorded as traditional medicine for diabetes [7]. It is also worth noting that a number of drugs currently used to treat diabetes are historically derived from

plants. These include metformin (derived from *Galega officinalis*) [8] and 4- hydroxyisoleucine that is derived from *Trigonella feonum-feacum*) [9].

Trigonella foenum-graecum is cultivated throughout India and in certain regions of China. Its seeds are used as condiment in India, a supplement to wheat and maize flour for bread-making in Egypt, and one of the staple foods in Yemen. Its seeds are also be used as herbal medicine in many parts of the world for their carminative, tonic and aphrodisiac effects. Various reports have demonstrated that *Trigonella foenum-graecum* (fenugreek) seeds can lower blood glucose and cholesterol in type 1 and type 2 diabetics and experimental diabetic animals [10, 11]. However, the effects of fenugreek seeds, we intended to investigate the effects of aqueous extract of *Trigonella foenum-graecum* seeds on Lactate dehydrogenase (LDH) activity of blood, liver and pancreas of normal and alloxan induced diabetic mice.

MATERIAL AND METHODS:

Plant material: *Trigonella foenum-graecum* seeds were collected from Arakkonam, Vellore District, Tamil Nadu, India. Collected seeds were shade-dried, cleaned and finely powdered and used for extraction.

Extraction of Aqueous Plant Material: Aqueous extract of *T. foenum-graecum* seed powder was prepared by grinding 300mg of dried seeds in 3ml of glass distilled water. 0.5 ml of this solution was administered to each set of ten animals. Freshly prepared extracts alone were administered.

Chemicals: Alloxan monohydrate was purchased from SD Fine chemicals (Mumbai, India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA, SRL and Qualigens (India).

Animals: Healthy mature male mice with bodyweight ranging from 25 to 30 grams were selected and maintained in the cages. The mice are fed with commercial pellets supplied by Lipton, India. Food and water were provided ad libitum.

Induction of Diabetes and Study Design: The blood glucose levels of normal male mice were determined and allowed to fast overnight. A single intra- peritoneal (i.p.) injection of alloxan monohydrate with a dosage of 120 mg / kg body weight in physiological saline was given [12]. This dosage was prepared because it produced maximum glucose levels. Mice with glucose levels ranging between 200 mg / dl and 350 mg / dl were considered severely diabetic and use for estimations of blood glucose at 1st, 3rd, 5th and 7th day after administration of alloxan.

The animals were divided in to four groups of five each.

- Group I: Control mice with normal saline (5ml/animal).
- Group II: Mice with oral administration of TFSE (50mg/animal).
- Group III: Alloxan induced diabetic mice (120 mg/kg body weight).
- Group IV: Mice treated with TFSE (50mg / animal) after alloxan treatment.

The dosage to be most effective was 50mg / animal (0.5 ml of extract). Animals were segregated after 1st, 3rd, 5th and 7th day after administration of seed extract and the samples were collected. The same procedure was followed for alloxan induced diabetic animals. One ml of peripheral blood (PB) was collected from the mice in sterile screw capped glass vials containing EDTA by using sterile disposable syringes.

The Lactate dehydrogenase (LDH) activity of blood, liver and pancreas were estimated by following the method of King, 1965. The values were expressed as µ moles of pyruvate formed /min/mg protein for tissues and for blood the values were expressed as µ moles of pyruvate formed /min/ml protein.

RESULTS:

Blood LDH Activity: Glucose metabolism involves several enzymes among which LDH is one of the key terminative enzyme in the sequence of reactions involved in anaerobic glycolysis promoting the breakdown of glucose to lactate and is essential for the production of ATP molecules. Hence, LDH activity was determined in all the four experimental groups along with that of control animals. The values obtained on blood LDH are shown in **table 1**.

A careful perusal of the results given reveals that blood LDH activity in the control animals varied between 115.14 ± 0.02 µ moles of pyruvate formed/min/ml of protein and 124.10 ± 0.03 µ moles of pyruvate formed/min/ml of protein. When alloxan administered animals were studied, the values ranged between 129.90 ± 0.02 µ moles of pyruvate formed/min/ml of protein and 213.56 ± 0.02 µ moles of pyruvate formed/min/ml of protein in the 1st and 7th day experimental animals indicating a sharp increase in blood LDH activity.

However, when *T. foenum graecum* seed extract was administrated to diabetic animals the blood LDH activity in the 1st day was 121.51 ± 0.01µ moles of pyruvate formed/min/ml of protein; in the 3rd day it was 117.21 ± 0.02 µ moles of pyruvate formed/min/ml of protein; in the 5th day it was 119.16 ± 0.02 µ moles of pyruvate formed/min/ml of protein and in the 7th day it was 113.38 ± 0.02 µ moles of pyruvate formed/min/ml of protein revealing the signs of return to normalcy. As the main source of blood LDH has been liver, it is of interest to investigate LDH activity of liver tissue.

TABLE 1: BLOOD LDH ACTIVITY OF MALE MICE

Groups	Blood LDH (µ moles of pyruvate formed / Min/ Ml of protein)			
	Experimental periods			
	1st day	3rd day	5th day	7th day
I Control (Received saline water)	119.91±0.03	117.85±0.03	124.10±0.01	115.14 ±0.02
II *Trigonella foenum- graecum* only	117.91* ±0.02	113.50* ±0.02	118.31* ±0.02	114.95* ±0.01
Change in %	1.67	3.69	4.67	0.17
III Alloxan	129.90** ± 0.02	180.61** ±0.02	210.21** ±0.02	213.56** ±0.02
Change in %	8.33	53.25	119.16** ±0.02	85.48
IV Alloxan + *Trigonella foenum-graecum*	121.51** ±0.01	117.21** ±0.02	119.16** ±0.02	113.38** ±0.02
Change in %	1.33	0.54	3.98	1.53

* Not significant ** significant at 0.05 level ± standard deviation (S.D.)

Liver LDH Activity: The blood LDH activity might reflect in the LDH activity of tissues like liver and pancreas. The results of liver LDH activity are given in **table 2**. When the alloxan was administered to normal control animals the liver LDH activity clearly indicating a gradual increase from 1st to 7th day experimental animals. All the values were statistically significant when compared to control animals. However, when *T. foenum graecum* seed extract was given to diabetic animals on the 1st day, the liver LDH activity was 306.21 ± 0.04 µ moles of pyruvate formed/min/mg of protein, which decreased to 271.34 ± 0.04 µ moles of pyruvate formed/min/mg of protein and in the 7th day experimental animals. It is of interest to note that the LDH activity declined significantly after the administration of *T. foenum graecum* seed extract.

TABLE 2: LIVER LDH ACTIVITY OF MALE MICE

Groups	Liver LDH (µ moles of pyruvate formed / Min/ Ml of protein)			
	Experimental periods			
	1st day	3rd day	5th day	7th day
I Control (Received saline water)	291.01±0.01	283.91±0.02	281.03±0.02	289.56±0.02
II *Trigonella foenum- graecum* only	289.51* ±0.02	281.01* ±0.02	279.14* ±0.02	286.14* ±0.02
Change in %	0.52	1.02	0.67	1.18
III Alloxan	464.98** ± 0.03	470.91** ±0.06	471.81** ±0.03	480.34** ±0.04
Change in %	59.78	65.87	67.89	65.89
IV Alloxan + *Trigonella foenum-graecum*	306.21** ±0.04	280.82** ±0.03	279.97** ±0.04	271.34** ±0.04
Change in %	5.22	0.54	3.98	1.53

* Not significant **significant at 0.05 level ± standard deviation (S.D.)

Pancreatic LDH activity: The results of LDH activity of the pancreatic tissue are given in **table 3**. A careful examination of the data reveals that the pancreatic LDH activity ranged between 175.01 ± 0.03 µ moles of pyruvate formed/min/mg of protein and 194.14 ± 0.02 µ moles of pyruvate formed/min/mg of protein in the control animals. The difference was insignificant. In the alloxan administered experimental animals, the pancreatic LDH was increased from control animals. When *T. foenum graecum* seed extract was a given to diabetic animals the pancreatic LDH reduced. It is evident that all the experimental animals returned to normalcy after the administration of *T. foenum graecum* seed extract to diabetic animals.

TABLE 3: PANCREATIC LDH ACTIVITY OF MALE MICE

Groups	Pancreatic LDH (μ moles of pyruvate formed / Min/ Ml of protein)			
	Experimental periods			
	1st day	3rd day	5th day	7th day
I Control (Received saline water)	83.92±0.02	79.36±0.03	80.11±0.02	81.32±0.02
II *Trigonella foenum- graecum* only	81.22* ±0.01	76.54* ±0.06	73.30* ±0.02	70.92* ±0.10
Change in %	3.22	3.55	8.50	12.79
III Alloxan	130.01** ± 0.05	135.49** ±0.34	158.61** ±0.04	170.37** ±0.07
Change in %	54.92	70.73	97.99	109.51
IV Alloxan + *Trigonella foenum-graecum*	10.69	84.02** ±0.05	92.54** ±0.04	73.40** ±0.04
Change in %	92.89** ±0.0	5.87	15.52	9.74

* Not significant ** significant at 0.05 level ± standard deviation (S.D.)

DISCUSSION: Lactate dehydrogenase (LDH) is an associate enzyme of glucose metabolism. It is reasonable to expert changes in LDH activity correlated with the changes in glucose content of tissues in this study after alloxan administration. The results obtained in the present investigation support such as assumption. When alloxan was given the LDH activity showed a tremendous increase along with glucose. The patterns of LDH activity revealed a close relationship with the cell metabolic changes. Similar observations have been reported [14, 15]. Recently, Mansour *et al*., 2002 have also reported an increase in LDH activity in alloxan induced diabetic rats.

The observations on liver LDH lend support to such a view. On administering alloxan to the animals, the liver and pancreatic tissues of experimental animals showed an increase in LDH activity. Brech *et al*., (1970) provide a clue to this observed increase in LDH activity. They noticed a shift in the location of the enzyme activity from mitochondria to cytosal from where LDH might have leaked out to blood. Such cellular leakage and loss of functional integrity of the cell membrane in the liver tissue might be the reasons of increase noticed in blood LDH activity.

The possibility of presence of such factors in *T. foenum graecum* seed extract is indicated in the work [18]. The aqueous extract of *T. foenum graecum* seeds, *Pterocarpus marsupium* bark and *Ocimum sanctum* leaves have been shown to exert hypoglycemic / antihyperglycemic effect in experimental as well as clinical setting. They suggested the presence of 4 – hydroxyisoleucine could stimulate insulin secreting properties of endocrine pancreas. It is possible that 4 – hydroxyisoleucine present in *T. foenum graecum* seed extract could be the substance which has the property of powerfully stimulating insulin secretion at all levels of organization.

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