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Effect of Fenugreek (Methi) on Cortical Thickness of the Thymus in Streptozotocin-Induced Diabetic Rats

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Abstract:

Objective: *To find out microscopically whether* Trigonella foenumgraecum *(fenugreek seeds/methi seeds) has got any preventive role against the lowering of cortical thickness of the thymic lobules in diabetes mellitus.*

Design: *An experimental study on Long Evans rats which were divided into three equal groups depending on their different shorts of dietary feeding and drug treatment.*

Setting: *Anatomy department of IPGMR (Institute of Post Graduate Medicine and Research) at present BSMMU (Bangabandhu Sheikh Mujib Medical University) and BIRDEM (Bangladesh Institute of Research and Rehabilitation in diabetes, Endocrine & Metabolic Disorders).*

Subjects: *Fifty eight healthy young Long Evans rats of either sex weighing 72 to 174gm aged between 50 to 60 days were used in this study.*

Main outcome measures: *Variation of cortical thickness of the thymic lobules in different groups of rat.*

Result: *Cortical thickness in the nondiabetic control group, which ranges from 30.17 to 36.99. and the mean was 34.83 ± 0.60. In diabetic control group the cortical thickness ranges from 17.78 to 26.46 and the mean was 21.85 ± 1 On the other hand, in the fenugreek- treated diabetic rats the cortical thickness ranges from 25.71 to 32.95 and mean cortical thickness was 30.49 ± 0.75.*

Conclusion: *Fenugreek showed a tendency of acting against lowering of the cortical thickness of the thymic lobule of Streptozotocin-induced diabetes mellitus. However, further investigations are recommended for establishing fenugreek as a safe, useful effective agent to preserve the cortical thickness improving the diabetic condition by acting as antidiabetogenic agent.*

Key words: *Diabetes mellitus, Differential lymphocyte count, Fenugreek, Thymus.*

Introduction:

High prevalence of diabetes mellitus is now a major health problem. At present in Bangladesh the number of diabetics registered to different health clinic and institutes among which only Bangladesh Institute of Research and Rehabilitation in diabetes, Endocrine & Metabolic Disorders (BIRDEM) has registered 1,78,015 cases in 1997, which is more than 15,000 higher than that of previous year. Although it is understandable that many of the diabetic patients especially in the rural area have not registered themselves to any diabetic clinic or hospitals and many other still remain undiagnosed.

In the treatment of diabetes mellitus, synthetic oral hypoglycaemics have various side effects and contraindications, has led to scientists to search for alternatives and many natural products indigenous to various parts of the world. A large number of herbal products have been used to treat the polyurea. Some of these are in the usual food

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list of the people concerned. Scientific studies also revealed the hypoglycaemic properties in many of these herbal products. Among these herbal products fenugreek is a leguminous herb, cultivated in the Indian subcontinent, in the Mediterranean region and in the North Africa.[1] It is also named as 'methi' in this subcontinent which has the hypoglycaemic effect due to its alkaloid 'trigonelline' was reported as early as in 1948 by Fournier, who showed the hypoglycaemic effect in animals[2], in humans NIDDM[1,3,4] and in IDDM patients.[5]

As because diabetes mellitus causes lowering in cortical thickness of thymus gland, it may be hypothesized that fenugreek (Methi) being an antidiabetic agent may also be used to minimize the lowering in cortical thickness by inhibiting death of cortical thymocytes in cortico-medullary junction of the thymus.

Materials and Methods:

A total number of 58 young rats of either sex of long Evans strain were used in this experiment. They were young rats of 50 to 60 days old, weighing between 72 and 174gm. Among them 10 rats were treated with vehicle (citrate buffer solution at the pH 4.5) only used as non-diabetic control rats (Group A) and 20 rats were treated with vehicle and Streptozotocin found as diabetes, 10 of which were treated as diabetic control group (Group B) and rest 10 (1 died, 1 escaped) were treated again with defatted fenugreek (methi) powder at a dose of 1.25gm in 10ml of demineralized water per kg body weight per day to control the diabetes mellitus and was called as fenugreek treated diabetic group (Group C).

After sacrifice (on the 13th day from the day of STZ/ Vehicle injection) of the rats, the thymus gland was dissected carefully and for this study a good section of right thymic lobe of each rat was stained with toluidine blue. Then the lobe was approximately divided into four quadrants as shown in Fig.-1. Three largest lobules were selected from each quadrant for measurement of cortical thickness. Where there were less than three lobules available, the desired number was selected by adding from the next quadrants, if the lobule had more than three.

The measurement was taken by an ocular micrometer. Then the diameter of the lobule and the thickness of the medulla were measured in three dimensions cortex as seen in Fig.1 For diameter of each lobule three transverse diameter of the medulla and six thickness readings of the cortex were made. Then for each diameter reading the two thickness readings were combined and expressed as the percentages of the corresponding diameter. Thus for each lobule 3 cortical thickness expressed as percentages of its diameter were obtained the average of which was taken as the value for that particular lobule. In this way a total of 12 values (3`4) were calculated for 4 quadrants of each section. The average of these 12 values was taken as the cortical thickness as a percentage of lobular diameter for a particular section (i.e. for a particular rat). Three mean values were then calculated for 3 groups of rat.

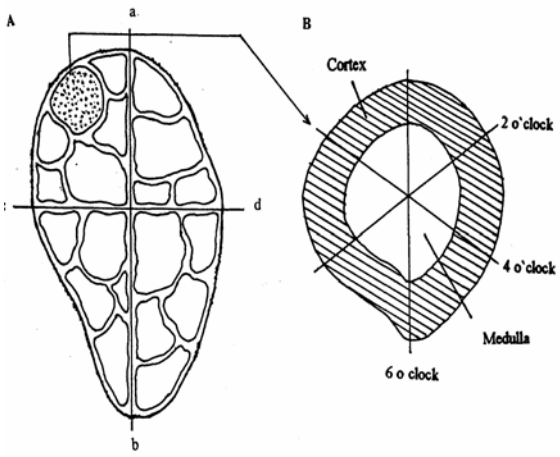

Fig. 1: *Procedure ol estimating cortical thickness ol a lobule. A. Diagrammatic representation of dividing a thymic section into four quadrants by two lines ab and cd crossing each other at right angles through the approximate centre of the section. The diagram shows many lobules. B. An enlarged view of the stippled lobule of A. The cortex is shown hatched. Three straight lines through the approximate centre of the lobule show the positions of the superimposed ocular micrometer: 12 - 6 o'clock, 2 - 8 o'clock and 4 - 10 o'clock along which cortical thickness was calculated.*

Observations and results:

Table-I shows the cortical thickness on the day of sacrifice of the rat (on day 13 from STZ/Vehicle injection). The cortical thickness was expressed as a percentage of the corresponding lobular diameter of the thymus.

Table-I

Thickness of the thymic cortex as a percentage of corresponding lobular diameter of the thymus on the 13th day

Group	Number of ats	Cortical thickness (%)	
		Range	Mean ± SE
A (Nondiabetic control)	10	30.17 - 36.99	34.83 ± 0.60
B (Diabetic control)	10	17.78 - 26.46	21.85 ± 1.0
C (Fenugreek-treated diabetic)	8	25.71 - 32.95	30.49 ± 0.75

Statistical analyses for significance of differences:

Cortical thickness as percentages of corresponding lobular diameters of the thymuses, compared through unpaired 't' test:

Group B vs A : P = 0.000*

Group C vs B : P = 0.000*

Group C vs A : P = 0.059

* Significant at 5% level (P £ 0.05).

In the nondiabetic control group the mean cortical thickness was 34.83 ± 0.60. In diabetic control group the mean cortical thickness was 21.85 ± 1.0 and in fenugreek-treated diabetic group the mean cortical thickness was 30.49 ± 0.75.

Statistical analysis shows that the cortical thickness as the percentage of corresponding lobular diameter in the non-diabetic group was significantly lower than that of the value in diabetic control group (P=0.000). On the other hand the cortical thickness in the fenugreek-treated diabetic group was significantly higher than that of the diabetic control group (P=0.000) but more or less nearer to the value in the non-diabetic group (P=0.059).

Discussion:

In this study, Streptozotocin-induced diabetes in rats was associated with a marked reduction in thymic volume. Drugs and pathological conditions impairing insulin production and secretion by pancreatic â-cells caused diabetes mellitus, which results in lymphocytes depletion in thymic cortico-medullary junction. Atrophy of the rat thymus gland occurs after the onset of both drugs induced and genetically determined diabetes mellitus. This atrophy is due to loss of thymocytes from the gland.[6-9]

Chatamara el al., has found that, histologically the cortex of the thymus was clearly reduced in width by the third day after the induction of diabetes with STZ. By seventh day, this change were pronounced with thickening of connective tissue matrix with some degree of hyaline change of fibrous tissue. By the ninth day the cortex was greatly reduced. After twentieth day there was no clear distinction between cortex and medulla.[6]

It has found in STZ-treated rat thymus that, the width of the cortex obviously became narrower and the cortical lymphocytes near to the cortico-medullary junction appeared to be reduced selectively.[7]

Warley and Morris showed that due to atrophy, the thymus got one third of the weight of the control animals within 10 days. Marked degeneration occurred in the cortical region.[8]

Tabata et al., has also found that in their histological findings, the cortical region of the thymus of diabetic rat was obviously reduced in size. Cortical lymphocytes present in the vicinity of the cortico-medullary junction appeared to be reduced selectively. Similar changes was not seen in insulin-treated diabetic rats.[9]

In diabetic rat the thymus was severely atrophic. Cortex and medulla was markedly reduced in width and thymocytes were depleted. The cortico-medullary boundary was ill defined and there was fibrosis of the medulla. Insulin and Insulin-like growth factor are responsible to minimize the cortical lymphocyte death in the cortico-medullary junction.[10]

Islam MN has also observed that fenugreek can act against streptozotocin-induced diabetes to prevent reduction of size of pancreatic islets in addition to reduction of blood glucose level. He even suggested possible regeneration of â -cell with fenugreek treatment of STZ-induced diabetes in rats. As defatted fenugreek powder has been used as the anti diabetogenic agent in this experiment, it has found that the diabetic condition of the rats has improved with possible regeneration of the â-cell of the pancreas to resume the insulin production as in normal condition.[11]

In case of diabetes mellitus there will be a massive thymocyte death at the onset of the disease. Potassium level is known to be reduced in uncontrolled diabetes as ion fluxes are dependent on insulin. Potassium is required for the correct functioning of RNA in thymocytes. Insulin can potentiate DNA synthesis in lymphocytes. Hence lack of insulin in diabetes mellitus, where it is spontaneous or drug induced like STZ, will cause gross lymphocytic depletion.[12]

In this study, it is found that in non-diabetic control group the mean relative cortical thickness of the thymic lobule is significantly higher than that of the diabetic control group (P=0.000). Again in fenugreek-treated diabetic group the mean relative cortical thickness of the thymic lobule was significantly higher than that of the diabetic group (P=0.000) and more or less similar to the non-diabetic control group (P=0.059). So it is comparable to the other studies.[6-10] From this study it may be concluded that defatted fenugreek preserve cortical thickness of the thymic lobule by minimizing the cortical lymphocyte death near the cortico-medullary junction in the diabetic rat thymus by the possible regeneration of the â-cell of the pancreas to resume insulin production as in normal condition.

Conclusion:

Defatted fenugreek showed a tendency of acting against the lowering of cortical thickness of the thymic lobule in diabetes mellitus. However, further investigations are recommended for establishing fenugreek as a safe, useful effective agent to preserve the cortical thickness improving the diabetic condition by acting as antidiabetogenic agent.

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