Experimental Material

THE EFFECT OF GINKGO BILOBA EXTRACT ON MACROPHAGE PHAGOCYTIC ACTIVITY IN EXPERIMENTAL DIABETES

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SUMMARY : Forty-four Swiss albino rats aged two months, weighted 214.00 ± 31.68 g (mean ± SD), were used in these experiments. They were divided into four equal groups as control, alloxan-diabetic, diabetic+GbE and control+GbE groups. After the onset of experimental period, diabetic+GbE and control+GbE groups received ginkgo biloba extract (GbE) and the other groups were given saline solution for ten weeks. Diabetic and diabetic+GbE groups were made diabetic by injecting alloxan on the 16th day. At the end of the experimental period, number of particles ingested by hundred macrophages, was counted under light microscope. The mean phagocytic activity of diabetic group was significantly decreased compared with control group ($p<0.05$). But the mean phagocytic activities of diabetic+GbE and control+GbE groups were not significantly different from those of the control group.

Key Words : Diabetes, GbE, phagocytosis.

INTRODUCTION

Diabetes mellitus has been described as a disease which has negative effects on immune system functions as well as on other systems. Numerous experimental studies based on clinical observations showed that phagocytic functions of macrophages and mitogenic activity of lymphocytes were decreased in diabetes (1,2). Investigations carried out to understand the pathogenesis of these changes have shown that the changes in the cholesterol/phospholipid ratio of the cell membrane due to the diabetic affections (3) cause hypercholesterolemia. This has been associated with a decrease in membrane fluidity, thus altering several functions including the cation transport mechanisms, Na-K ATPase activity etc (4-8). Consequently, since phagocytosis is one of the properties of cell membrane, the decrement in fluidity also affects this important function of the macrophage (9). The process of phagocytosis consists of binding, signaling, and responding to the signal and ingestion (10).

The other factors altering the membrane fluidity are oxygenated free radicals which generate a peroxidation cascade permitting the damage to travel to distant sites within the cell and to the organelles (11,12). In normal conditions, free radicals in the cell are balanced by protective mechanisms without damaging the cell functions (11,12). But enzymes which serve an important role in the protective mechanisms are decreased in diabetic conditions as reported in the previous papers (13-18). Thus the increased amount of oxygenated free radicals play a significant role in the development of irreversible tissue damage in diabetic patients (16,18).

Especially in the last decade, an increasing amount of attention has been focused on antioxidants that are able to prevent oxidative stress (19-21). Therefore, in the present

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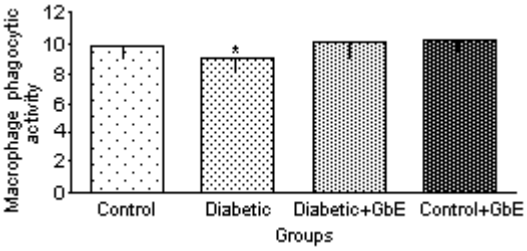
Figure 1: Phagocytic activities of peritoneal macrophages, *p<0.05.



investigation, ginkgo biloba extract (GbE) was used to determine the role of antioxidants on the impairment of phagocytic activity encountered in diabetes. GbE is one of the oldest natural therapeutic agents known in traditional Chinese Medicine for about 5000 years. This extract is particularly rich in glycosides of the benzopyrone family and possesses the capacity to very actively entrap oxygenated free radicals (22,23).

The present study was undertaken to examine the effect of GbE on the alterations of phagocytic functions of macrophages in the experimental diabetes (Figure 1).

MATERIAL AND METHODS

Forty four Swiss albino rats (two months old), weighed 214.00 ± 31.68 g (mean±SD), were used in this study. They were equally divided into four groups: control, alloxan-diabetic, diabetic+GbE and control+GbE.

The experimental diabetes was induced in the rats by administration of 5 mg alloxan per 100 g of body weight intravenously through the caudal vein. One hour afterwards of 2 ml 5% glucose for 24 hours was given ed lib .The blood glucose levels animals were monitored by determination of glycemia after 48 hours and were maintained below 30 mM/L by injection of 0.5-1 IU insulin. Treatment with GbE started 15 days before injection of alloxan and the treatment consisted of oral administration of 10 mg/kg/day given through a gastric tube early the morning in fasting animals, was begun 15 days before induction of the diabetes and continued throughout the duration of evolution. The rats in the control and the diabetic groups were gavaged with saline solution during the same period of time.

At the end of two months of diabetic period, animals were deprived of food for 18 h and then prepared for the experimental procedure after ether anesthesia. 10 ml Krebs phosphate buffer solution (pH:7.4) was injected into the peritoneum. The abdomen was operated from the linea alba and after 3 minutes peritoneal message was applied after which peritoneal fluid was drained through a cannula during the rest of the procedure. 0.5 ml of this fluid which includes macrophages were incubated at 37°C for an hour with 0.5 ml 1% active carbon particles. Number of particles ingested by hundred macrophages were counted under light microscope (x100) and the mean value was calculated macrophage. This value was regarded as representing phagocytic activity. To test the viability of macrophages they were exposed to 0.2 trypan blue.

Amount of glucose in the blood which was taken from the rats by cardiac puncture at the end of experiment and was measured according to Folin-Wu method (24).

Student's t test was used in statistical analysis.

Table 1: Blood glucose values of studied rats (mM/L).

	Glucose Values		
	Begining	2 wk after DM	8 wk after DM
Control	4.62 ± 0.57	5.38 ± 0.47	5.70 ± 1.71
Diabetic	4.95 ± 0.73	18.98 ± 3.80***	21.09 ± 3.82***
Diabetic+GbE	4.87 ± 0.80	17.78 ± 3.95***	20.84 ± 3.38***
Control+GbE	4.98 ± 1.01	5.56 ± 0.91	6.01 ± 5.57

***p<0.05.

Table 2: The means and standard deviations of macrophage phagocytic activities and viabilities of four groups.

Macrophage phagocytic activity (count/per cell)		Viability (%)
Control	10.01 ± 1.11	92.70 ± 4.64
Diabetic	8.90 ± 1.27*	88.78 ± 5.02
Diabetic+GbE	10.10 ± 1.28	87.68 ± 6.92
Control+GbE	10.07 ± 0.34	90.83 ± 5.85

*p<0.05.

RESULTS

Blood glucose values of rats, at the beginning and two and eight weeks after onset of diabetes are summarized in Table 1. Both these values and clinical symptoms of the rats (plyuria, polydipsia, polyphagia) showed an effective diabetic state.

The mean and standard deviation of phagocytic activities and viabilities of four groups are shown in Table 2. The difference between phagocytic activities of diabetic and control groups was significant ($p<0.05$). However phagocytic activities of diabetic+GbE and control+GbE groups were not different from those of the control group.

DISCUSSION

It was noted that glucose levels of plasma were significantly higher in the diabetic groups than in the control group. This result is consisted with previously published data (13-16).

One of the important factors which plays a significant role in the etiopathogenesis of diabetes is superoxide radicals (25). Because cells were damaged as a result of intracellular defense mechanisms do not act functionally against free radicals due to effect of diabetes. Therefore phagocytic activity was found to be decreased in diabetes (1,2). On the other hand, decreased macrophage activity in diabetic rats returned to the control group's activity with the effects of ginkgo biloba extract (GbE). However phagocytic activity of macrophage was not affected by GbE. Based on this result, it may be concluded that diabetic patients has insufficient immune system.

It was shown in the previous experimental studies (19,20) that administration of antioxidants decrease the disruption of phagocytic activity of macrophages caused by oxygenated free radicals. In the present study, alterations in macrophage phagocytic activity of diabetic rats was returned to control value by GbE.

In conclusion, our result indicate that GbE was effective at reducing lipid peroxidation in experimental diabetes. It can thus regulate the cell functions against the injurious effects of superoxide radicals on membrane functions.

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