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Research Article

EFFECT OF ETHYL ACETATE EXTRACT OF BLACK TEA ON CARDIOVASCULAR COMPLICATIONS ASSOCIATED WITH STREPTOZOTOCIN DIABETIC RATS

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ABSTRACT
Diabetes mellitus (DM) is associated with an increased incidence of cardiovascular events and microvascular complications. These complications contribute to the morbidity and mortality associated with the disease. There is increasing evidence supporting a role for Matrix MetalloProteinases (MMPs) and their inhibitors (tissue inhibitors of matrix metalloproteinases—TIMPs) in the atherosclerotic process. The main cause of vascular complications is increase in collagen-4, Gelatinase A and B content of extracellular matrix of cells which lead to cell wall thickening in DM. The collagen content is regulated by MMP (Matrix MetalloProteinases) enzymes. The up-regulated levels of MMP-2 and MMP-9 enzymes are responsible for the increased collagen content in extracellular matrix, which further carries micro & macrovascular complications. The involvement of MMP-2 and MMP-9 enzymes in Diabetic Complications was determined by several scientists well before. The inhibitory effect of Theaflavins on Matrix Metalloproteinases was studied by some scientist on cancerous tumours. It has been reported in the literature that Teaflavins (Theaflavin) in Black Tea are inhibitors of MMP-2 & 9. So, here it was hypothesized that Black Tea which contains Theaflavin can be used for the treatment of DM Complications by inhibition of MMP 2 & 9. The efficacy was measured on Diabetic Cardiac Complications which are the development of a coronary microangiopathy and decrease of diastolic and systolic functions of the left ventricle were measured.
KEY WORDS – Diabetes mellitus, Diabetic Cardiac Complications, Matrix Metalloproteinases, Theaflavins

INTRODUCTION

Diabetes mellitus is likely to become one of the most prevalent and economically important diseases of the 21st century largely owing to an increasing incidence of type 2 diabetes mellitus (DM) in the developed nations and many of the developing nations. Diabetes is the paradigm of a condition that necessitates a multidisciplinary approach to its management and treatment.[1, 2]

Diabetes mellitus is associated with an increased incidence of cardiovascular events and microvascular complications. These complications contribute to the morbidity and mortality associated with DM. There is increasing evidence supporting a role for matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of matrix metalloproteinase—TIMPs) in the atherosclerotic process. However, the relationship between MMPs/TIMPs and diabetic angiopathy is less well defined.

Hyperglycemia directly or indirectly (e.g., via oxidative stress or advanced glycation products) increases MMP expression and activity. These changes are associated with histologic alterations in large vessels. On the other hand, low proteolytic activity of MMPs contributes to diabetic nephropathy.

Within atherosclerotic plaques an imbalance between MMPs and TIMPs may induce matrix degradation, resulting in an increased risk of plaque rupture. Furthermore, because MMPs enhance blood coagulability, MMPs and TIMPs may play a role in acute thrombotic occlusion of vessels and consequent cardiovascular events. Some drugs can inhibit MMP activity. However, the precise mechanisms involved are still not defined. Further research is required to demonstrate the causative relationship between MMPs/TIMPs and diabetic atherosclerosis. It also remains to be established if the long-term administration of MMP inhibitors can prevent acute cardiovascular events.[3, 4]

DM is associated with an increased incidence of peripheral arterial disease (PAD), but the true prevalence is difficult to determine. This is because PAD can be asymptomatic as a result of diabetic peripheral neuropathy blunting the perception of pain. According to the Framingham study, DM increases the risk for intermittent claudication by 2–3-fold. In addition to CHD and PAD, DM adversely affects the cerebro-vascular circulation, increasing the risk for stroke by 2–3- fold. Therefore, DM promotes generalized atherosclerosis and the related morbidity and mortality.

There may also be an increase in atherosclerotic renal artery stenosis. In contrast, there may be a decreased risk of abdominal aortic aneurysm (AAA) in patients with DM. Nevertheless, there is an increased risk for aortic occlusive disease.[5]

Hyperglycemia and MMPs: Glucose can modulate the production, expression, and activity of MMPs in specific cell lines. Hyperglycemic cultures of endothelial cells (ECs) simultaneously enhance the activity and expression of MMP-1, MMP-2, and macrophage-derived MMP- 9.

Diabetic Retinopathy and MMPs: Apart from the role of MMPs in diabetic macrovascular complications, these changes may play a role in DM-related microvascular complications. A recent study50 provides a rationale for the association between microangiopathic complications, such as retinopathy, nephropathy, and peripheral neuropathy and MMP-2 in patients with DM1. After a 5-year follow-up, MMP-2 activity remained higher in diabetic patients than in healthy control subjects. Moreover, retinas from diabetic patients had increased concentrations of activated MMP-2 and MMP-9 in comparison to "control eyes." In particular, increased levels of both proenzyme and activated MMP-2 and MMP-9 were identified in the basement membranes of new nonfunctional retinal capillaries.

Diabetic Microangiopathy and MMPs: MMPs degrade surrounding ECM, facilitate EC migration, promote EC sprouting, and induce new vessel growth. DM may adversely influence neoangiogenesis in response to lumen occlusion and ischemia. Studies from rat hind-limb ischemia models suggest that in diabetic conditions, MMP activity is inhibited and thus angiogenesis is prevented.[6]

The aim of this research is to qualify Black tea as reducer of Diabetic Cardiac Complications. Flavonoids (polyphenols) are present as major constituents in all form of the Tea. Black tea is the fermented form of the green tea. Theaflavins, as Flavonoids, are principal components present in Black Tea.[7, 8, 9]

MATERIALS AND METHODS

Materials

Black Tea Leaves (Flowery Orange Pekoe) were purchased from Girnar Food and Beverages Pvt. Ltd., Kurla, Mumbai, India. Streptozotocin was procured from Sisco Research Lab, India. Urethane was purchased from Sigma Aldrich Chemicals, Germany. Glucose oxidase–peroxidase (GOD/POD) glucose kit was purchased

from Erba, India. All other chemicals were purchased from Merck (India). Ethyl acetate extract of Black tea was formulated as 0.5% sodium CMC suspension. STZ was freshly dissolved in ice cold citrate buffer (pH 4.5) solution.

Animals

Male Wistar rats (150-210g) were purchased from the Haffkine Institute, Mumbai, India and were housed at a temperature of 25 ± 1^0C and relative humidity of 45 to 55% in a clean environment under 12:12h light and dark cycle. The animals had free access to food pellets and filtered water was made available *ad libitum*. The research protocol was approved by Institutional Ethical Committee (IEC) of School of Pharmacy and Technology Management, NMIMS University Mumbai, constituted under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Extraction of Black Tea

The flavonoid content of black tea was extracted by Soxhlet Extraction method. The dried leaves of Black tea crushed to powder and 100 mg of powdered black tea then extracted with 600ml of Ethyl Acetate. The siphon off cycle was continued up to 72 hrs. After extraction the remaining solvent was evaporated by rotary evaporator. Dark greenish black sticky extract was obtained.

Induction of Diabetes

A single dose (55 mg/kg, i.p.) of Streptozotocin (STZ) was used for induction of diabetes in rats. Age matched control rats received the equal volume of vehicle. Diabetes was confirmed after 48 h of STZ injection. Plasma glucose levels were estimated using GOD/POD kit and rats with plasma glucose level > 350 mg/dl were considered for further studies.

Experimental design and drug treatment

Two weeks after the diabetic induction treatments were given for further two weeks (3^{rd}, and 4^{th} weeks). After 2 weeks, normal and diabetic rats were randomly divided into experimental groups and treated with drugs as follows: Diabetic Group 1 was treated with distilled water (DW, 4 ml/kg, p.o.), Diabetic Group 2 was treated with ethyl acetate extract of black tea 100mg/kg, 0.5% carboxymethyl cellulose suspension (CMC, 1 ml/kg, p.o.), Diabetic Group 3 was treated with ethyl acetate extract of black tea 50mg/kg, 0.5% carboxymethyl cellulose suspension (CMC, 1 ml/kg, p.o.), Normal Group 4 was untreated.

Measurement of Hemodynamic Parameters

Diabetic Cardiopathy is directly related with change in Haemodynamic parameters. There is decrease in diastolic and systolic function. Therefore the haemodynamic measurement would be the best parameter to describe the diabetic cardiopathy. The haemodynamic parameters measured were: Blood Pressure (Mean Arterial Pressure, Systolic Pressure, Diastolic Pressure) and Cardiac Cycle (RR intervals, QRS intervals, Heart Rate, QTc intervals).[10]

Measurement of ECG

Cardiac cycle parameters i.e. ECG was measured for all four group animals. Animal was anaesthetized by giving Urethane 1.25 gm/kg intra-peritoneal for long induction. After anaesthesia animal was kept on its back i.e. on posterior side. The electrode probes provided by PowerLab Data Acquisition system were attached to the paw of animal. Negative probe was attached to the right front paw and positive probe to the left rear paw, and neutral probe was attached to the right rear paw. Then the PowerLab Data Acquisition system put on and ECG was recorded. This procedure was followed for all the animals for the entire groups. The ECG graph was then analysed for different ECG parameter such as RR intervals, QRS wave, Heart Rate, QTc intervals.

Measurement of Blood Pressure

After anaesthetized with Urethane 1.25 mg/kg intra-peritoneal animal was kept on its back i.e. on posterior side. The carotid artery was cannulated by giving small incision at right neck position. The cannulated artery was then attached to pressure valve provided by IWOX Data Acquisition system for blood pressure measurement. Recorded blood pressure then analysed for different parameters.

RESULTS

Blood Pressure Measurement

Blood pressure parameters are decreased (see Table 1) in the diabetic group compared to normal group shows that the functionality of heart in diabetic group is decreased.

The graphs of Mean Arterial Pressure (MAP), Systolic pressure, and Diastolic pressure are shown in Figure 1.

The treatment group i.e. group treated with ethyl acetate extract of black tea 100mg/kg and 50mg/kg show good improvement in blood pressure parameters.

MAP in normal control group is 102.90 ±1.24, while in DB control it is decreased to 86.43 ±6.1. When the diabetic animals are treated with ethyl acetate extract of black tea, MAP is brought back to normal i.e. 104.51 ±1.3 and 114.75 ±0.57 with 100mg/kg and 50 mg/kg.

The similar situation is observed for Systolic pressure and Diastolic pressure, they also back to normal level when Diabetic animal were treated with Black Tea extract.

Cardiac Cycle Measurement

Electrocardiogram describes the electrical activity of heart. By examining the pattern of waves and the time interval between cycle and parts of cycles, information about the state of myocardium and the cardiac conduction system is obtained.

Here also, the cardiac cycle parameters are compared between different groups. The decreased in Heart Rate in diabetic group shows the abnormal cardiac cycle. The group treated with ethyl acetate extract black tea show well improvement in cardiac cycle parameters. The comparison between different groups for RR intervals, Heart Rate, and QRS intervals are shown in Figure 2, 3, and 4.

Heart rate in normal group is 356.94 ±26.99, while in DB control it is decreased to 216.73 ±35.37, when the diabetic animal were treated with ethyl acetate extract of black tea, Heart Rate is brought back to normal i.e. 344.67 ±8.73 and 366.10 ±15.22 with 100mg/kg and 50 mg/kg. The similar thing is observed with RR intervals, QRS intervals and QT interval.

DISCUSSION

DM amplifies the risk for vascular events; this is why DM is considered a CHD equivalent. There is an increasing interest in MMPs because evidence supports a role for these enzymes in the atherosclerotic process. Although DM accelerates atherosclerosis, there is limited research concerning the role of MMPs. These enzymes are also responsible for macrovascular and microvascular complications.[4,5]

In our study we evaluated the effect of Theaflavin present in the Black Tea on cardiovascular complications of diabetes. The effect of Black tea is evaluated on Cardiac Haemodynamic parameters. Measured values and plotted graphs in result shows that ethyl acetate extract of black tea has normalised the haemodynamic parameters which was deviated in diabetic animals.

The presence of flavonoid content in ethyl acetate extract of Black tea is clearly identified by the TLC method. Theaflavins, as Flavonoids, are principal components present in Black Tea. These extract was found to be safe up to 2000mg/kg dose when given orally, according to OECD AOT 425 guidelines.

The MAP which is 102 ±1.24 mmHg in normal group is decreased to 86.43 ±6.17mmHg. It shows that heart's ability to maintain normal blood pressure is decreased in diabetic conditions. When diabetic rats were treated with ethyl acetate extract of Black tea MAP is come back to normal levels i.e. 104 ±51mmHg and 114.75 ±0.57mmHg with 100mg/kg and 50mg/kg dose of extract

respectively (Table 1). The same phenomenon is observed with systolic pressure and diastolic pressure parameters of blood pressure. The RR interval which is 0.168 ±0.012 s in normal group is increased to 0.27 ±0.048 s. This increase in RR interval clearly indicates that decrease in heart rate, and this can be seen by viewing Table 2 This phenomenon specifies that, in diabetic rats the ability of heart to pump with regular intervals is reduced up to certain levels.

When diabetic rats were treated with ethyl acetate extract of black tea the RR interval along with heart rate and QRS interval have been also normalised. The RR interval which was 0.278 ±0.048 in diabetic group is normalised in Treatment group i.e. 0.178 ±0.001 and 0.168 ±0.010 as compared to normal group 0.168 ±0.012.

This effect of ethyl acetate extract of Black tea on haemodynamic parameters of Diabetic heart is not dose dependent, because the diverse effect of two different doses i.e. 100mh/kg and 50mg/kg cannot be clearly define, this can be seen from the Tables and Graphs plotted in Result.

These effects of Black tea on Diabetic heart are due to the presence of Theaflavins in it. These Theaflavins are inhibitors of MMP-2 and MMP-9 enzymes[8]. These enzymes are reportedly causable factors for many of the Diabetic Complications, which are up-regulated in Diabetes.

Further experiments are needed on complete extraction of pure Theaflavins and identification of specific Theaflavin molecule for inhibitory effect on MMP enzymes.

CONCLUSION

The effect of EA extract of Black tea on Cardiac Complications of Diabetes is well studied. The involvement of MMP-2 and MMP-9 enzymes in Diabetic Complications was determined by several scientists well before. The inhibitory effect of Theaflavins on Matrix Metalloprotienases was studied by some scientist on cancerous tumours.

The dose independent effect of ethyl acetate extract of Black tea is evaluated. This extract is able to reduce the Cardiac Complications of STZ Diabetic Rats. The presence of Theaflavins (Flavonoids) in EA extract of Black Tea is well determined through TLC method.

So, it can be concluded that Theaflavins have inhibitory effect on MMP enzymes responsible for many of the Diabetic Complications at some extent. But, there is a need for further study on identification and purification of specific Theaflavin responsible for the inhibitory effect on MMP enzymes responsible for Diabetic Complications.

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Table I: Measured Blood Pressure Parameters for the entire group

Animal Group	Mean Arterial Pressure average (mmHg)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
Normal	102.90 ±1.24	112.41 ±5.53	95.551 ±2.18
DB Control	86.43 ±6.17	95.114 ±5.60	75.510 ±6.84
100mg/kg	104.51 ±1.35*	115.190 ±2.89*	96.781 ±1.27*
50mg/kg	114.75 ±0.57*	126.299 ±3.03*	96.800 ±0.95*

* p<0.05

Table II: Measured Cardiac Cycle parameters for all the Groups

Animal Group	Cardiac Cycle Parameter			
	RR Interval (s)	Heart Rate (BPM)	QRS Interval (s)	QT Interval (s)
Normal	0.168 ±0.012	358.94 ±26.99	0.017 ±0.003	0.074 ±0.01
DB Group	0.278 ±0.008	219.73 ±25.37	0.018 ±0.002	0.078 ±0.015
DB + 50 mg/kg	0.178 ±0.001*	344.67 ±8.73*	0.013 ±0.002	0.041 ±0.036
DB + 100 mg/kg	0.168 ±0.010*	366.10 ±15.22*	0.014 ±0.002	0.030 ±0.005

*p < 0.05

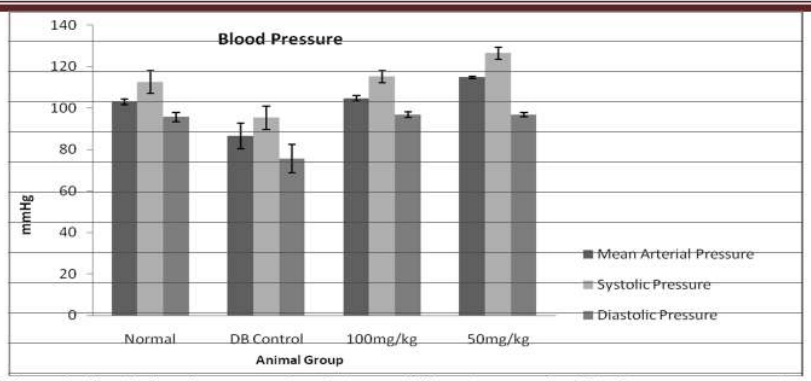


Figure I: Graph showing comparison between different groups for Blood pressure parameters

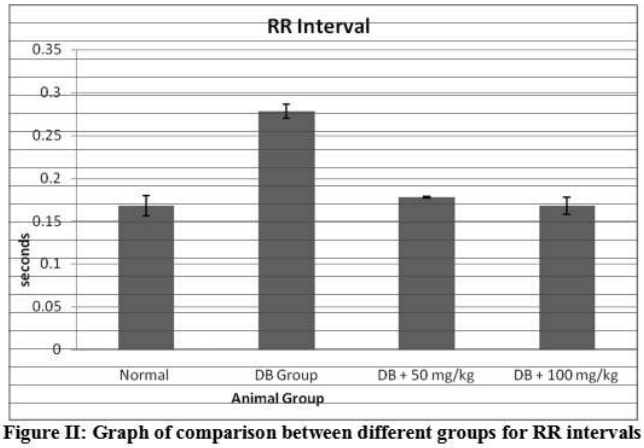


Figure II: Graph of comparison between different groups for RR intervals

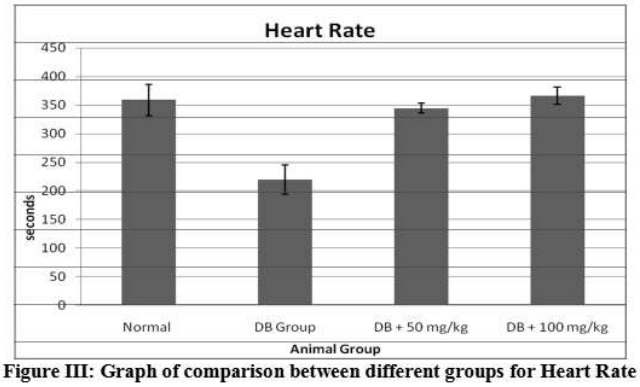


Figure III: Graph of comparison between different groups for Heart Rate

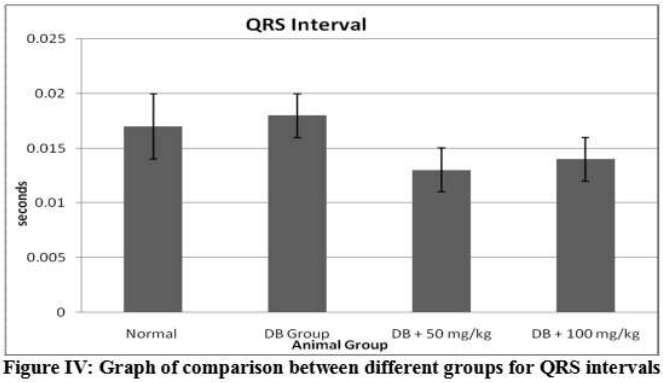


Figure IV: Graph of comparison between different groups for QRS intervals

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