BIOCHEMICAL STUDIES ON THE EFFECT OF PHENOLIC COMPOUNDS EXTRACTED FROM *Myrtus communis* IN DIABETIC RATS

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ABSTRACT

Phenolic compounds , extracted from the leaves of M.communis *were administered to streptozotocin induced diabetic rats @ 400mg/ kg body weight and 800mg/ kg body weight. Biochemical estimations namely serum glucose, cholesterol, triglycerides , HDL, LDL, AST , ALT, BUN, creatinine, total proteins, albumin and globulin were carried out. Rats received 800mg of extract showed marked antihyperglycemic response while rats received 400 mg showed only moderate response. In addition, the values of other biochemical parameters in diabetic rats received 800 mg were similar to that of control rats.*

Of various plants, *Myrtus communis* (commonly known as myrtle) which is grown abundantly throughout the borders of Mediterr

anean has been widely reported for its antihyperglycemic properties (Elfellah *et al*.,1984 and Aylin sepici *et al*., 2004). However, little attention has been paid to the therapeutic use of this evergreen shrub. There are different types of chemical agents used to produce diabetes mellitus in experimental animals. Streptozotocin injection is a well established means of inducing diabetes in the animal model by the destruction of beta cells of the pancreas (Hardman *et al*., 2001)

The present work was undertaken to evaluate the antihyperglycemic effects of phenolic compounds extracted from the leaves of *M.communis.*

MATERIALS AND METHODS

The method described by Elfellah *et al*., (1984) was adopted for isolation and quantification of the extract containing phenolic compounds from the leaves of *M. communis.* Male albino Wistar rats weighing 200 to 250 grams were chosen for the present study. All the animals were maintained as per the recommended standards (NRC, 1996). They were housed in polypropylene cages and fed on standard pellet diet with water given adlibitum. Streptozotocin was purchased from Sigma Chemicals, USA. Biochemical kits and other reagents used were purchased from Randox, England.

Extract of phenolic compounds was dissolved in distilled water and administered orally twice daily through intragastric tube at the dose rates of 400mg and 800mg / kg body weight for 28 days.

The rats were injected intraperitoneally with streptozotocin, freshly dissolved in 0.01 rnol/L citrate buffer (pH 4.5) at a dose rate of 65 mg/ kg body weight as a single dose for induction of diabetes. Animals which had reached a steady state of hyperglycemia after 10 to 14 days were chosen for further studies.

A total number of 36 rats were used for the present study. They were divided into 6 groups each comprising 6 numbers. The total period of our present study was of 28 days duration.

Group 1: Non-diabetic rats received no treatment.
Group 2: Diabetic rats received no treatment.
Group 3: Diabetic rats received glibenclamide @ 5mg/ kg body weight
Group4: Non-diabetic rats received phenolic compound @ 800mg/kg body weight
Group 5: Diabetic rats received phenolic compound @ weight ., 400mg/kg bodyweight
Group 6: Diabetic rats received extract of phenolic compound @ 800mg / kg body weight

Blood samples were collected once in a week for 28 days by intra orbital sinus method using capillary tubes after partly anesthetizing the rats. Blood glucose was determined by GOD-POD method (Trinder, 1969), serum total cholesterol was determined by CHOD-PAP method (Allain *et al*., 1974) and serum triglycerides concentration was determined by GPO-PAP method (Kaplan and Lavemel, 1983). Serum HDL and LDL concentrations were determined by the method described by Rifai and Warnick (1994). Blood urea was determined by diacetyl monoxine method (Wybenga, 1971) and serum creatinine was determined by alkaline picrate method (Toro and Ackermann, 1975). AST (GOT) and ALT (GPT) were determined by Reitman and Frankel (1957) method. Total proteins, albumin and globulin were determined by modified Biuret and Dumas method (Varley, 1980).

RESULTS AND DISCUSSION

The blood glucose levels recorded in all the six groups are shown in Table-1. Diabetic control rats recorded a marked increase in blood glucose levels throughout the period of study when compared with normal. Similar elevated values of blood glucose were also recorded in alloxan induced diabetic rats by Ramalingam and Leelavinothan (2005). Diabetic rats treated with 800 mg of extract produced stastistically significant ($P<0.05$) antihyperglymemic effect showing values towards normal at the end of the trial while rats treated with 400 mg could produce only moderate response. Elfellah *et al*(1984) also reported antihyperglycemic effect in streptozotocin induced diabetic mice treated with extract of *M.communis*. Rats treated with glibenclamide showed values towards normal as in non-diabetic control rats. Rats received only phenolic compound showed values as recorded in normal rats.

The values of total cholesterol and triglycerides recorded are shown in Table 1. Diabetic rats showed a progressive increase in total cholesterol and triglycerides levels when compared to the normal rats. Diabetic rats treated with 800 mg of phenolic compounds and rats treated with glybenclamide alone showed a progressive decline in level towards normal while diabetic rats treated with 400 mg showed only moderate decline. Our findings of elevated values of cholesterol and triglycerides recorded in diabetic rats was indicative of abnormal fat metabolism that is commonly encountered in diabetes mellitus. In diabetic patients, glucose metabolism progressively diminishes and the metabolism rapidly turns to utilization of fatty acids for energy purposes
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The HDL and LDL cholesterol values recorded in all the six groups are shown in Table-1. Diabetic rats showed a progressive decrease in HDL levels and a corresponding increase in LDL levels when compared to the normal rats indicating abnormal fat metabolism encountered in diabetes mellitus. Diabetic rats treated with 800 mg of extract as well as diabetic rats treated with glibenclamide alone maintained their values within normal limits. However rats treated with 400 mg showed only a moderate response.

The AST and ALT values recorded in all the six groups are shown in Table-2. Diabetic control rats recorded a progressive increase in both the enzymatic activities when compared to the normal rats indicating hepatic damage. Diabetic rats treated with 800 mg of phenolic compounds showed values towards normal as noticed in rats treated with glibenclamide. Rats treated with 400 mg showed a moderate increase in both the enzyme activities.

The BUN and creatinine values recorded in all the six groups are shown in Table-2. Diabetic control rats recorded a progressive increase in both BUN and creatinine levels when compared to to the normal rats indicating renal damage.Diabetic rats treated with 800 mg of phenolic compounds showed values towards normal as noticed in rats treated with glybenclamide. Rats treated with 400 mg showed a moderate decline in levels towards normal.

The values of total proteins , albumin and globulin recorded in all the six groups are shown in Table-3. Diabetic rats though recorded a normal level of total proteins showed a drastic decrease in albumin and a corresponding increase in globulin when compared to the normal rats indicating hepatic damage. Rats treated with 800 mg of extract showed values towards normal as observed in rats treated with glibenclamide. However rats treated with 400mg showed only moderate response.Non diabetic rats treated with phenolic compounds @ 800 mg showed no biochemical alterations indicating its non toxicity.

CONCLUSION

Phenolic compounds when administered @800mg/kg body weight could produce a remarkable antihyperglycemic effect than administered @400mg. It is suggested that further isolation of active ingredient of phenolic compound present in *M.communis* that produces antihyperglycemic effect should be carried out.

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Table-1 Serum Glucose, Cholesterol, Triglycerides, HDL and LDL levels' in normal, diabetic and treated rats

Days	1					7					14					21					28				
	G	C	TG	HDL	LDL	G	C	TG	HDL	LDL	G	C	TG	HDL	LDL	G	C	TG	HDL	LDL	G	C	TG	HDL	LDL
	117[a] ± 0.42	68.7[a] ± 0.76	63.5[a] ± 0.76	*31.5*[a] ± 1.25	31.0[a] ± 0.88	111[a] ± 0.76	61.67[a] ± 0.75	65.67[a] ± 1.37	*325*[a] ± 0.76	31.6[a] ± 0.94	113[a] ± 0.57	75.83[a] ± 0.94'	62.66[a] ± 1.14	32.5[a] ± 0.76	*31.1*[a] ± 0.94	115[a] 1 0.59	65[a] ± 0.89	68[a] ± 0.76	331[a] 0.96	31.3[a] ± 1.19	106[a] ± 1.13	68.67[a] 1.01	66[a] ± 0.76	32.5[a]± 0.66	31.8[a] ± 1.27
Gr2	380[b] ± 0.70	[b]67.3± 1.05	70.2[b] ± 0.94	32.8[b] 0.946	34[b] ± 0.66	460[b] ± 0.87	76.17[b] ± 1.65	74.83[b] ± 1.34	28.5[b] ± 0.766	36.05[b] 0.85	480[b] ± 0.78"	91.5[b] ± 1.66	75.33[b] 0.87	21.5[b] 0.76	67[b] ± 0.85	472[b] ± 0.57	110.8 3 [b] ± 1.07	76.66[b] ± 1.04	17.16[b] ± 0.59	84.3[b] ± 1.19	495[b] ± 0.41	126.6[b] ± 1.42	97.8[b] ± 0.47	116.5[b] 0.98	66.67[b] ± 0.1
Gr 3	381[e] ± 0.76	90.20[c] ± 0.94	70.66[b] ± 0.876	33f[b] 0.856	34[b] ± 0.996	161[d] ± 0.76	82.17[c] ± 0.94	68.83[c] ± 1	*33.33*[c] ± 0.87	33.33[c] ± 0.87	129[e] ± 0.76	77.83[c] ± 1.13	67.83[c] ± 0.94	33.5[a] ± 0.88	331[c] 1.17	149[d] ± 0.76	76[c] ± 1.06	66.51 1.05	33.5[c] ± 0.76	36.12[c] ± 1	143[c] ± 0.76	75.66[c] ± 1.04	63.83[c] ± 1	32.33[c] ± 0.75	33[c] ± 0.98
G r4	121[f] ± 0.78	68.8[d] ± 0.94	60.5[c] ± 0.76	34.3[a] ± 0.85	32[c] ± 0.76	120[e] ± 0.76	71.5[d] ± 0.76	59.4[d] ± 0.79	35[d] ± 1.31	32.67[c] ± 0.71	121[f] ± 0.76	66.5[d] ± 1.71°	59.5[d] ± 0.77	35.33[d] ± 1.22	31.83[d] ± 0.94	113[e] ± 0.76	66.5[d] ± 1.17	58.66[d] ± 0.86	33[d] ± 0.91	31.66[d] 0.98	117[f] ± 0.87	65.1[d] ± 1.80	58.4[d] ± 0.76	31.1[d] ± 0.70	32.33[d] ± 0.76
Gr5	391[c] ± 0.60	111.5[e] 1.17	87.4[d] ± 0.76	32.8[b] ± 0.946	33[d] ± 0.91	251[c] ± 0.87	95.83[e] ± 1.39	86.8[e] ± 0.59	32.83[d] ± 0.59	*33*[d] ± 0.96	240[c] ± 0.76	88.66[e] ± 1.37	84[e] ± 1.56	32.5[e] ± 1.25	56.6[d] ± 0.71	236[c] ± 0.76	86.67[e] ± 0.98	80.33[d] 1 0.96	28.1[a] ± 0.94	53.83[d] 1.27	230[c] ± 0.76	78.20[e] ± 0.94	74.83[e] ± 0.76	23.5[a] ± 0.84	46.33[d] ±O.87
Gr 6	421[d] ± 0.76	78.3[f] ± 1.35	85.33[e] ± 0.88	31.7[c] ± *1.11*	34[b] ± 88	160[d] ± 0.70	7Z67[b] ± 1.32	81.5[f] ± 0.98	34[d] ± 1.06	34[d] ± 0.96	121[d] ± 0.57	77[c] ± 0.72'	72.5[f] ± 0.76	32.83[c] ± 0.94	34.6[e] ± 0.57	149[d] ± 0.59	731[f] 0.96	70.67[e] ± 0.87	32.8[a] 0.95	34.21[e] 1.07	131[d] ± 0.98	73[f] ± 1.69	62.5[e] ± 0.76	31.16[f] ± 0.86	34.6[e] ± 0.49

Mean values with same super script do not differ significantly ($p > 0.05$)

Table -2 BUN, CREATININE, ALT AND AST levels in normal, diabetic and treated rats

	1				7				14				21				28			
Days	**BUN**	**Cr**	**ALT**	**AST**	**BUN**	**Cr**	**ALT**	**AST**	**BUN**	**Cr**	**ALT**	**AST**	**BUN**	**Cr**	**ALT**	**AST**	**BUN**	**Cr**	**ALT**	**AST**
Gr1	11.2 ^{a}±	1.1 ^{a} ±	17 ^{a} ±	41.5 ^{a}±	11.5 ^{a}±	0.98 ^{a}±	17.3 ^{a}±	46.17^{a}±	11.17^{a}±	0.81 ^{a}±	17.5 ^{a}±	41.5 ^{a}±	12 ^{a}±	0.78 ^{a}±	18.66^{a}±	41.16^{a}±	12.3 ^{a}±	0.88 ^{a}±	17.5 ^{a}±	47 ^{a}±
	0.48	0.04	0.57	0.43	0.43	0.04	0.42	0.59	0.48	O.O4	0.43	0.56	0.57	0.03	0.42	0.59	0.66	0.06	0.46	0.72
Gr2	11.2 ^{a}±	1.1 ^{a} ±	47.8 ^{b} ±	81.5^{b}±	16.17^{b}±	0.98^{b}±	52^{b}±	81.67^{b}±	22.5^{b}±	1.08^{b}±	56^{b}±	82.83^{b}±	23.5^{b}±	1.33^{b}±	57.5^{b}±	85.33^{b}±	26^{b}±	1.81^{b}±	62.17^{b}±	85.5^{b}±
	0.48	0.04	0.48	0.76	0.60	0.07	L. 15	0.66	0.76	0.04	0.73	0.47	0.76	O.O3	0.76	0.42	0.36	0.05	0.94	0.47
Gr3	11.3 ^{c}±	1.05 ^{a} ±	63 ^{d} ±	80.3^{d}±	11.66^{a}±	1.03 ^{a}±	47^{d}±	77 ^{c}. ±	12.5 ^{c}±	1.18^{b}±	33.5^{e}±	73.17^{c}±	12 ^{a}±	10.03^{c}±	37.07^{e}±	70.33^{c}±	10.8 ^{c}±	1 ^{c}±	33 ^{c}±	54 ^{c}±
	0.66	0.04	0.96	0.42	0.49	0.05	0.58	0.59	0.76	0.03	0.76	0.59	0.60	0.03	0.59	0.42	0.57	0.03	0.96	0.57
Gr4	13.2 ^{b}±	1.03 ^{a} ±	17 ^{a} ±	45.7±	12.3 ^{c}±	1.01 ^{a}±	16.17 ^{a}	42.83^{d}±	12 ^{c}±	1.05^{b}±	17.33^{a}±	40.83^{a}±	11.3 ^{c}±	1.2 ^{c}±	16^{f}±	37.83^{d}±	11.67^{d}±	1.08^{d}±	17.16^{a}±	37.67^{d}±
	0.6	0.03	0.57	0.71	0.66	0.04	0.59	0.94	0.57	0.04	0.66	0.59	0.49	0.02	0.57	0.78	0.66	0.02	0.59	0.66
Gr 5	13.86 ^{b}	1.12 ^{a} ±	36.5 ^{c} ±	81.16±	12.3 ^{c}±	1.2 ^{c}±	36.5 ^{c}±	79.3^{f}±	12 ^{c}±	1.01^{b}±	53.67^{c}±	79.5^{d}±	12.5 ^{a}±	0.71 ^{a}±	61.66^{c}±	77.5^{e}±	12.83^{e}±	1.68^{e}±	55.66^{d}±	72.5^{e}±
	± 0.6	0.05	0.76	0.65	0.88	0.05	0.76	0.88	0.57	0.05	0.83	0.98	0.42	O.O5	0.49	0.88	0.47	0.04	1.25	0.76
Gr 6	14.8 ^{d} ±	0.78 ^{b} ±	47^{b}±	81.3±	13 ^{c}±	0.8 ^{a}±	37 ^{c}±	78 ^{c}±	12.5 ^{c}±	1.2 ^{c}±	37^{d}±	71^{e}±	11.1 ^{c}±	1.01 ^{c}±	63^{d}±	62.5^{f}±	11.8^{d}±	1.08^{c}±	33 ^{c}±	51.83^{b}±
	0.8	0.051	0.57	0.49	0.57	0.03	0.73	0.58	0.57	0.03	0.72	0.51	0.47	0.04	0.57	0.76	0.59	0.04	0.96	0.59

Mean values with same super script do not differ significantly ($p > 0.05$)

Table- 3 Total Proteins, Albumin and Globulin (g %) in normal, diabetic and treated rats

Days	1			7			14			21			28		
	TP	**AL**	**GL**	**TP**	**AL**	**GL**	**TP**	**AL**	**GL**	**TP**	**AL**	**GL**	**TP**	**AL**	**GL**
Gr 1	$6.18^{a} \pm 0.07$	$4.18^{a} \pm 0.08$	$2^{a} \pm 0.04$	$6.2^{a} \pm 0.09$	$4.1^{a} \pm 0.58$	$2.1^{a} \pm 0.06$	$6.25^{a} \pm 0.08$	$4.13^{a} \pm 0.04$	$2.12^{a} \pm 0.05$	$6.01^{a} \pm 0.13$	$4.13^{a} \pm 0.06$	$1.98^{a} \pm 0.07$	$6.0^{b} \pm 0.07$	$4.06^{a} \pm 0.05$	$2.06^{a} \pm 0.06$
Gr 2	$6.23^{a} \pm 0.10$	$3.15^{b} \pm 0.04$	$3.08^{b} \pm 0.08$	$6.2^{a} \pm 0.05$	$3.1^{b} \pm 0.06$	$3.1^{b} \pm 0.05$	$6.4^{b} \pm 0.10$	$3.05^{b} \pm 0.04$	$3.35^{b} \pm 0.07$	$6.7^{b} \pm 0.09$	$3.05^{b} \pm 0.04$	$3.65^{b} \pm 0.07$	$5.48^{b} \pm 0.08$	$2.0^{b} \pm 0.07$	$3.48^{b} \pm 0.04$
Gr 3	$5.3^{b} \pm 0.09$	$3.08^{b} \pm 0.05$	$2.22^{c} \pm 0.06$	$5.45^{c} \pm 0.09$	$3.3^{b} \pm 0.06$	$2.15^{a} \pm 0.06$	$5.73^{c} \pm 0.10$	$3.6^{c} \pm 0.06$	$2.13^{a} \pm 0.06$	$5.55^{c} \pm 0.10$	$3.8^{c} \pm 0.06$	$1.73^{e} \pm 0.06$	$5.75^{a} \pm 0.06$	$3.9^{a} \pm 0.04$	$1.75^{c} \pm 0.06$
Gr 4	$5.92^{c} \pm 0.07$	$3.98^{b} \pm 0.05$	$1.94^{a} \pm 0.08$	$5.86^{b} \pm 0.06$	$3.93^{a} \pm 0.06$	$1.393^{a} \pm 0.05$	$6.15^{d} \pm 0.10$	$3.95^{d} \pm 0.07$	$2.2^{a} \pm 0.08$	$6.7^{d} \pm 0.09$	$3.98^{c} \pm 0.07$	$2.73^{c} \pm 0.06$	$5.9^{d} \pm 0.06$	$3.96^{a} \pm 0.06$	$1.94^{a} \pm 0.06$
Gr 5	$5.07^{b} \pm 0.09$	$3.05^{b} \pm 0.04$	$2.02^{a} \pm 0.06$	$5.25^{c} \pm$ 0.1O	$3.08^{b} \pm 0.06$	$2.15^{a} \pm 0.07$	$5.17^{d} \pm 0.09$	$3.12^{b} \pm 0.05$	$2.03^{a} \pm 0.06$	$5.38^{c} \pm 0.06$	$3.3^{d} \pm 0.04$	$2.08^{d} \pm 0.06$	$5.46^{b} \pm 0.07$	$3.4^{c} \pm 0.04$	$2.06^{a} \pm 0.06$
Gr 6	$5.7^{c} \pm 0.08$	$3.07^{b} \pm 0.03$	$2.63^{d} \pm 0.09$	$5.73^{b} \pm 0.10$	$3.15^{b} \pm 0.07$	$2.58^{b} \pm 0.07$	$6.12^{c} \pm 0.06$	$3.87^{d} \pm 0.04$	$2.25^{a} \pm 0.07$	$6.16^{a} \pm 0.06$	$3.9^{c} \pm 0.06$	$2.26^{d} \pm 0.04$	$6.25^{f} \pm 0.06$	$3.93^{a} \pm 0.06$	$2.31^{b} \pm 0.05$

Mean values with same super script do not differ significantly ($p > 0.05$)