IJBPAS, January, 2017, 6(1): 108-124

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTI-DIABETIC AND ANTI-HYPERLIPIDEMIC ACTION OF AQUEOUS ETHANOLIC EXTRACTS OF *MENTHA SPICATA* (LEAVES), *PLUMERIA ALBA* (LEAVES) AND *NYMPHAEA ALBA* (FLOWERS AND RHIZOMES)

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Received 28th Aug. 2016; Revised 4th Oct. 2016; Accepted 6th Nov. 2016; Available online 1st Jan. 2017

ABSTRACT

Objective: Glucose being the fuel of body and brain needs the availability of insulin around the clock. Any impairment in the functionality of insulin leads to long lasting metabolic disorder called diabetes mellitus which can be described as a group of disorders i.e. hyperlipidemia, cardiomyopathy, nephritis, fatty liver and weight loss. Patients need such therapy which maintains the blood glucose level according to the needs of body. None of the commercially available anti-diabetic medicines are hundred percent efficacious and free of side effects. This study was conducted to explore the beneficial effects of three indigenous plants in lowering glucose and lipid levels. **Methodology:** In this study aqueous ethanolic extracts of *Mentha spicata* (leaves), *Plumeria alba* (leaves) and *Nymphaea alba* (flowers and rhizomes) were evaluated individually in 200mg/kg and 400mg/kg doses against alloxan (130mg/kg i.p.) induced hyperglycemic rats. Blood glucose levels were determined periodically on 1st, 3rd, 5th, 7th, 9th, 11th and 13th day of study and at 15th day, animals were sacrificed to get blood for determination of serum cholesterol, triglycerides, LDL, HDL and VLDL. **Results:** The results indicated that the crude extracts reduced blood glucose level, serum cholesterol, triglycerides, LDL and VLDL while body weights and HDL levels were increased. Two way ANOVA test was applied which declared that extracts in dose of 400mg/kg produced significant ($P<0.001$) results. **Conclusion:** Based on results it was concluded that the crude extracts

possess not only anti-diabetic activity but also show marked anti-hyperlipidemic potential. However, extensive studies are needed to be conducted to identify the active moieties of extracts and to explore the molecular basis of their mechanism of action.

Keywords: Alloxan, Diabetes Mellitus, Reactive Oxygen Species, Hyperlipidemia, Hyperglycemia and Anti-oxidant

INTRODUCTION

A significant numbers of deaths have been reported every year due to diabetes mellitus. Reactive oxygen species produced in body may contribute to increased risks of neuropathy, stroke, vascular diseases and myocardial infarction like complications in diabetic patients **[1]**. Peroxidation of lipids is commenced by free radicals which further promote the glycation of several proteins **[2]** inactivate enzymes and produce structural and functional alterations in membranes. This results in long lasting hyperglycemia along with other disorders like hyperlipidemia **[3]**. The substance that scavenges the free-radicals has great potential in relieving those adverse processes in the body **[4, 5]** and addition of anti-oxidants and associated supplements in anti-diabetic therapy prevents the further worsening of diabetes mellitus **[6]**. Studies have shown that plants and herbs enriched with vitamins (C and E), tannins and flavonoids prevent the oxidative damage of β cells by inhibition of certain hydrolytic and oxidative enzymes **[7]**. Patients need such therapy which maintains the blood glucose level according to the needs of body. None of the commercially available anti-diabetic medicines are hundred percent efficacious and free of side effects **[8]**. Currently trend is shifting towards the use of conventionally available methods of diabetic controls. Use of plants, herbs and natural substances having anti-oxidant potentials are preferred over all the things due to their toxic free multidimensional therapeutic effects **[9]** and a huge list of plants is available which have been investigated for their anti-diabetic actions **[10, 11]**. The main goal of this study was to investigate the anti-diabetic activity of aqueous ethanolic extracts of *Mentha spicata* (fresh leaves), *Plumeria alba* (fresh leaves) and *Nymphaea alba* (flowers and rhizomes) due to the fact that these plants are highly enriched with anti-oxidant species.

MATERIALS AND METHODS

Chemicals and equipments: Glucometer (Accu Chek), DPPH (Sigma), ascorbic acid (Sigma). glucometric strips (Accu Chek), diagnostic kits (for lipid profile, by Human

company), distilled water, ethanol, xylene, lidocain, paraffin, chloroform, glimepiride and alloxan monohydrate. All the chemicals of analytical grade were purchased from the Sigma. Glimepiride was donated by CCL Pharmaceuticals on special request. Lidocaine was purchased from local pharmacy. Digital electronic weighing balance, vortex mixer, rotary evaporator, spectrophotometer, centrifuge machine, merck microlab300, refrigerator and microscope.

Animals: White albino rats of either sexes weighing 150-200g were selected for research work. All the animals were provided by the animal house of the Riphah Institute of Pharmaceutical Science (Riphah International University Lahore). Animals were kept under standard temperature controlled and hygienic environment. The standard temperature (25±2°C) and humidity (45-55%) was maintained in animal house along with 12/12 hours light and dark cycle. Before initiation of work the rats were acclimatized for one week during which they were handled with care to make them friendly with environment. The animal had free access to their feed and plenty of water. The Ethical clearance for animal handling was obtained from the Research and Ethical Committee of Riphah Institute of Pharmaceutical Science (Riphah International University Lahore) with reference number RIU-01-06-15.

Plant material: The fresh leaves of *Mentha spicata* **(**Spearmint) and *Plumeria alba* and flowers and rhizomes of *Nymphaea alba* were collected in the month of November from the local fields of Lahore, Pakistan. Fresh plant materials were dried separately under shade at room temperature by changing their sides on filter paper twice daily to avoid fungal contamination. The dried materials were ground to coarse powder with grinder machine and soaked separately into 70:30% aqueous ethanolic solvent and obtained semi solid extracts of three plants after soaking, filtration and solvent evaporation processes.

Anti-oxidant Activity: The method of Yan was followed to investigate the scavenging activity of aqueous ethanolic extracts of *Mentha spicata* (fresh leaves), *Plumeria alba* (fresh leaves) and *Nymphaea alba* (flowers and rhizomes) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method **[12, 13]**. A 0.004% methanolic solution of DPPH was made by precisely weighing 0.004g of DPPH and putting it into volumetric flask. Then few ml of methanol were added into the flask, mixed well and finally made the volume 100ml with

methanol. Properly covered it with aluminum foil and kept the solution in dark place. This was labeled as stock solution of reagent. Then the concentration of ascorbic acid in distilled water was made. 0.025g of ascorbic acid was dissolved in 5ml of distilled water and labeled as standard stock solution. Then 0.025g of aqueous ethanolic extract of each plant was dissolved separately in 5ml methanol solution in the concentration of 5µg/µl and labeled as stock solution of plants. First the absorbance of methanol against reagent stock solution was measured at 517nm and it was considered as blank. Different concentrations of standard solution along with 200µl of each plant extract were taken in test tube. 2ml of reagent solution were added in each test tube and was incubated for 30 minutes at dark place for the completion of reaction. Then the absorbance of various concentrations and standard against the blank were measured at 517nm with UV spectrophotometer.

The percentage (%) of scavenging activity of the DPPH free radical was measured by using the following equation.

$$\frac{\text{Absorbance of blank - Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Phytochemical analysis: Crude extract of plant was analyzed for the estimation of saponins, carbohydrates, alkaloids, proteins, amino acids, glycosides, tannins, steroids, flavonoids and phenol **[14]**.

Pharmacological Studies:

Induction of diabetes: The saline solution of alloxan at the dose of 130mg/kg was administered to 12 hour fasted rats by Intra peritoneal route. The 10% glucose solution and free access to food was given to rats after 30 mints to avoid fetal hypoglycemia. The blood glucose level was checked after 72 hours. Rats with the blood glucose level more the150 were considered as diabetic. They were separated and regrouped for further studies.

Anti-diabetic activity: For estimation of anti-diabetic activity of a selected plant extracts, the albino rats of either sexes weighing 150-200g were divided into equal nine groups with six rats in each. Group-1 received Normal Saline 5ml/kg/p.o. daily. Group-2 received alloxan monohydrate 130mg/kg/i.p in normal saline solution once at the time of induction. Group-3 received saline alloxan solution 130mg/kg i.p once, followed by the treatment of glimepiride 4mg/kg/p.o for 14 days. Groups-4-9 received saline alloxan solution 130mg/kg i.p. once, followed by administration of crude extracts (200mg /kg/p.o and 400mg/kg/p.o) respectively, for 14 days. Blood glucose of rats was checked at zero, 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 11^{th}

and 13th day at different time interval i.e. (Zero, half, one, two, four, six and eight hours)**.**

Blood sampling for biochemical analysis: At 15th day, the animals were anesthetized with Chloroform and scarified to expose the heart, took 3ml of blood from each rat, directly from the heart with cardiac puncture through sterile disposable syringe and poured the blood in the centrifuge test tubes which were kept at room temperature for 30 minutes. The blood was centrifuged at 2500 rpm for 15 minutes. The serum was collected from centrifuge test tubes into appendrof tubes with the help of micropipette. Serum biochemical parameters were estimated by using diagnostic kits i.e. (cholesterol, triglyceride, LDL and HDL) by analyzing the sample, blank, reagent and standard in Merck microlab.

Toxicity Studies: Swiss albino mice (25-30g) were divided in three groups and Groups-I-III were given aqueous ethanolic extract of *Mentha spicata, Plumeria alba* and *Nymphaea alba* respectively, in different doses starting from 500mg/kg/p.o in increasing order by doubling the dose after every 24 hour and the dose was determined at which 50% of the animal started to die and the LD_{50} values were determined **[15]**.

RESULTS

Aqueous ethanolic extracts of all three plants were made and their %age yields were calculated as shown in table 1.

Phytochemical Study: Phytochemical screening of aqueous ethanolic extract of *Mentha Spicata, Pulmeria alba* and *Nymphaea alba* showed the presence of various secondary metabolites which are given below in table 2.

Solubility study

Solubility of all three plant extracts were checked in different solvents in the ratio of 1mg extract into 1ml solvent (1:1), 5mg into 5ml (5:5) and 10mg into 10ml (10:10) for making the oral preparation for animal and shown in table 3. All three plants were well soluble in distilled water and DMSO while *Plumeria alba* was insoluble in ethanol and normal saline and *Mentha spicata* was insoluble in ethanol only.

Anti-oxidant Study: Anti-oxidant studies indicated that %age scavenging of different free radicals is increased significantly ($P \leq 0.001$) by the use of all three plant extracts in comparison to standard (Ascorbic acid). As expressed in table 4, at low concentrations *Nymphaea alba* showed better results ($P \leq 0.001$) in comparison of other two plant extracts but with increasing

concentrations the scavenging activity by other plants is also increased.

Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats: Experimental studies showed the blood glucose levels (mg/dl) in normal control, disease control, standard control and experimental control (Ms200mg/kg, Ms400mg/kg, Pa200mg/kg, Pa400mg/kg, Na200mg/kg and Na400mg/kg) groups at different time intervals (0-hr, 2-hrs, 4-hrs, 6-hrs and 8-hrs) on 1st, 3rd, 5th, 7th, 9th, 11th and 13th day of experimental study, also shown in tables. The results indicated that there was marked elevation in blood glucose level in disease control group which was administered with alloxan monohydrate 130mg/kg/i.p. Normal control group which was given normal diet indicated the normal blood glucose levels (mg/dl). The results indicated that the standard control group which was administered with Glimepiride (4mg/kg/p.o) significantly ($P<0.001$) reduced the blood glucose levels at various time intervals.

The tabular results indicated that the blood glucose levels of Ma200 and Ma400mg/kg at different time interval on 1st, 3rd, 5th, 7th, 9th, 11th and 13th day respectively, were significantly ($P<0.001$) less than that of disease control group. Ma400mg/kg reduced blood glucose level, more efficiently as compared to Ma200mg/kg. Ma200 and Ma400mg/kg produced the results with highest level of significance, having $P<0.001$.

Pa200 and Pa400mg/kg at different time intervals on 1st, 3rd, 5th, 7th, 9th, 11th and 13th day significantly reduced the blood glucose levels ($P<0.001$) as compared to disease control and normal control group. Pa400mg/kg showed marked reduction in blood glucose (mg/dl) as compared to Pa200mg/kg. Pa 200 and Pa 400mg/kg produced the results with highest level of significance, having $P< 0.001$ as compared to disease control group.

Blood glucose values for Na200 and Na400mg/kg at different time interval on 1st ,3rd ,5th ,7th ,9th, 11th and 13th day significantly ($P<0.001$) reduced the blood glucose levels as compared to disease control and normal control group. Na400mg/kg was shown to have more efficacies in reduction of blood glucose levels in albino rats as compared to Na200mg/kg.

Results indicated that hypoglycemic effect was not obtained immediately after administration of respective dose of standard and experimental extracts i.e. at zero time intervals irrespective of day of treatment.

Hypoglycemic effect appeared to occur usually at 4th to 6th hour after administration of dose and maximum effects were seen at 8th hour sampling in most of instances. It is also cleared that the effect of experimental extract doses remained only for half of the day and again blood glucose level was elevated as the effect of treatment vanished. If we individually describe the result, then at day 1st treatment, it is cleared that effects were obtained gradually with increasing time span i.e. minimum at 0-hr, increasing from 2-hrs to 6-hrs and maximum at 8-hrs. At this level, if we compare the effects of all the plant extracts with each other than it is cleared that Ms400mg/kg and Na400mg/kg have maximum blood glucose lowering effects. At day 3rd it is cleared that again effects were obtained gradually with increasing time span. Comparison of all treatment groups indicated that maximum beneficial effects were obtained by Ms400mg/kg and Na400mg/kg at 8th hour of treatment. At day 5th, 7th and 9th, again same pattern of results were obtained. At day 11th maximum effects were seen at 6th hour treatment while at 13th day the results were quite variable. Standard control and Ms200mg/kg, Ms400mg/kg, Pa200mg/kg and Pa400mg/kg followed the same above described pattern while Na200mg/kg and Na400mg/kg showed continuous ups and downs in blood glucose levels at different time intervals.Comparative studies depicted that maximum blood glucose lowering effects were obtained by Na400mg/kg and Ms400mg/kg after standard control drug which produced marked significant ($P<0.001$) effects. Details of all results are shown in table 5-11 and graphical representation is made in figure 1.

Effect of Aqueous Ethanolic Extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on Body Weights (g) in Albino Rats: The effect of standard drug and ethanolic extracts on body weight was also studied. The mean weights of individual group are shown in table 12 along with their SEM values. The weights of animals were determined before and after the treatment. The results indicated that the weights of the rats of normal control groups were elevated gradually during the study period. The weights of disease control group were reduced during the study period from 273.16±13.40g to 267.00±12.87g. Standard control drug had no any effect on body weights of rats. The study also indicated that Ma200mg/kg, Ma400mg/kg, Pa200mg/kg, Pa400mg/kg and Na200mg/kg had no any marked effect on body weights of the animals. The body weight remained almost same throughout the study period. However,

Na400mg/kg significantly (P<0.001) reduced the body weights at the end of study as shown in figure 2.

Effect of Aqueous Ethanolic Extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on Serum Cholesterol (gm/dl), Triglycerides (mg/dl), LDL (mg/dl), VLDL and HDL (mg/dl) Level in Albino Rats. At 15th day the animals were sacrificed by giving them chloroform and blood was taken by cardiac puncture. 3ml of blood was taken from each rat and it was allowed to get clot for half an hour. After that all the blood samples were centrifuged to get serum which was subjected to biochemical tests for the analysis of Serum Cholesterol (gm/dl), Triglycerides (mg/dl), LDL (mg/dl), VLDL and HDL (mg/dl) Levels. The results so obtained were tabulated in table 13 after application of one way ANOVA as statistical tool and also expressed graphically. Crude extracts of all three plants i.e Ma200mg/kg, Ma400mg/kg, Pa200mg/kg, Pa400mg/kg, Na200mg/kg and Na400mg/kg significantly (P<0.001) reduced the level of serum cholesterol, Triglycerides, LDL and VLDL levels but serum HDL levels were significantly (P<0.001) elevated in all these groups.

Table 1: %age yield of aqueous ethanolic extract of *Mentha spicata, Plumeria alba* and *Nymphaea alba.*

Extracts	Weight of dried powder	%age yield
Mentha spicata Extract (Ms)	500 g	30.55%
Plumeria alba Extract (Pa)	500 g	27.73%
Nymphaea alba Extract (Na)	500 g	35.19%

Table 2: Phytochemical analysis of aqueous ethanolic extract of *Mentha Spicata, Plumeria alb* and *Nymphaea alba.*

No	Test Name	*Mentha Spicata*	*Plumeria alba*	*Nymphaea alba*
1	Carbohydrates	Present	Absent	Present
2	Glycosides	Present	Present	Present
3	Alkaloids	Absent	Present	Present
4	Phenols & tannins	Absent	Absent	Present
5	Protein	Present	Absent	Absent
6	Flavonoids	Present	Present	Present
7	Terpenoids	Present	Present	Present
8	Steriods	Present	Present	Present
9	Saponins	Present	Absent	Present

Table 3: Stability studies of aqueous ethanolic extract of *Mentha spicata, Plumeria alba* and *Nympheae alba.*

	Distilled Water	Ethanol	Normal saline	DMSO
Mentha spicata Extract (Ms)	Yes	No	Yes	Yes
Plumeria alba Extract (Pa)	Yes	No	No	Yes
Nympheae alba Extract (Na)	Yes	Yes	Yes	Yes

Table 4: Percentage anti-oxidant activity of aqueous ethanolic extracts of *Mentha Spicata*(Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) by DPPH assay

		Percentage (%) Scavenging effect			
No	Concentrations	Ascorbic acid	*Mentha spicata*	*Plumeria alba*	*Nymphaea alba*
1	0.02µg/µl	62.66±1.45	75.00±2.88**	55.00±2.5^{ns}	47.66±1.45***
2	0.04µg/µl	72.66±1.45	61.33±0.88 *	70.00±1.15^{ns}	56.00±0.57 ***
3	0.08µg/µl	84.33±0.88	68.66±3.9 ***	64.00±2.08 ***	71.33±1.45 **
4	0.16µg/µl	85.00±2.88	68.33±0.88***	69.00±1.00***	73.33±1.20*
5	0.32µg/µl	90.00±2.88	70.66±1.76***	73.00±1.15***	77.33±0.88**
6	0.64µg/µl	96.33±0.57	76.66±0.88***	77.00±2.64***	84.33±2.33**

Table 5: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 1st

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	432.00± 2.47	98.50± 2.59	101.33± 3.77	109.00± 5.34	107.00± 5.37
2	Disease Control	339.00± 10.21	423.66± 18.26	432.33± 12.26	375.33± 13.19	379.83± 14.73
3	Standard Control	352.00± 9.35	297.16± 5.86	291.33± 4.34	254.33± 3.74	141.66± 4.23
4	Ms 200mg/kg	356.00± 13.40 **	352.00± 8.24 *	279.33± 20.20 ***	264.50± 9.73 ***	211.83± 7.58 ***
5	Ms 400mg/kg	423.66± 8.86 *	333.66± 9.83 ***	269.66± 9.70 ***	210.50± 3.19 ***	192.50± 3.92 ***
6	Pa 200mg/kg	407.00± 9.03 ns	415.83± 11.44 ns	389.16± 17.84 ns	323.50± 7.60 ns	278.00± 7.09 ***
7	Pa 400mg/kg	396.00± 10.07 ns	364.00± 9.56 ns	306.16± 19.78 ***	264.16± 11.02 ***	259.16± 13.64 ***
8	Na 200mg/kg	430.33± 36.28 ns	436.16± 15.35 ns	365.83± 15.89	327.16± 8.2 ns	285.00± 14.60 ***
9	Na 400mg/kg	105.00± 14.33 ns	394.00± 6.52 ***	358.66± 16.96 *	289.33± 13.73 **	176.66± 4.69 ***

Table 6: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 3rd

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	99.00± 3.67	103.83± 4.28	103.16± 2.99	108.33± 4.55	106.16± 5.28
2	Disease Control	498.16± 12.02	420.33± 6.22	347.00± 14.90	404.00± 8.00	403.50± 5.23
3	Standard Control	533.66± 16.50	418.33± 20.39	298.16± 6.30	216.50± 7.72	140.16± 7.62
4	Ms 200mg/kg	479.50± 8.22 ns	463.00± 9.07 ns	392.00± 5.79 ns	317.83± 10.27 *	235.00± 8.29 ***
5	Ms 400mg/kg	353.83± 23.16 ***	313.00± 24.03 ns	267.33± 24.28 *	207.33± 17.31 ***	147.50± 12.14 ***
6	Pa 200mg/kg	446.66± 19.51 ns	389.83± 10.21 ns	370.83± 20.57 ns	329.33± 18.25 ns	292.66± 13.73 ***
7	Pa 400mg/kg	401.16± 21.44 **	381.66± 17.38 ns	280.66± 20.27 ns	266.00± 12.00 ***	211.50± 8.29 ***
8	Na 200mg/kg	469.50± 15.67 ns	459.00± 16.52 ns	419.00± 20.29 ns	353.00± 13.91 ns	272.83± 9.49 ***
9	Na 400mg/kg	456.00± 18.24 ns	378.16± 10.69 ns	330.33± 11.97 ns	260.00± 12.26 ***	156.66± 7.35 ***

Table 7: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 5th

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	104.66±4.12	101.00± 4.66	103.50± 3.93	107.16±2.73	103.83± 2.32
2	Disease Control	417.00±23.22	429.50± 23.96	421.66± 20.05	366.50±14.02	367.50± 19.34
3	Standard Control	336.83±5.79	302.66± 5.49	291.33± 4.34	253.00±4.53	156.50± 10.24
4	Ms 200mg/kg	390.50±29.17 ns	401.16± 24.32 ns	342.00± 27.60 ns	284.66±26.45 ns	228.66± 18.66 ***

5	Ms 400mg/kg	387.50±17.79^{ns}	373.50±18.48^{ns}	293.50±14.96***	230.00±12.63***	191.16±4.23***
6	Pa 200mg/kg	430.00±21.58^{ns}	425.33±29.80^{ns}	397.83±23.19^{ns}	329.50±17.90^{ns}	279.66±23.56^{ns}
7	Pa 400mg/kg	400.83±13.05^{ns}	369.83±12.31^{ns}	326.33±13.63^{ns}	272.00±11.71^{ns}	250.16±12.04**
8	Na 200mg/kg	405.00±39.40^{ns}	423.00±16.85^{ns}	381.16±19.89^{ns}	343.33±14.25^{ns}	276.16±14.56^{ns}
9	Na 400mg/kg	444.66±19.13***	414.16±20.94***	379.50±17.94^{ns}	310.83±4.64***	174.00±10.17***

Table 8: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 7th

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	105.00±2.47	98.50±2.59	101.33±3.77	109.00±5.34	107.00±5.37
2	Disease Control	422.00±18.23	423.66±18.26	421.66±20.05	368.83±17.63	379.83±14.77
3	Standard Control	355.66±24.73	297.16±5.86	291.33±4.34	254.33±3.74	141.66±4.23
4	Ms 200mg/kg	346.50±18.83^{ns}	352.00±8.24^{ns}	279.33±20.20***	261.16±12.75**	211.83±7.58***
5	Ms 400mg/kg	334.33±25.87^{ns}	317.00±24.33**	260.83±16.10**	205.83±7.18***	192.50±3.92***
6	Pa 200mg/kg	435.33±18.83^{ns}	425.66±19.13^{ns}	389.16±17.84^{ns}	319.83±9.91^{ns}	271.83±12.44**
7	Pa 400mg/kg	390.33±24.61^{ns}	340.16±19.07^{ns}	306.16±19.79**	259.16±14.24**	259.16±13.64**
8	Na 200mg/kg	412.66±41.24^{ns}	441.00±18.80^{ns}	376.16±22.46^{ns}	337.16±15.34^{ns}	285.00±14.60**
9	Na 400mg/kg	436.00±19.19^{ns}	410.33±20.50^{ns}	358.66±16.96^{ns}	289.33±13.73^{ns}	168.00±9.43***

Table 9: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 9th

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	105.33±3.09	98.50±2.59	99.50±3.73	108.16±4.84	107.00±3.18
2	Disease Control	411.16±9.95	413.33±16.07	405.83±14.77	359.66±13.12	368.66±11.16
3	Standard Control	369.50±60.29	375.66±36.92	291.33±4.34	245.66±4.65	211.16±20.47
4	Ms 200mg/kg	421.16±33.18***	412.33±34.43***	366.83±38.26***	321.50±38.55***	285.00±25.18***
5	Ms 400mg/kg	419.50±26.93***	400.16±27.17***	314.83±21.76***	232.66±9.75^{ns}	198.33±7.35^{ns}
6	Pa 200mg/kg	388.00±23.56***	381.50±27.11***	354.16±28.25***	299.33±19.67***	281.66±20.50***
7	Pa 400mg/kg	415.66±21.21***	383.16±23.12***	339.83±20.02***	281.33±15.37***	238.00±10.56^{ns}
8	Na 200mg/kg	407.16±43.11***	432.16±23.86***	383.83±23.15***	334.00±12.25***	303.83±14.38***
9	Na 400mg/kg	269.00±41.65**	360.50±24.81***	306.00±31.95***	249.00±27.40*	233.00±34.48^{ns}

Table 10: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 11th

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	105.66± 5.30	114.33± 5.04	109.50± 5.46	107.66± 3.25	103.83±4.26
2	Disease Control	447.00± 22.70	407.50± 30.01	436.83± 23.32	429.66± 20.13	480.50±17.22
3	Standard Control	381.83± 45.84	340.83± 54.85	381.33± 43.97	187.33± 22.81	320.83±88.50
4	Ms 200mg/kg	416.16±*** 35.49	426.33±*** 40.95	339.16±** 50.20	293.50±ns 47.78	393.83±46.12***
5	Ms 400mg/kg	409.50±*** 31.17	354.66±** 46.74	306.33±* 29.63	224.66±ns 13.02	278.83±42.58ns
6	Pa 200mg/kg	387.66±*** 23.53	311.83±* 30.18	344.66±** 29.35	280.66±ns 17.37	352.50±49.61**
7	Pa 400mg/kg	390.00±** 25.06	355.00±*** 32.79	326.66±*** 28.80	289.66±* 12.80	295.33±35.47ns
8	Na 200mg/kg	437.33±*** 23.85	386.83±*** 35.45	398.66±*** 29.17	318.16±* 14.06	372.00±44.81***
9	Na 400mg/kg	369.33±*** 21.42	258.83±ns 36.91	297.00±ns 32.44	183.66±ns 9.40	314.50±70.53*

Table 11: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at day 13th

No	Groups	0-hr	2-hrs	4-hrs	6-hrs	8-hrs
1	Normal Control	106.66± 3.95	103.16± 5.36	103.66± 5.07	110.33± 3.58	109.00±4.46
2	Disease Control	430.16± 13.33	421.00± 16.74	397.00± 21.95	366.33± 6.13	370.33±18.63
3	Standard Control	362.83± 57.27	357.66± 28.12	327.83± 17.41	255.66± 10.27	193.16±24.08
4	Ms 200mg/kg	390.00±ns 27.20	369.33±ns 25.15	337.83±ns 19.89	267.00±* 7.72	237.50±11.84*
5	Ms 400mg/kg	357.33±ns 22.13	336.33±ns 22.75	318.66±ns 12.31	222.00± 6.74***	205.83±12.07**
6	Pa 200mg/kg	418.16±ns 16.08	410.33±ns 15.63	375.50±ns 29.59	309.50±ns 21.93	278.33±24.11ns
7	Pa 400mg/kg	426.66±ns 13.54	384.50±ns 15.08	361.66±ns 9.84	278.16±ns 10.43	243.83±17.47**
8	Na 200mg/kg	397.50±ns 40.69	422.66±ns 23.63	410.33±ns 25.82	338.16±ns 23.69	300.00±19.85ns
9	Na 400mg/kg	229.00±*** 25.50	276.66±*** 53.33	371.40±ns 25.55	296.66±ns 25.57	262.33±25.35*

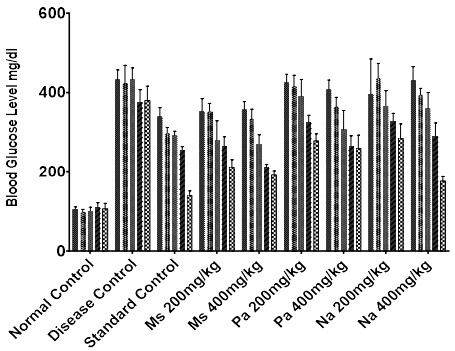
Table 12: Effect of Aqueous Ethanolic Extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on Body Weights (g) in Albino Rats

No	Groups	Before Treatment	After Treatment
1	Normal Control	257.33±3.14	263.83±2.93
2	Disease Control	273.16±13.40	267.00±12.87
3	Standard Control	247.50±15.37	243.66±15.53
4	Ms 200mg/kg	185.67±11.91***	181.50±12.21**
5	Ms 400mg/kg	171.50±10.66***	167.16±10.26**
6	Pa 200mg/kg	186.67±11.78**	182.66±10.88**
7	Pa 400mg/kg	203.00±14.22*	197.00±13.40*
8	Na 200mg/kg	206.33±17.45*	198.66±17.11*
9	Na 400mg/kg	231.16±20.57ns	206.50±21.27ns

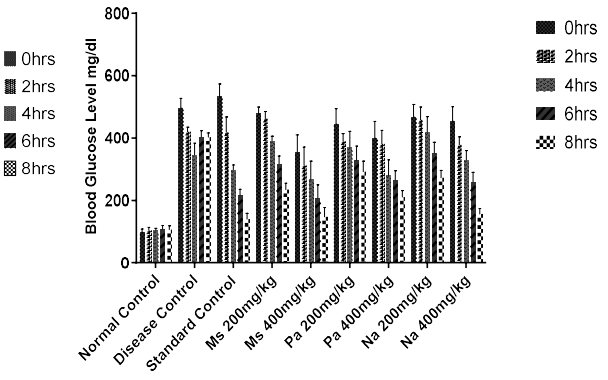
Table 13: Effect of Aqueous Ethanolic Extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on Serum Cholesterol (gm/dl), Triglycerides (mg/dl), LDL (mg/dl), VLDL and HDL (mg/dl) Level in Albino Rats.

No	Groups	Serum Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)	Serum LDL (mg/dl)	Serum VLDL (mg/dl)	Serum HDL (mg/dl)
1	Normal Control	100.30±2.17	96.00±1.39	69.00±1.57	16.00±0.36	33.67±0.66
2	Disease Control	205.71±3.41	155.71±1.17	139.50±2.15	43.67±0.61	18.17±0.47
3	Standard Control	139.50±1.40	115.50±1.25	75.17±1.24	15.67±0.33	37.83±1.51
4	Ms 200mg/kg	159.30±1.92***	145.51±2.04*	93.50±1.17**	27.17±0.60***	28.17±0.60***
5	Ms 400mg/kg	146.70±1.62***	133.51±1.56**	77.50±1.76***	23.33±0.21***	28.50±0.56***
6	Pa 200mg/kg	152.50±2.66***	136.71±1.60***	80.83±1.30***	23.17±0.30***	28.00±0.96***
7	Pa 400mg/kg	141.21±1.81***	136.72±0.66***	75.67±1.22***	21.83±0.40***	28.67±1.25***
8	Na 200mg/kg	142.23±2.33***	131.00±1.39***	72.33±0.80***	19.17±0.47***	33.67±1.33***
9	Na 400mg/kg	132.35±2.47***	119.20±1.44***	67.50±2.04***	17.83±0.40	33.67±0.84***

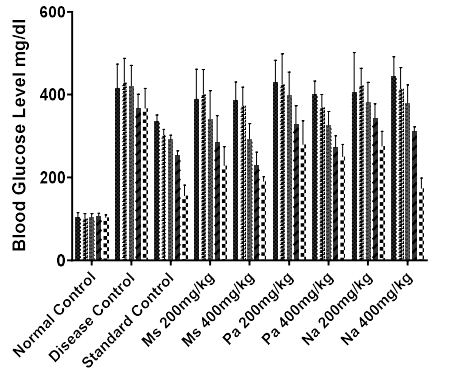
A. (At day 1st)



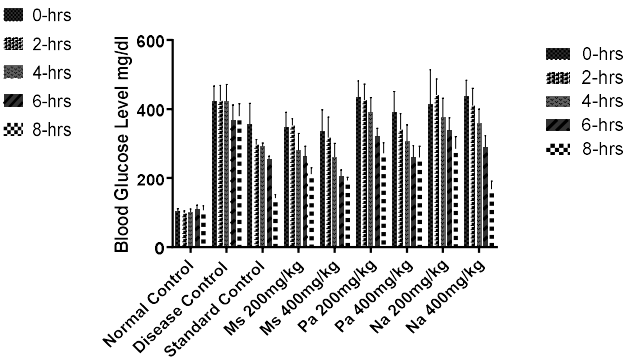
B.(At day 3rd)



C.(At day 5th)



D.(At day 7th)



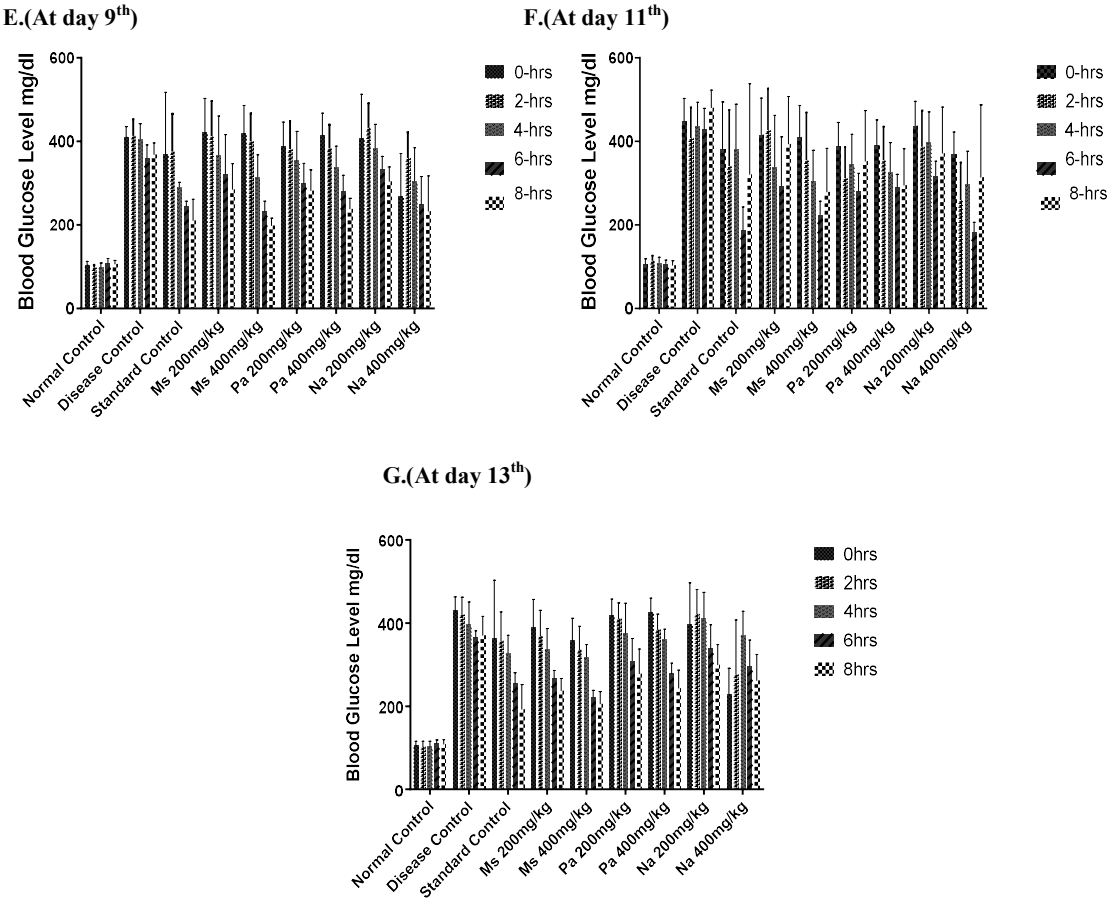


Figure 1: (A to G): Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on blood glucose level (gm/dl) in albino rats at different days

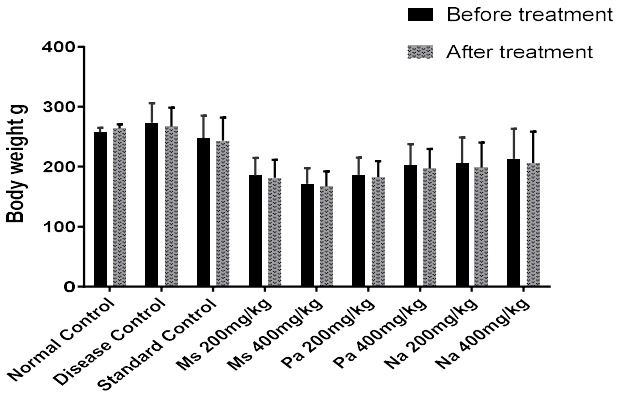


Figure 2: Effect of aqueous ethanolic extracts of *Mentha Spicata* (Ma), *Plumeria alba* (Pa) and *Nymphaea alba* (Na) on body weights (g) in albino rats

DISCUSSION

The DPPH assay of our study indicated that the aqueous ethanolic extracts of *Mentha spicata* (fresh leaves), *Plumeria alba* (fresh leaves) and *Nymphaea alba* (flowers and rhizomes) are highly enriched with anti-oxidant species. DPPH method used in this study was also utilized by Oldham and

Bowen which proved the accuracy of this method for the estimation of percentage scavenging effect **[16]**. Anti-diabetic action of three plant extracts is most probably due to the presence of anti-oxidants present in plants. These anti-oxidants scavenge the free radicals and reactive oxygen species (ROS) which was also proved by Premanth that anti-diabetic activity of plants increases due to the suppression of ROS, like superoxide dismutase and glutathione peroxidase which may cause increase in level of antioxidant enzymes **[17]**. Moreover other phytochemicals like saponins, tannins and alkaloids present in our plant extracts are responsible in lowering blood glucose levels. Pervious study on *Gymnema sylvestre* leaves indicated that the same anti-diabetic effects were produced by the use of that plant due to the presence of saponins, alkaloids and flavonoids **[18]**. However the presence of Carbohydrates, Proteins, terpenes, steroids, tannins, flavonoids, glycosides, phenols and alkaloids also contribute to hypoglycemic effects. These metabolites stimulate the glucose utilization by increasing insulin secretion from beta cells and reduce the blood glucose concentration in body **[19]**. The photochemical study of *Plumeria alba* confirms the presence of glycosides, alkaloids, flavonoids, terpenoids and steroids by conducting various chemical tests. The work of Radhaalso reported that the presence of following secondary metabolites i.e. flavonoids, terpenoids, glycosides and steroids which are highly responsible for their medicinal uses against diabetes **[20]**. According to Kamtchouing *et al*. the flavonoids reduce the serum blood glucose and stimulate beta cells for insulin production **[21]**. While glycoside terpenoids and steroids also has inhibitory effect on elevated glucose level in blood and control the diabetes. Carbohydrate increases the glucose tolerance and insulin release, phenol and flavonoids increase the utilization of insulin and reduce glucose level, alkaloids inhibit alpha-glucosidase by decreasing glucose transport through the intestinal epithelium **[22]**. So it is confirmed that the hypoglycemic action of plants is due to the presence of these beneficial metabolites. It is proposed that the presence of flavonoids, alkaloids, glycosides, carbohydrates, phenols, steroids and tannins in aqueous ethanolic extract of *Mentha spicata*, *Plumeria alba* and *Nymphaea alba* show better lipid control in albino rats. A study by Saravanan proposed that flavonoids increase the activity of lecithin acyltransferase, which is important for the control of lipid by affecting the free cholesterol and thus increase the level of high

density lipids **[23]**. Saponin causes the mal-absorption of cholesterol in intestine and bile acid reduces the extra circulation of cholesterol by increasing its metabolism into sterol. The fast fecal bile acid excretion is balanced through the stimulation of cholesterol synthesis from liver and hence produces hypo-lipidemia. So the marked antihyperlipidemic activity achieved with the use of aqueous ethanolic extract of *Mentha spicata*, *Plumeria alba* and *Nymphaea alba* is due to the presence of certain phytochemical constituents which lower the serum lipid profile by above described mechanism.

CONCLUSION

On the basis of above described results, it is concluded that aqueous ethanolic extracts of *Mentha spicata* (fresh leaves), *Plumeria alba* (fresh leaves) and *Nymphaea alba* (flowers and rhizomes) have antidiabetic activity but still extensive studies are needed to be conducted on these extracts to explore the qualitative and quantitative details of all of the hidden phytochemical agents.

ACKNOWLEDGEMENT

The credit goes to Riphah International University Lahore campus that provided us with all facilities and resources to conduct this research.

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