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Original Article

Hypoglycemic and Hypolipidemic Effect of Active Compounds (Glucokinin or Plant Insulin) from *Bauhina variegate* L. in Alloxan Induced Diabetic Mice

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ABSTRACT

Bauhinia variegata L. belongs to the family Leguminosae. This study has employed an *in vivo* evaluation of leaves (petroleum ether and methanol extract) and methanol extract and flowers extraction in mice at concentrations (200 and 400 mg / kg) for (LM1 and LM2) and (200mg /kg)for F given intra peritoneal for 10 days after inducing diabetes mellitus type 2 by alloxan (200mg/1kg body weight). The serum was isolated from blood by cardiac puncture for the biochemical tests, including glucose, cholesterol(ch), Triglyceride(TG),high density lipoprotein(HDL) ,low density lipoprotein(LDL) and very low density lipoprotein(VLDL). From the observations, it was concluded that the reduction of blood glucose levels in diabetic rats were found to be dose dependent and also dependent on duration of action. So that the Plant insulin (Glucokinin) may be responsible for some of the actions at plant extracts for their antidiabetic properties without toxicity.

Key words: *Bauhinia variegata*, Antidiabetic *activity, Hyperglycemia, Hypolipidemic*

INTRODUCTION

Herbal medicines have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years and continue to play a major role in the primary health care of about 80% of the world's inhabitants [1]. The leaves of *Bauhinia variegata* a plant widely utilized for the management of diabetes showed partial sequence identity to bovine insulin. Bodakhe et al.[2]found that the extract of the stem bark of *B. variegata* shows hepatoprotective activity against carbon tetra chloride (CCl_4) induced hepatotoxicity in Sprague Dawley rats at the dose of 100 and 200 mg/kg. Oral administration of ethanolic extract decrease the level of AST, ALT, ALP, GGT, total lipids & increase the level of total protein which increase during the hepatotoxicity and decrease the level of total proteins. Velraj et al.[3] shown that ethyl acetate (EAESOF) and ethanolic (EESOF) extract of *Scindapsus officinalis* fruit were evaluated for antidiabetic activity on blood glucose level, lipid profiles in alloxan induced diabetic rats. EAESOF & EESOF (200 mg/kg) and Glibenclamide (10mg/kg) were administered orally in alloxan (120 mg/kg, i.p.) induced diabetic rats. The maximum reduction in blood glucose was observed in EAESOF and EESOF (160.8, 96.7 mg/dl) at the dose of 200 mg/kg on 21st day respectively. The EAESOF and EESOF showed the significant effect ($p<0.005$) in the various biochemical parameters like protein, triglycerides, cholesterol and total lipid levels. The aim of current study is to evaluate the hypolipidemic and hypolipidemic effect of methanol extract of B. *variegate* leaves and flowers in normal and alloxan induced diabetic mice.

MATERIALS AND METHODS

Bauhinia leaves and flowers were collected from garden AL-Nahrin University in November 2015 and flowers during May 2015**.** It has been diagnosed by the College of Science / Baghdad University. The leaves and flowers were washed and dry and then crushed by electric grinder to powder then stored in an air tight container until used. The extracts active compounds (methanolic extracted (LM1) ; Petroleum ether and methanol (LM2) and Petroleum ether and methanol Flowers extract (F1) were papered according to Al-Jumaily and Fakhri,[4] and AL- Shahat,[5].

Animals: The animals used were mice which were bought from drug control center in Baghdad. The mice kept in the animal house, fed with milk,

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bread. The were 80 mice (male and female) divided into 8 group.

Group1: Control group
The mice of this group received normal mice feeds (bread, milk, water). After feeding them for one week their body weights and fasting serum blood glucose levels were taken other parameters.

Group2: Diabetic mice without treatment
At the end of first week of experiment, alloxan was injected into the mice of control group (positive) and they formed group 2. The mice were confirmed diabetic after of their fasting serum blood glucose level. Two weeks after injection of alloxan .Amice was considered to be diabetic if it had fasting serum blood glucose where level >115 mg\dl other parameters were also taken and recorded.

Group3: Diabetic mice after treatment with petroleum ether and methanol extract (LM1 400)
At the end of 1 week of induction of diabetes into the mice of group2 they were given sample1 o.1 ml 1g\ml orally for period of 10 days and thus they formed the mice of group3, at the end of week their fasting serum blood glucose levels was estimated and recorded.

Group 4: Diabetic mice after treatment with methanol extract (LM2 400)
At the end expiration of 1week of induction of diabetes into the mice of group 2 they were given with 0.1 ml sample 2 1g\ml orally for 10 days of group 4**.**

Group 5: Diabetic mice after treatment with petroleum ether and methanol extract (LM1 200)
At the expiration of 1 week of induction of diabetes into the mice of group 2 they were given 0.1 ml sample1 0.5g\ml orally for 10 days and thus they formed the mice of group 5, at the end of weeks their fasting blood glucose level were estimated and recorder.

Group 6: Diabetic mice after treatment with methanol extract (LM2 200)
At the expiration of week of induction of diabetes into the mice group 2. They were given 0.1 ml sample 0.5g/ml orally for 10 days and thus they formed the mice of group6, at the end of weeks their fasting blood glucose level were estimated and recorder.

Group 7: Diabetic mice after treatment with flowers extract (F200): At the expiration of week of induction of diabetes into mice of group 2 they were give 0.1 ml sample 0.5g\ml orally for 10 days and thus they formed the mice of group7, at the end of weeks their fasting blood glucose level were estimated and recorder

Group 8: Diabetic mice after treatment with insulin
At the expiration of week of induction of diabetes into mice of group2 they were give 0.1 ml insulin 0.5g\ml orally for 10 days and thus they formed the mice of group8, at the end of weeks their fasting blood glucose level were estimated and recorder.

Biochemical Assays: Determination of Serum Glucose Concentration according to Young *et al.*, [6], and **Calculations *as: Serum*** glucose concentration (mg/dL) = $\frac{A_{Sample}}{A_{S\tan dard}} \times C_{S\tan dard}$

Biochemical Analysis: On the tenth day of experiment the animals were sacrificed and blood was collected from various groups by puncturing the retro-orbital plexus, kept aside for half an hour for clotting. Serum was separated by centrifuging the blood samples at 6000 rpm for 20 minutes and stored in the refrigerator until analyzed. The serum was analyzed for various biochemical parameters such as of total cholesterol assay according to Srinivasan and Ramarao [7], triglycerides (Etuk,[8] and HDL and LDL cholesterol (Brunton et al.[9]). Kit supply from Biomaghreb (tunis) company. Statistical analysis of data was performed on the mean values has been calculated using unpaired Students't-test. Multiple group comparisons are made using analysis of variance (ANOVA) [10]. P-values less than 0.05 have been considered a significant for all data showed in results.

RESULTS AND DISCUSSION

After a week from the onset of the injections, Alloxan selectively destroys the insulin secreting beta-cells of the pancreas. With the maintained hyperglycemia, the mice showed polyuria, polydipsia, polyphagia, glycosuria and weight loss; although the clinical signs related to the diabetes mellitus were not obvious. Therefore, the induced diabetes mellitus was confirmed by the continuous hyperglycemia, glycosuria and clinical signs. The results present in figure (1) table (1) that show the serum glucose level were low ($p < 0.01$) insulin treatment diabetic mice (group 3) as compared to control positive and negative. (Group1 and 2). Diabetic mice were treatment with (LM1400) showed a significant ($p<0.01$) decreased in the serum glucose level as compared with control positive and negative (group 1and 2). Diabetic mice were treatment with (LM2 400) showed a significant ($p<0.01$) decreased in the serum glucose

level as compared with control positive and negative (group 1 and 2). Diabetic mice were treatment with (LM1 200) showed a significant ($p<0.01$) decreased in the serum glucose level as compared with control positive and negative (group1and 2). Diabetic mice were treatment with (LM2 200) showed a significant ($p<0.01$) decreased in the serum glucose level as compared with control positive and negative (group1 and 2). Diabetic mice were treatment with (F200) showed a significant ($p<0.01$) decreased in the serum glucose level as compared with control positive and negative (group1 and 2). These results agreed with the study of diabetes induced by Streptozotocin resulted in a significant elevation in blood glucose level in comparison with the control group after administration of aqueous methanolic (2: 3) extract of *S. mahagoni* seed to the diabetic rabbits for 21 days [11].

A significant reduction in blood glucose level was noted this was close to the control level .These results also agreed with the study of the protective effect of plant compound idopyranose isolated from the plant, *Vitex negundo* evaluated against streptozotocin-induced diabetics. Wister rats showed significant reduction in blood glucose [12]. Also the study agreed with another researcher Shafiq [13] which used the resveratrol which isolated from *vitis vinifera* and their derivatives employed an *in vivo* in female rabbits at concentration (1 mg/kg) gave orally for 42 days after inducing diabetes mellitus type II by alloxan. Serum glucose level showed significant decrease ($P<0.05$) in blood.

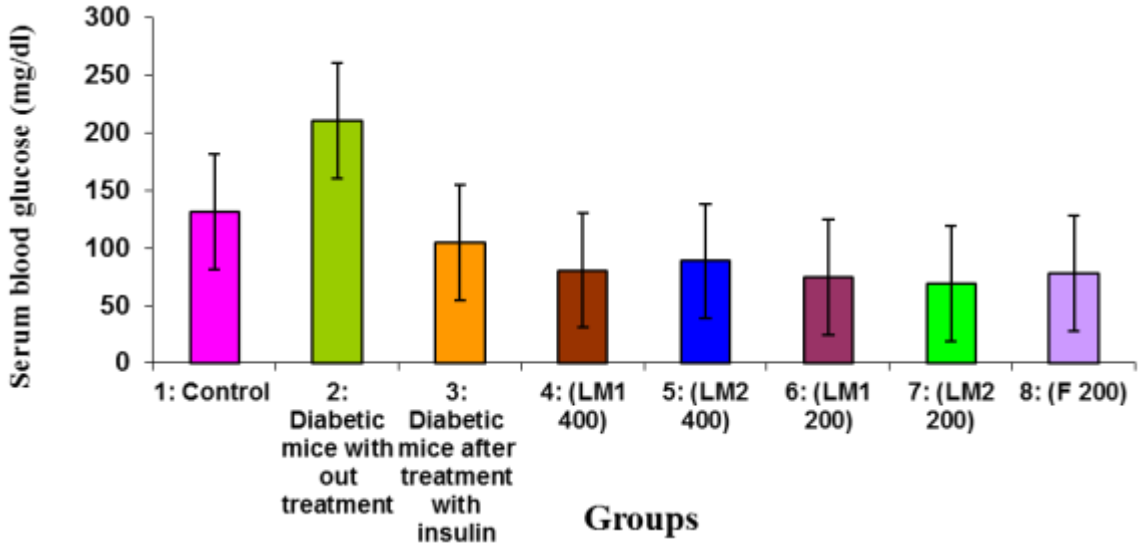

Figure (1): The effect of different extract of *Bauhinia variegata* L. leaves and flowers on serum blood glucose concentration (mg/dl).

Group One – Control negative
Group two-control positive
Group three-Diabetic mice after treatment with insulin.
Group four- Diabetic mice after treatment (LM1 400).
Group five- Diabetic mice after treatment (LM2 400).
Group six- Diabetic mice after treatment (LM1 200).
Group seven - Diabetic mice after treatment (LM2 200).
Group eight - Diabetic mice after treatment (F 200).

The effect of different extract of *Bauhinia variegate* L. leaves and flowers on lipid profile state: Lipids are substances that are insoluble in water (hydrophobic) but soluble in organic solvent such as alcohol, chloroform, ether, acetone, hexane, and benzene. Lipids are composed of different percent of the element: carbon (C), hydrogen (H), and oxygen (O) in order to provide the highest amount of energy, and these element constitute the basic units of lipids in the blood. [14]

Cholesterol

The best known steroid, 2/3 of total cholesterol is esterifies with Fatty acids (FA), in practice total cholesterol. It is present in diet and mainly synthesis in liver (the body can synthesis up to 1 gram of cholesterol per a day, while only 0-40mg per a day is absorbed from food and small intestine, it is either excreted unchanged into bile or converted to bile acids[14].

The results were present in table(1) and figure (2) show ($p < 0.01$) the Diabetic mice were treatment with insulin increased in level cholesterol as compared with control negative(group), and decreased in level cholesterol in serum as compared with control positive (group 2). Diabetic Mice were treatment with (LM1 400) (group4) showed ($p< 0.01$) increased in level cholesterol as compared with control negative (group 1).and decreased in level cholesterol in serum as compared with control positive (group 2). Diabetic mice were treatment with (LM2 400) (group5) showed equal in level cholesterol as compared with control negative (group 1) and decreased in level cholesterol in serum as compared with control positive (group 2). Diabetic mice were treatment with (LM1 200) (group6) showed increased in level cholesterol as compared with control negative (group 1) and decreased in level cholesterol in serum as compared with control positive (group 2). Diabetic Mice were treatment with (LM2 200) (group7) showed increased in level cholesterol as compared with control negative (group 1) and decreased in level cholesterol in serum as compared with control positive (group 2).

Diabetic Mice were treatment with (F 200) (group 8) showed increased in level cholesterol as compared with control negative(group 1) and decreased in level cholesterol in serum as compared with control positive (group 2). The results for the diabetic and lipid profile of LM1 and LM2 and F treated groups showed significant decrease ($P>0.01$), when compared with control negative and positive groups. Based on the results obtained from this study, it may be concluded that the methanol flower and leaf extract of LM1 and LM2 and F has hypoglycemic properties and was able to alleviate elevations in lipid profile and oxidative stress induced by Alloxan in mice.And also Al-Jumaily and Auokty [15] found that flaxseed contains secoisolariciresinol (lignans) which decrease serum lipid profile and lowers the risk of atherosclerosis in cholesterol fed rabbits may be through the inhibition of lipid peroxidation and antioxidant activity.

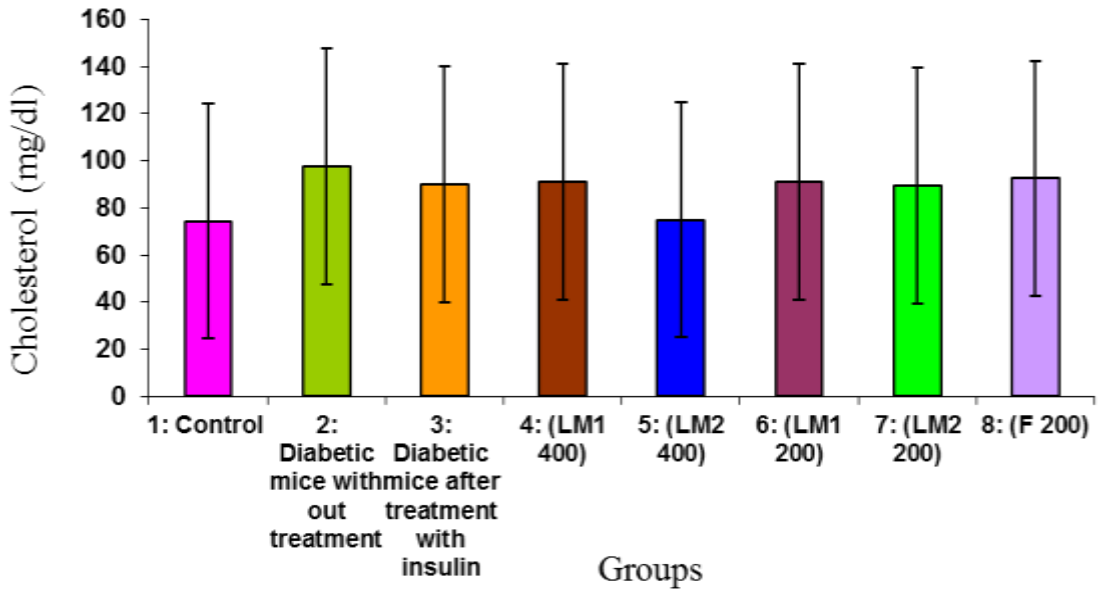

Figure (2): The effect of different extract of Bauhinia variegate L. leaves and flowers on cholesterol (mg/ml).

Serum Triglyceride level: Figure (3) and table (1) show the results of mean value of serum triglyceride level (mg/dl) before and after treatment. The serum triglyceride level were significantly ($P \leq 0.01$) increase in mean value insulin treatment Diabetic mice (178 mg/dl) as compared with negative control group (group1) amount of (76.96 mg/dl) and decrease as compared with control positive group(group2) amount of (130.33 mg/dl). Diabetic Mice were treatment with (LM1400) shown a significant ($p < 0.01$) increase in level triglyceride serum amount of (171 mg/dl)

as compared with control negative and decrease as compared with control positive. Diabetic Mice were treatment with (LM2400) showed ($p < 0.01$) a significant increase in level triglyceride serum amount of (134 mg/dl) as compared with control negative and decrease as compared with control positive. Diabetic Mice were treatment with (LM1200) showed ($p < 0.01$) a significant increase in level triglyceride serum amount of (170 mg/dl) as compared with control negative and decrease as compared with control positive. Diabetic mice (Group 7) were treatment with (LM2 200) showed ($p < 0.01$) a significant increase amount of (121 mg/dl) in level triglyceride serum as compared with control negative and decreased as compared with control positive. Diabetic Mice were treatment with (F 200) showed ($p < 0.01$) a significant increase in level triglyceride serum amount of (131 mg/dl) as compared with control negative and decreased as compared with control positive. These results agreed with another research which they showed that serum triglyceride level elevated significantly in the diabetic control group after treatment with the *S. mahagoni* seed in comparison with the control group [11]. Also Shafiq [13] shown when used resveratrol and their derivatives to treated diabetic male rabbits and Akah *et al*.,[16] when used to treated diabetic rabbits with aqueous leaf extract of *V. amygdalina*.

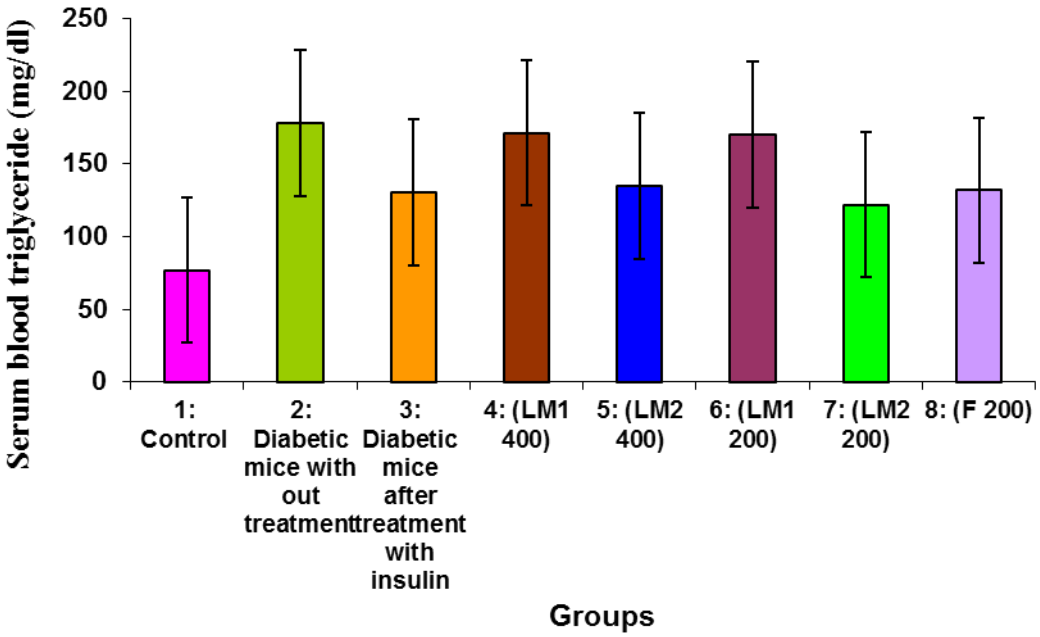

Figure (3): The effect of different extract of *Bauhinia variegate* L. leaves and flowers on Serum blood triglyceride (mg/dl).

Serum High density lipoprotein (HDL) level: The results presented in table (1) figure(4) shown ($p < 0.01$) that serum HDL level were decreased in insulin Diabetic mice treatment as compared with control positive and negative. Diabetic Mice were treatment with (LM1 400) showed ($p < 0.01$) increased in serum HDL level as compared with control positive and negative. Diabetic Mice were treatment with (LM2 400) showed($p < 0.01$) increased in serum HDL level as compared with control positive and decreased as compared wih control negative. Diabetic Mice were treatment with (LM1 200) showed ($p < 0.01$) increased in serum HDL level as compared with control positive and decreased as compared wih control negative Diabetic Mice were treatment with(LM2 200) showed ($p < 0.01$) increased in serum HDL level as compared with control positive and negative. Diabetic Mice were treatment with (F 200) showed ($p < 0.01$) increased in serum HDL level as compared with control positive and negative. (2-main types HDL2, HDL3) also referred to as (good cholesterol) it account for 20-30%of total cholesterol .levels it are inversely correlated with CHD risk, they are small size particles with high density contain mainly cholesterol and PL, they transport cholesterol from peripheral tissue to the liver, normal range of HDL in female > male [17].

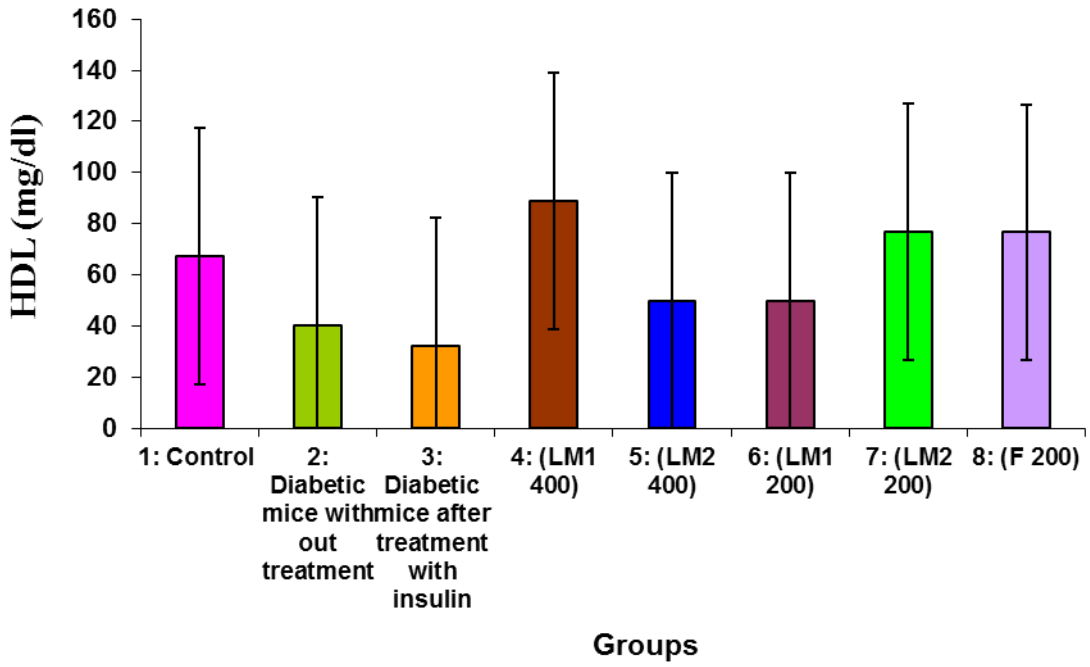

Figure (4): The effect of different extract of Bauhinia variegate L. leaves and flowers on HDL (mg/dl).

Low density lipoprotein (LDL): The results presented in table (1) and figure (5) showed that the mean value of serum LDL level (mg/dl) before and after treatment. Diabetic mice treatment with insulin have shown a significant ($p \leq 0.01$) increase in mean value of serum LDL (31.01 mg/dl) as compared to negative control group (group 1) amount of (14.96 mg/dl) and positive group (group 2) amount of (1.60 mg/dl). Diabetic Mice were treatment with (LM1 400) showed ($p \leq 0.01$) increased in serum LDL level (32.50 mg/dl) as compared with control positive and negative. Diabetic Mice were treatment with (LM2 400) showed ($p \leq 0.01$) decreased in serum LDL level (2.13 mg/dl) as compared with control positive and increased as compared with control negative.

Diabetic Mice were treatment with (LM1 200) showed ($p \leq 0.01$) increased in serum LDL level (7.53 mg/dl) as compared with control positive and decreased as compared with control negative. Diabetic Mice were treatment with (LM2 200) showed($p \leq 0.01$) increased in serum LDL level (12.78 mg/dl) as compared with control positive and decreased as compared with control negative. Diabetic Mice were treatment with (F 200) showed ($p \leq 0.01$) increased in serum LDL level (11.93 mg/dl) as compared with control positive and decreased as compared with control negative. Also referred to as (bad cholesterol) it account for 60-70% of total serum cholesterol it is the most atherogenic lipoprotein ,they are cholesterol rich particles formed form LDL by the removal of more TG & apolipoprotein ,transport cholesterol to the cells[18].

VLDL (intermediate density lipoprotein): Moderate size particles consist mainly of TG (endogenous TG) synthesis mainly in liver and to a lesser extent in small intestine (transport from liver to the cells [19]. The results presented in table (1) figure(6) have shown a significant ($p \leq 0.01$) decreased in mean value of serum VLDL level (26.08 mg/dl) with Diabetic mice treatment with insulin as compared with control positive group 2 (35.80 mg/dl) and decreased as compared with control negative (group 1) (15.39 mg/dl). Diabetic mice were treatment with (LM1 400) showed a significant ($p \leq 0.01$) decreased in mean value serum VLDL level (34.23 mg/dl) as compared with control positive and increased as compared with control negative. Diabetic mice were treatment with (LM 2 400) ($p \leq 0.01$) decreased in serum VLDL level (26.90 mg/dl) as compared with control positive and increased as compared with control negative. Diabetic mice were treatment with (LM1 200) showed ($p \leq 0.01$) decreased in serum VLDL level (34.00 mg/dl) as compared with control positive and increased as compared with control negative. Diabetic mice were treatment with (LM 2 200) showed($p \leq 0.01$) decreased in serum VLDL level (24.41 mg/dl) as compared with control positive and increased as compared with control negative. Diabetic mice were treatment with (F 200) showed ($p \leq 0.01$) decreased in serum VLDL level (26.90 mg/dl) as

compared with control positive and increased as compared with control negative.

Interestingly, most of the studies with different plant extracts in diabetic rats were in agreement with our result Administration of extracts of S. monostachyus showed significant decrease ($p<0.05$) in TC, LDL, VLDL, and TG. This alleviation denotes the anti-hyperlipidaemic potential of S. monostachyus. S. monostachyus in reversing the elevation of LDL-c, TC, VLDL-c, TG and decreased HDL-c in hyperlipidaemic rats agrees with the findings of Adaramoye et al. [20] for diabetic and hyperlipidaemic rats.

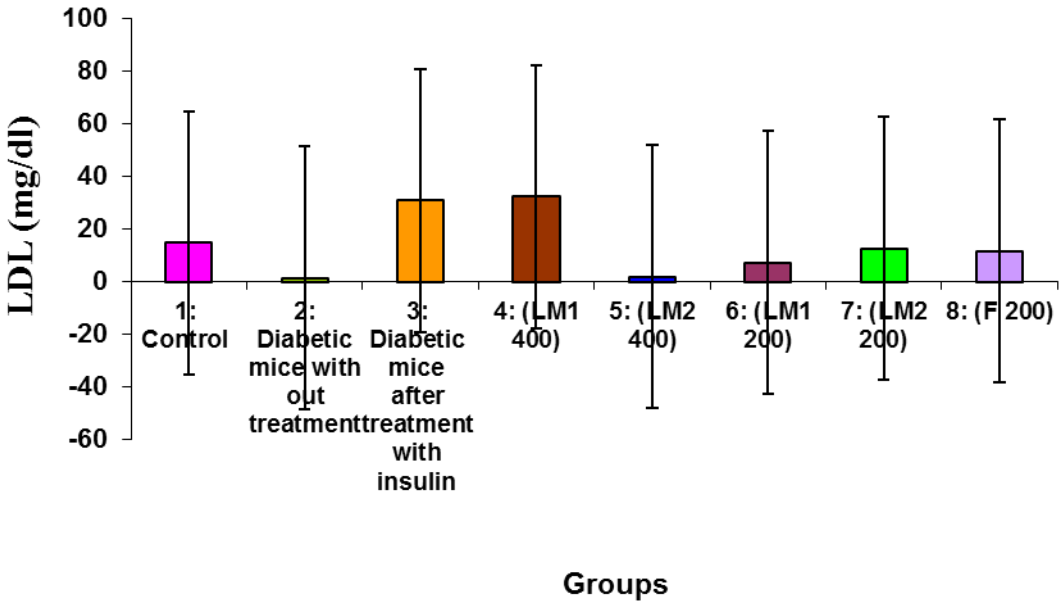

Figure (5): The effect of different extract of *Bauhinia variegate* L. leaves and flowers on LDL (mg/dl).

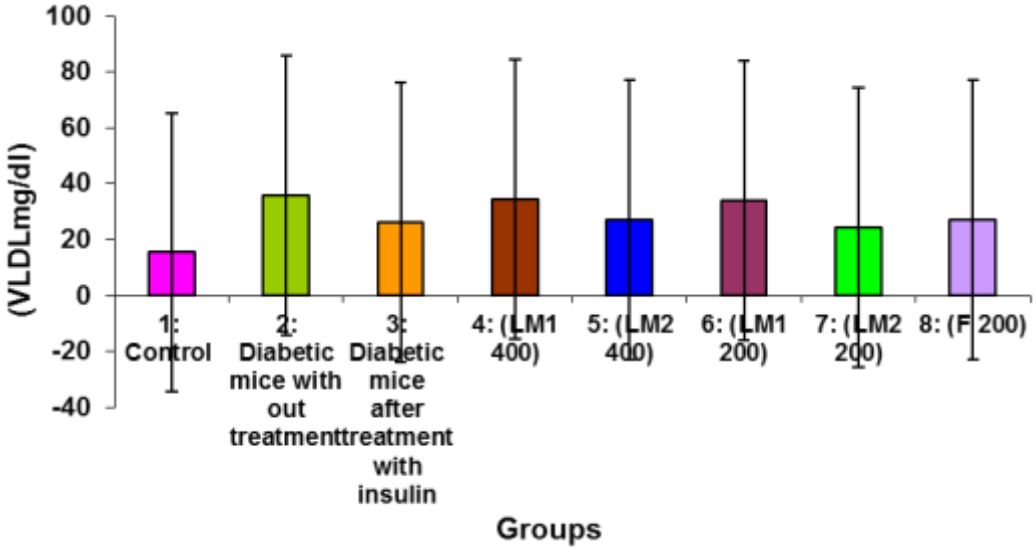

Figure (6): The effect of different extract of *Bauhinia variegata* L. leaves and flowers on VLDL (mg/dl).

Table (1): Effect of Different doses of *Bauhinia variegate* L methanolic (LM1; LM2 and Flowers) extracts on the mean serum blood in treated mice.

Groups	Serum blood glucose (mg/dl)	Cholesterol (mg/dl)	Serum blood triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group one	131.67 ± 2.40 b	74.50 ± 5.19 b	76.96 ± 8.28 c	67.40± 0.00 b	14.96± 0.36 b	15.39± 1.65 c
Group two	210.33± 3.92 a	97.67 ± 1.30 a	178.00± 22.51 a	40.33± 0.88 cd	1.60± 0.35 d	35.80± 1.65 a
Group three	105.06 ± 3.97 c	89.83 ± 0.60 a	130.33 ± 2.59 b	32.46± 0.29 d	31.01± 0.86 a	26.08± 0.00 b
Group four	80.67 ± 18.98 d	91.30 ± 1.04 a	171.15± 11.31 a	89.00± 0.58 a	32.50± 3.57 a	34.33± 2.26 a
Group five	88.67 ± 1.67 cd	75.00 ± 8.66 b	134.67 ± 2.60 b	50.00± 9.09 c	2.13± 0.12 d	26.90± 0.52 b
Group Six	74.53 ± 6.22 d	91.20 ± 1.64 a	170.00 ± 3.17 a	50.00± 0.00 c	7.53± 0.50 c	34.00± 2.31 a
Group Seven	68.90 ± 3.87 d	89.67 ± 0.72 a	121.70 ± 2.31 b	76.83± 1.01 b	12.78± 0.40 b	24.41± 0.09 b
Group eight	77.93 ± 3.55 d	92.50 ± 1.73 a	131.70 ± 8.12 b	76.67± 0.93 b	11.93± 1.22 b	26.90± 1.50 b
LSD value	22.897 **	11.186 **	29.956 **	9.820 **	4.192 **	6.366 **

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