SJIF Impact Factor: 3.458

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 2.
Issue: 5.
Page N. 102-107
Year: 2018

Original Article www.wjahr.com

THE EFFECTS OF MULBERRY FRUITS (*MORUS ALBA* L.) EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

[1]Md. Mahmudul Hasan, [2]Mst. Ismat Ara Begum and *[1]Shahanaz Khatun

[1]Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.
[2]Department of Animal husbandry and Veterinary Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh.

Received date: 17 July 2018 **Revised date:** 07 August 2018 **Accepted date:** 28 August 2018

Corresponding author: Shahanaz Khatun

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.

ABSTRACT

Objective: To investigate the protective role of *Morus alba* L. fruits against alloxan induced diabetic rats. **Methods:** Swiss albino rats were divided into five groups. The first group was used as non-treated control group, the second group was used as diabetic treated control group, the third group was diabetic treated mice plus methanolic extract of *Morus alba* L. fruits (200mg/kg body weight per day), the fourth group was diabetic treated mice plus ethanolic extract of *Morus alba* L. fruits (200mg/kg body weight per day) and the fifth group was diabetic treated mice plus glibenclamide. Serum indices related to diabetic, cardiac, renal and liver functions were determined to investigate the protective effect of *Morus alba* L. fruits on alloxan induced diabetic rats. **Results:** It revealed that the *Morus alba* L. fruits extract suplementation abrogated the alloxan induced elevation of glucose, total cholesterol, triglycerides, low density lipoprotein, urea, SGPT and SGOT. *Morus alba* L. fruits prevented the alloxan induced perturbation of serum high density lipoprotein cholesterol. **Conclusions:** The present study reported that the *Morus alba* L. fruits may be very useful for the improvement of the complications of alloxan induced diabetes.

KEYWORDS: *Morus alba*, glibenclamide.

INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is characterized by hyperglycemia resulting from defects in insulin action, insulin secretion or both.[1] In the year 2000, this rapid growing metabolic disorder affecting approximately 171 million and is projected to increase to 366 million by 2030.[2] The risk of diabetic complications are particularly cardiovascular disease (CVD) and peripheral vascular disease (PVD).[3] Complications such as coronary artery disease (CAD), stroke, blindness, neuropathy, renal failure etc are known to be associated with DM.[4] Diabetes has caused a tragedy in Bangladeshi poor people. Despite excellent potencies, several synthetic anti-diabetic drugs had offered unwanted therapeutic profiles marked by fluid retention, drug-induced hypoglycemia and increased rate o\f lactic acidosis, liver malfunctioning due to cirrhosis, weight gain and cardiac dysfunction.[5] Since antique era, plants containing medicinal properties are enormously used in treating diabetes throughout the world. Many scientific investigations have confirmed the efficacy of plant preparations, few of which are remarkably effective. It has been reported that some medicinal plant have to be useful in diabetes worldwide and have been used empirically in anti-diabetic, anti-hyperlipidimic remedies.[6]

Morus alba Lam. belongs to the family *Moraceae*, known as white mulberry. There are 24 species of *Morus*, with at least 100 varieties.[7] The species is native to northern China, and is widely cultivated in United States, Mexico, Australia, Argentina, India, Bangladesh and Pakistan.[8-13] They are grown to feed silkworms.[14,15] In Chinese medicine, *Morus alba* L. leaves, bark and branches have long been used.[16] In most European countries *Morus alba* L. are grown for fruit production.[17,18] It is reported that *Morus alba* L. fruit is a potential source of natural antioxidants such as total phenoliccs, vitamin E, vitamin C, β-carotene, coenzyme Q, flavonoids, polyphenol oxidase.[19] The fruits are a rich source of essential amino acids such asisoleucine, leucine, threonine, lysine, valine, phenylalanine, tyrosine, tryptophan, histidine,

methionine and cysteine.[20] In traditional Chinese medicine, the fruit is used to improve eyesight and protect against liver damage.[21] The fruits have been reported to act as anti-cholesterol, antioxidative and anti-obesity and anti tumour agent.[22–25] It is also reported that *Morus alba* L. leaf and fruit powder reduced CVD risk in mice.[26,27]

In the current literature there is not much data concerning the effect of *Morus alba* L. fruit extract on the glucose level, lipid parameters, urea and enzymes, which are abnormally altered due to DM. Therefore, the present study has been planned to investigate how the *Morus alba* L. fruit ethanol and methanol extract influences glucose level, lipid parameters, urea and enzymes in alloxan induced diabetic rats and to compare it with glibenclamide as a reference standard.

MATERIALS AND METHODS

Chemicals and kits

Alloxan monohydrate, (germany) methanol, ethanol (Merck, India) reagent and glucose, total cholesterol (TC), triglyceride (TG), SGPT, SGOT, urea, and high density lipoprotein cholesterol (HDL-C) precipitation reagent were used for this study.

2.2 Animal Maintanance

Adult healthy Albino rats (4 weeks of age) with average body weight 130-160g were collected from the Animal Resource Division of ICDDR'B Mohakhali, Dhaka, Bangladesh. They were individually housed in polycarbonate cages and wood-cube bedding (six mice per cage) in well-ventilated rooms under hygienic conditions. The animals prior to use were acclimatized for 7 d and were divided into five equal groups, the first group was named as non-treated control group, the second group was named as diabetic treated control group, the third group was diabetic treated mice plus methanolic extract of *Morus alba* L. fruits (200mg/kg body weight per day), the fourth group was diabetic treated mice plus ethanolic extract of *Morus alba* L. fruits (200mg/kg body weight per day) and the fifth group was diabetic treated mice plus glibenclamide. Diabetic rats were treated by Glibenclamide at a dose of 0.5mg/kg body weight. The doses were selected on the basis of previously published reports and experiments were conducted for 3 weeks before sacrifice. Rats were maintained with 12 h: 12 h light dark cycle with available supply of distilled water and food. Ethical permission was obtained from the Institute of Biological Sciences, Rajshahi University, Bangladesh (101/320/IAMEBBC/IBSC).

Induction of diabetes

Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of alloxan (90 mg/kg body weight) in a 0.1M sodium citrate buffer (pH-4.5). The age-matched control rats received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after alloxan administration. The development of hyperglycemia in mice was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 48 hours after alloxan administration, with a Portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose level $\geq$ 11.0 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered diabetic and included in the study.

Preparation of *Morus alba* L. fruits powder

Fresh young *Morus alba* L. fruits were collected from Rajshahi University Campus, washed with tape water properly and then sun dried. Finally fruit powder was obtained by grinding and kept it at 4 °C with sealed plastic packet until experiment to avoid the contamination of microorganism.

Preparation of Extract

The powder of *Morus alba* L. fruits (200gm) was mixed with ethanol and methanol (95%) separately in a 600 mL flask with mild shaking. The flask was closed with cotton plug and aluminum foil at 48 hours at room temperature. The extract was filtered through Whatman filter paper (No.1), concentrated using a rotary evaporator at low temperature (40-50°C). The extract was preserved in airtight container and kept at 4°C until further use.

Collection of sample and preparation of serum

Blood specimens of rats were collected from thoracic arteries after anaesthetization with diethyl ether. For coagulation, blood was kept about 30 min at room temperature then centrifuged at 4 000 r/min for 15 min at 4 °C. Then serum were drawn off and stored at -80 °C until the experiments were performed.

Biochemical assay

The biochemical analyzer (CHEM-5 V3, Erba, Mannheim, Germany) was used for the measurement of serum indices by using the commercially available kits according to manufacture's protocol. glucose, TC, TG, HDL, urea LDL-cholesterol (LDL), VLDL were measured; SGPT, SGOT activities were determined by the kits (Human, Germany). All the serum samples were analyzed in triplicate and then the mean values were taken.

Statistical Analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) followed by Tukey's honestly signify cant difference (HSD) post hoc test ($p < 0.05$). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($p < 0.001$). These were carried out using Microsoft office Excel 2010 and SPSS version 16.0 program (IBM Corporation, New York, USA)

RESULTS

It is reported that alloxan induces an increase of the serum glucose level both in human and rats.[28] Diabetes is associated with high blood glucose level for a prolonged period. In this experiment, we also found that alloxan caused an increase of the serum glucose levels upto 22.02±0.83 mmol/dl i.e., the rats became diabetic. The administration of MEMA (methanol extract of *Morus alba*) & EEMA (ethanol extract of *Morus alba*) produced significant changes in the high blood glucose level in Alloxan induced diabetic rats. Comparing the blood sugar level in diabetic rat, both the extract supplementation rats showed significant (P<0.05) reduction of blood glucose which was as near as glibenclamide administered rats (Table 1). From 3rd to 21th days, MEMA & EEMA administration group's glucose levels maintained 5.12% -43.83% and 5.05-43.46% reduction than that of diabetic control group. Whereas, in case of glibenclamide the reduction was 12.32%-59.23% than that of diabetic control group (P< 0.001). *Morus alba* L. fruits extract decrease serum glucose levels by indicating its ability of possessing glucose lowering effects.

Table 1: Effects of EEMA & MEMA on serum glucose level (mmol/L) in Alloxan induced diabetes in rat.

Groups	Group-1	Group-11	Group-111	Group-1V	Group-V
Initial day	5.94±0.21	20.01±0.47	21.87±0.46	20.18±0.32	22.02±0.83
3 day	5.82±0.11	21.25±0.52	21.04±0.43	22.17±0.52	21.12±0.81
6 day	5.95±0.18	21.7±0.47	20.41±0.38^{b}	20.64±0.23^{c}	17.18±0.75^{a}
9 day	5.89±0.17	22.5±0.47	17.67±0.24^{b}	18.06±0.20^{c}	15.22±0.77^{a}
12 day	5.90±0.25	22.93±0.58	15.18±0.28	15.56±0.17^{c}	13.74±0.4
15 day	5.84±0.27	23.48±0.72	14.29±0.31^{b}	14.64±0.39	11.36±0.78^{a}
18 day	5.97±0.12	24.14±0.44	13.8±0.30^{b}	14.12±0.45^{c}	10.60±0.54^{a}
21 day	5.91±0.13	22.87±0.35	13.04±0.26^{b}	13.20±0.26^{c}	9.82±0.42^{a}

Blood glucose level in the treated rats were significantly different from diabetic control group with Glibenclamide $^{a}P<0.001$, where as MEMA & EEMA were $^{b}P<0.05$, $^{c}P<0.05$.

Elevated level of TG is very often associated with cardiovascular diseases,[29] and cardiovascular disease is one of the major causes of diabetic mortality.[30] In this study, we determined the serum TG levels in the five groups of experimental rats. The TG levels (mean±SD) of the control, diabetic treated contrl group, diabetic treated rats plus methanolic extract of fruits, diabetic treated rats plus ethanolic extract of fruits and diabetic treated rats plus glibenclamide were (1.45±0.08), (2.04±0.12*), (1.63±0.21), (1.68±0.27), and (1.21±0.03) mmol/L, respectively (Table 2). The MEMA & EEMA administration also demonstrated the reduction of TG 20% and 17.64% in diabetic rat, respectively.

The serum TC levels were (5.27±0.11), (6.18±0.13), (4.78±0.54), (4.81±0.35) and (4.38±0.12^{a}) mmol/L for the control, diabetic treated control group, diabetic treated rats plus methanolic extract of fruits, diabetic treated rats plus ethanolic extract of fruits and diabetic treated rats plus glibenclamide respectively (Table 2). Atherosclerosis risk is inversely related to circulating levels HDL-C and low HDL-C levels are independent predictive marker of cardiovascular risk.[31] In this study, we evaluated the serum HDL-C levels in the five groups of experimental rats which were (0.32±0.01), (0.25±0.02), (0.37±0.02), (0.35±0.01) and (0.27±0.02) mmol/L for control, diabetic treated control group, diabetic treated rats plus methanolic extract of fruits, diabetic treated rats plus ethanolic extract of fruits and diabetic treated rats plus glibenclamide respectively (Table2). We have found that *Morus alba* L. fruits extract significantly (P<0.05) protected the lowering tendency of HDL-C levels in alloxan induced diabetic rats.

LDL level was significantly reduced (P<0.05) for MEMA & EEMA treatment 26.03% and 25.31% in diabetic rats (Table 2). VLDL level was also reduced (P<0.05) for MEMA & EEMA treatment 19.56% and 17.39% in diabetic rat respectively (Table 2).

Dysfunction of kidney is one of the major health effects of the long term diabetes, and elevated levels of serum urea have been reported to be associated with renal dysfunction and excessive protein catabolism.[32,33] We have found that in alloxan induce diabetic rats serum urea levels increased significantly (P<0.05) in comparison with control. The admionistration of *Morus alba* L. fruits extract significantly (P<0.05) protected alloxan-induced elevation of serum urea levels (Table 2).

Table 2: Effects of EEMA & MEMA on biochemical parameter in diabetic rat after 21 days treatment.

Group-11Groups	Parameters					
	Total Cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL (mmol/L)	HDL (mmol/L))	VLDL (mmol/L)	Urea (mg/dl)
Group-1	5.27±0.11	1.45±0.08	4.66±0.14	0.32±0.01	0.64±0.02	40.09±0.02
Group-11	6.18±0.13*	2.04±0.12*	5.53±0.18*	0.25±0.02*	0.92±0.05*	46.21±0.21
Group-111	4.78±0.54**	1.63±0.21	4.09±0.47**	0.37±0.02**	0.74±0.05**	42.32±2.02
Group-1V	4.81±0.35**	1.68±0.27	4.13±0.36**	0.35±0.01**	0.76±0.13**	43.01±0.32
Group-V	4.38±0.12[a]	1.21±0.03	3.87±0.15[a]	0.27±0.02	0.44±0.02[a]	39.99±0.11

Values were expressed as mean ± SD. In column wise comparison symbol * indicated that the values are statistically significant from general control group at P<0.001. In column wise comparison symbol ** indicated that the values are statistically significant from diabetic control group at P< 0.05. The value is statistically significant from diabetic control group at P<0.05.

The liver transaminases SGOT and SGPT are useful biomarkers of liver injury in a patient with some degree of intact liver function.[34,35] In order to confirm the hepatocellular degeneration of hepatic tissue, activities of SGOT and SGPT were then estimated. Usually the serum levels of all these enzymes are increased in liver injury. We observed that these enzyme activities were significantly increased (p<0.05) in diabetic mice group when compared to the control group. A significant (p<0.05) lower levels of these altered enzymatic activities were observed in the of *Morus alba* L. fruits extract MEMA & EEMA treated diabetic rats (Table-3).

Table 3: Effect of MEMA & EEMA on serum SGPT and SGOT of experimental rats.

Group	SGPT (U/L)	SGOT (U/L)
Group-1	53.60±2.26	38±3.21
Group-11	68.80±1.91*	90.00±2.77*
Group-111	50±1.46**	73.29±3.04**
Group-1V	51.00±1.52**	75.80±3.20**
Group-V	32.60±3.72**	54.00±2.23**

Serum SGPT and SGOT in the treated mice were significantly different from normal and diabetic control groups at P<0.001; * indicated the difference from normal group; whereas ** indicated the difference from Diabetic control group.

DISCUSSION

The present study evaluated the effect of two crude fruit extract EEMA & MEMA on aloxan induced diabetic mice and the results of the present investigation revealed that treatment with the *Morus alba* L. fruits significantly improve blood glucose tolerance. Due to the deleterious effects of diabetes on human body, there is an increasing interest in the development of preventive therapy for reducing various effectc of diabetes in human. *Morus alba* L. fruit is a safe natural antioxidant containing fruit and is found as a potential source of natural antioxidants such as total phenolics antioxidant, vitamin A, C, and E.[36,37]

In this study, the observed significant increase in of blood glucose level in diabetic rats could be due to the destruction of pancreatic β-cells by alloxan administration during diabetes. Sadighara, *et al*. reported that *Morus alba* leaves are useful for prevention of diabetes to be used as best choice of alternative medicine for treating diabetes [38]. Intriguingly, *Morus alba* fruit extract provided significant hypoglycemic effect to improve blood glucose tolerance in alloxan induced diabetic rats compared with alloxan induced diabetic control rats.

Deposition of cholesterol of the artery walls were removed by HDL-C and return them to the liver where they are broken down and eliminated from the body.[39] Therefore, decreased HDL-C observed in this study led us to make a hypothesis that HDL-C lowering effect of alloxan might be a key event for the development of alloxan-induced atherosclerosis. In our study, HDL-C level was significantly increased (P<0.001) following a significant decrease in LDL-C level (P<0.001) which is an indication of the reduction of the risk of coronary heart disease that are very common in diabetic patient. The level of HDL increased after administration of extract might be due to the presence of polyphenols in *Morus alba* L. fruit extracts.[40]

When the kidney tubules are prevented from removing the urea and other waste products from the blood, the blood urea becomes raised.[41] In this study, we found that increased serum urea levels in diabetic mice that might be an indication of the adverse effects of alloxan on kidney and liver. *Morus alba* L. fruits potentially inhibited the elevation of serum urea levels of diabetic rats.

Present study clearly indicated the ameliorating effects of *Morus alba* L. fruits *Morus alba* L. fruits on the changes of serum indices of diabetic rats. However, we did not clarify how *Morus alba* L. fruits showed the protective effect against alloxan action. One possibility was that the phenolic acids, the active ingredients of *Morus alba* L. fruits, might inhibit action by perturbation of the alloxan-mediated signal transduction pathways or by scavenging free radicals through its antioxidant property.

The SGOT and SGPT are the major serum hepatic enzymes that are used for liver function test. In liver damage, the elevated activities of these enzymes in serum are an indication. In this study, we found that in diabetic rats serum SGOT and SGPT activities were increased and all these results were consistent with the results showed by Islam et al.[42] The SGOT and SGPT level was increased in diabetic patient as an indication of the liver damage that back to their respective normal level after treatment with extract and glibenclamide that further strengthen the antidiabetic effect of the fruit extract. The SGOT and SGPT level in extract treated mice reduced significantly. These results indicated that *Morus alba* L. fruits had an effect on liver injury of diabetic ratys.

CONCLUSION

The present study revealed that *Morus alba* L. fruits reduce tne complications of diabetic rats. Thus, the results suggested that *Morus alba* L. fruits could be useful therapeutically in the future as herbal medicine for the treatment of diabetes.

ACKNOWLEDGEMENT

The authors would like to gratefully acknowledge National Science and Technology (NST), Dhaka Bangladesh, for their financial support and to the chairman, Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh.

REFERENCES

1. Schoenfelder, T., Cirimbelli, T.M., and Citadini, Z.V. Acute effect of *Tremamicrantha*on serum glukosa levels in normal and diabetic rats. *J. Ethnopharmacol*, 2006; 107(3): 456-459.
2. Shula R, Sharma SB, Purt D, Pabhu KM, Murthy PS, Medicinal plants for treatment of diabetes mellitus, Indian, J. Clin. Biochem, 2000; 15: 169 – 177.
3. Bajaj JS. Madan R. Diabetes in tropics and developing countries. IDF bull, 1993; 38(2): 56.
4. David MN. The pathophysiology of diabetic complications: How much does the glucose hypothesis explain? Ann. Intern. Med., 1996; 174(1 pt): 286 – 289.
5. Rosen CJ. Revisiting the rosiglitazone story–lessons learned. N Engl J Med, 2010; 363: 803-806.
6. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomed, 1995; 2: 133–169.
7. Orban, E.; Ercisli, S. Genetic relationships between selected Turkish mulberry genotypes (*Morus* spp) based on RAPD markers. *Gen. Mol. Res.*, 2010; *9*; 2176–2183.
8. *Zhengyi Wu, Zhe-Kun Zhou & Michael G. Gilbert (2013). "Morus alba". eFloras. Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA. Retrieved,* 27 June *2013.*
9. Suttie, J. M. (undated). FAO Report: *Morus alba* L.
10. Flora of North America, *Morus alba* Linnaeus, White mulberry, Russian mulberry, silkworm mulberry, mûrier blanc, moral blanco, 1753.
11. Biota of North America Program 2014 county distribution map.
12. Atlas of Living Australia, *Morus alba* L., White Mulberry.
13. SEINet, Southwestern Biodiversity, Arizona chapter photos, description, distribution map.
14. Arabshahi-Delouee, S.; Urooj, A. Antioxidant properties of various solvent extracts of mulberry (Morus Indica L.) leaves. Food Chem, 2007; 102; 1233–1240. [Cross Ref]
15. Sohn, B.-H.; Park, J.-H.; Lee, D.-Y.; Cho, J.-G.; Kim, Y.-S.; Jung, I.-S.; Kang, P.-D.; Baek, N.-I. Isolation and identification of lipids from the silkworm (Bombyx mori) droppings. J. Korean Soc. Appl. Biol. Chem, 2009; 52: 336–341. [CrossRef]
16. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effect on superoxide radicals. *Food Chem*, 1999; *64*: 555–559.
17. Ercisli, S. A short review of the fruit germplasm resources of Turkey. *Gen. Res. Crops Evol*., 2004; *51*: 419–435.
18. Gerasopoulos, D.; Stavroulakis, G. Quality characteristics of four mulberry (*Morus* spp.) cultivars in the area of Ghania Greece. *J. Sci. Food Agric*, 1997; *73*: 261–264.
19. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 2nd end, 1989.
20. Jiang, Y.; Nie, W.-J. Chemical properties in fruits of mulberry species from the Xinjiang province of China. Food Chem, 2015; 174, 460–466. [CrossRef] [PubMed]
21. Yang, X.; Yang, L.; Zheng, H. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food Chem. Toxicol, 2010; 48: 2374–2379. [CrossRef] [PubMed]
22. Kang, T.H.; Hur, J.Y.; Kim, H.B.; Ryu, J.H.; Kim, S.Y. Neuroprotective effects of the cyanidin-3-O-beta-D-glucopyranoside isolated from mulberry fruit against cerebral ischemia. Neurosci. Lett., 2006; 391: 122–126. [CrossRef] [PubMed]
23. Kim, A.J.; Park, S. Mulberry extract supplements ameliorate the inflammation-related hematological parameters in carrageenan-induced arthritic rats. J. Med. Food., 2006; 9: 431–435. [CrossRef] [PubMed]
24. Zhang, Z.; Shi, L. Anti-inflammatory and analgesic properties of cis-mulberroside a from Ramulus mori. Fitoterapia, 2010; 81: 214–218. [CrossRef] [PubMed]
25. Huang, H.-P.; Chang, Y.-C.; Wu, C.-H.; Hung, C.-N.; Wang, C.-J.. Anthocyanin-rich Mulberry extract inhibit the gastric cancer cell growth in vitro and xenograft mice by inducing signals of p38/p53 and c-jun. Food Chem, 2011; 129: 1703–1709. [CrossRef]

26. Harauma, A.; Murayama, T.; Ikeyama, K.; Sano, H.; Arai, K.; Takano, R.; Kita, T.; Hara, S.; Kamei, K.; Yokode, M. Mulbery leaf powder prevent atherosclerosis in apolipoprotein E-deficient mice. *Biochem. Biophys. Res. Commun*, 2007; *358*: 751–756.
27. Mohamed Y. Mahmoud. Natural Antioxidants Effect of Mulberry Fruits (*Morus nigra* and *Morus alba* L.) On Lipids Profile and Oxidative Stress in Hypercholestrolemic Rats Pakistan Journal of Nutrition, 2013; 12(7): 665-672.
28. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia, 2008; 51(2): 216–26.
29. Stalenhoef AF, de Graaf J. Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. Curr Opin Lipidol, 2008; 19: 355-361.
30. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC, Sowers JR. Diabetes and cardiovascular disease: a statement for health professionals from the American Heart Association. *Circulation*, 1999; 100: 1134 –1146.
31. Lesnik P, Chapman MJ. A new dimension in the vasculoprotective function of HDL: progenitor-mediated endothelium repair. Arterioscler Thromb Vasc Biol, 2006; 26: 965-967.
32. Wang JP, Wang SL, Lin Q, Zhang L, Huang D, Nq JC. Association of arsenic and kidney dysfunction in people with diabetes and validation of its effects in rats. Environ Int, 2009; 35: 507-11.
33. Tuot DS, Plantinga LC, Hsu CY, Jordan R, Burrows NR, Hedgeman E. Chronic kidney disease awareness is low among individuals with clinical markers of kidney dysfunction. Clin J Am Soc Nephrol, 2011; 6: 1838-1844.
34. *Johnston DE "Special considerations in interpreting liver function tests". Am Fam Physician, 1999;* 59*(8): 2223–30. PMID 10221307.*
35. McClatchey, Kenneth D. Clinical laboratory medicine. Lippincott Williams & Wilkins, 2002; 288. ISBN 978-0-683-30751-1. *Retrieved, 5 August 2011*.
36. Lin, J.Y. and C.Y. Tang, Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation, Food Chem., 2007; 101: 140-147.
37. Imran, M., H. Khan, M. Shah, R. Khan and F. Khan, Chemical composition and antioxidant activity of certain *Morus*species. J. Zhejiang, Univ-Sci., B. (Biomed. Biotechnol.), 2010; 11: 973-980.
38. Parisa Sadighara, Amir Moghaddam Jafari, Golamreza Jahed Khaniki, Nabi Shariati and Ali Akbar Lotfi Potential Therapeutic Effects of Morus alba Leaf Extract on Modulation Oxidative Damages Induced by Hyperglycemia in Cultured Fetus Fibroblast Cells Global Veterinaria, 2013; 10(1): 35-38.
39. Zhang Y, Zanotti I, Reilly MP, Glick JM, Rothblat GH, Rader DJ. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. Circulation, 2003; 108: 661-663.
40. Kanti, Bhooshan, Pandey and Syed Ibrahim Rizvi. Plant polyphenols as dietary antioxidants in human health and disease, 2009; 2(5): 270–278.
41. Ali N, Hoque MA, Haque A, Salam KA, Karim MR, Rahman A, Association between arsenic exposure and plasma cholinesterase activity: a population based study in Bangladesh. Environ Health, 2010; 9: 36.
42. Rahman M.S, Asaduzzaman M, Munira S, Hasan N, Rahman M.M, Hasan M, Begum M.M, Maniruzzaman M, Islam M, Khan M.M.H, Rahman M, Karim M.R and Islam M.A. Effects of *Coccinia Cordifolia* (Cucurbitaceae) Leaves extract on Hepatic and Cardiovascular Disease(CVD) markers in Streptozotocin induced diabetic albino rats, *International Journal Of Pharmacy & Technology*, 2015; *7(2): 9178-9189.*