Int. J. Biol. Res., 5(1): 7-10, 2017.

PROTECTIVE ROLE OF *MELANOTHAMNUS AFAQHUSAINII* AGAINST HYPERGLYCEMIA AND HYPERLIPIDEMIA IN ALLOXAN INDUCED DIABETIC RATS

Sheeba Wajid[1], Wajeeha Haider[1], Maria Azam[1], Amna Tariq[2], Khan Hira[1], Viqar Sultana[1] and Jehan Ara[2]

[1]*Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan*
[2]*Department of Food Science and Technology, University of Karachi, Karachi-75270, Pakistan*
[*]*Corresponding author's email: wajeehahaider@yahoo.co.uk*

ABSTRACT

Diabetes Mellitus (DM) is now considered as serious health issue around the globe. Seaweed also known as marine macro-algae plays a significant role against different diseases such as diabetes, cancer, anti-inflammatory, antithrombic. Present study investigated the anti-diabetic activity of ethanol and water extracts of *Melanothamnus afaqhusainii (MAH)*, red alga at 25mg/kg body weight in diabetic and normal rats. Female rats were administrated intraperitoneal injection of alloxan 100 mg/Kg and 50mg/ Kg body weight in two consecutive doses to induced diabetes, hypoglycemic effect of *M. afaqhusainii* was examined after 7 days of treatment and blood serum was collected and used for the analysis of the parameters such as blood glucose, serum cholesterol, serum TGs, serum LDL and serum HDL. Oral administration of ethanol and water extract resulted in a significant decrease ($p<0.05$) in serum glucose, in seaweed treated diabetic group when compared with its respective diabetic rats.

KEYWORDS: *Melanothamnus afaqhusainii*, Alloxan, Hyperglycemia, Hyperlipidemia.

INTRODUCTION

Diabetic mellitus (DM) is an endocrine disorder that is involved in the impairment of different organs (Cesur *et al*., 2007; Nogueira-Machao *et al*., 2003). DM can be classified as type I and type II. DM is associated with hyperglycemia and ineffectiveness of insulin`s action. Dietary carbohydrate are the main source of blood glucose and these carbohydrates are hydrolyzed by the action of α-amylase and α- glucosidase and ultimately absorbed in small intestine (Su *et al*., 2013). Therefore glycoside hydrolases inhibiting in the digestive organ results to delayed the glucose absorption and this approach has been promising for diabetes treatment (Levetan *et al*., 2013).

All over the world, millions of people have been diagnosed with DM and it has been predicted that the prevalence would be doubled by 2030 (Sarwar *et al*., 2010). In Pakistan, rate of diabetic mellitus increased from 7.6% to 11% in 2011, it is estimated to increase up to14 million (15%) by 2030. This health problem gives a challenge to health care policy makers and professionals in Pakistan (Bahada *et al*., 2014). Hyperglycemia have also been reported to induce hyperlipidemia, resulting in cardiovascular complications (Matheus *et al*., 2013).

Many researchers used edible plants for treating diabetes like garlic (*Allium sativa*), onion (*Allium cepa*) and *Momordica charantia* (Karela) (Kook *et al*., 2009; Joseph & Jini, 2013). Seaweeds or marine macro-algae are well known around the globe due to their high nutritional quality (Makkar *et al*., 2016). They also possesses various biological activities such as hepatic and reno-protective effect (Hira *et al*., 2016, 2017ab) and antioxidant activities (Tariq *et al*., 2011, 2015). Polyphenol-rich seaweed extracts has been reported to possess anti-diabetic effects via inhibiting α-amylase and α-glucosidase enzymes (Nwosu *et al*., 2010). Jia & Yin (2007) has been reported that natural seaweeds contain polysaccharides that have hypoglycemic effect. Present study describes the anti-diabetic activity of *Melanothamnus afaqhusainii*, a red alga in alloxan induced diabetic rats. This report also describes the protective role of alga in hyperlipidemic complications.

MATERIALS AND METHODS

Collection of seaweed and preparation of water and ethanol extracts: Seaweed was collected from Buleji, washed with tap water and dried under shed. The dried seaweed was grinded and extracted with distilled water to prepare water extract. The extract was lyophilized using Freeze dryer (Eyela, FD -1). For ethanol extract, powdered seaweed was extracted in distilled ethanol and concentrated under reduced pressure on rotary vacuum evaporator (Buchi R-200).

Animals: Albino-Wistar female rats (180-200g) were purchased from PCSIR, Karachi and housed in polypropylene cages and maintained at 22°C in a well-ventilated room. Tap water was supplied freely by means of inverted bottles.

Induction of diabetes: Alloxan monohydrates (Sigma) dissolved in normal saline was injected to rats intraperitoneally for two consecutive days in a dose of l00 mg/kg body weight and 50 mg/kg body weight. After 72 h, diabetes was confirmed with fasting blood glucose levels above 250 mg /dL. The blood glucose levels were checked by using glucose meter (Glucotrend 2(R) Roche, Serial No. 05146127001(2), glucose levels was checked at 3rd day after induction of diabetes.

Table 1. Effect of ethanol and water extracts of *Melanothamnus afaqhusainii* (MAH) on lipid profile of alloxan diabetic rats.

Treatments	Cholesterol	High density lipoprotein	Low density lipoprotein	Triglyceride
Normal control	78.66 ± 6.65^{b}	51 ± 3.60^{b}	16.13 ± 2.50^{c}	96.66 ± 6.65^{b}
Normal + MAH (25mg/kg) ethanol extract	47.66 ± 2.08^{e}	46.33 ± 0.57^{c}	12.86 ± 0.57^{c}	82.33 ± 2.08^{c}
Normal + MAH (25mg/kg) water extract	59 ± 5.29^{d}	63.33 ± 1.52^{a}	25.86 ± 1.52^{b}	91.33 ± 5.29^{b}
Diabetic control	115 ± 6.08^{a}	25.33 ± 2.08^{d}	47.33 ± 6.35^{a}	114.33 ± 4.61^{a}
Diabetic + MAH (25mg/kg) ethanol extract	80.33 ± 1.15^{b}	50.33 ± 2.08^{b}	11.26 ± 1.10^{c}	93.33 ± 3.21^{b}
Diabetic + MAH (25mg/kg) water extract	68.33 ± 2.88^{b}	42.66 ± 1.15^{c}	14.86 ± 0.94^{c}	91.33 ± 0.57^{b}

Data are presented as means ± SD (n=3). P-value<0.05 were consider significant

Table 2. Effect of ethanol and water extracts of *Melanothamnus afaqhusainii* (MAH) on liver and cardiac enzymes of alloxan diabetic rats.

Groups	Treatments	ASAT	ALAT	LDH	ALP
1.	Normal control	111.66 ± 4.6^{a}	35.66 ± 1.5^{b}	185.66 ± 4.9^{d}	146.33 ± 4.9^{d}
2.	Normal + MAH (25mg/kg) ethanol extract	105.66 ± 3.5^{d}	25.33 ± 2.5^{d}	303 ± 1^{c}	132 ± 3.5^{e}
3.	Normal + MAH (25mg/kg) water extract	93 ± 1^{e}	31.66 ± 1.5^{bc}	292.66 ± 10.4^{c}	186.66 ± 1.4^{c}
4.	Diabetic control	136.33 ± 3.2^{a}	42.66 ± 2.0^{e}	428.66 ± 6.8^{a}	307.33 ± 9.4^{a}
5.	Diabetic + MAH (25mg/kg) ethanol extract	27.66 ± 2.5^{b}	28 ± 2^{cd}	375 ± 11.2^{b}	156.33 ± 4.0^{d}
6.	Diabetic + MAH (25mg/kg) water extract	114.33 ± 1.5^{c}	33.33 ± 4.6^{b}	381.33 ± 9.0^{b}	240 ± 10.1^{b}

Data are presented as means ±SD (n=3). P-value<0.05 were consider significant

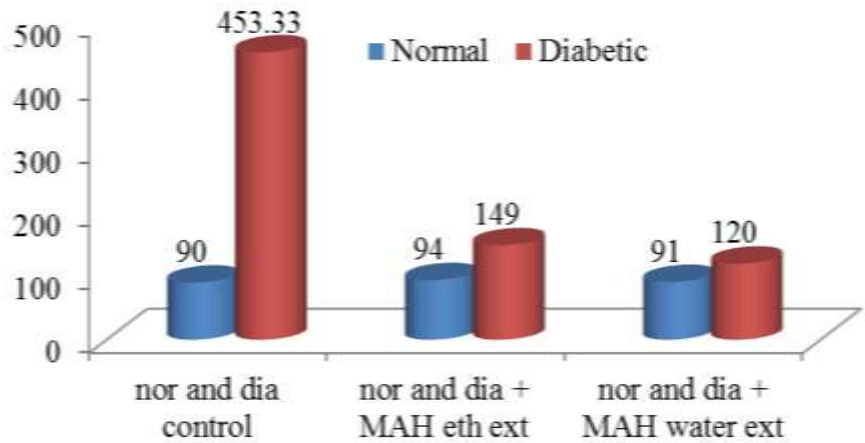

Fig. 1. Effect of ethanol and water extracts on normal and alloxan diabetic rats.
Series 1= normal, Series 2= diabetic

Experimental design

Rats were divided into i) normal control a) following models:

Group 1: Normal control

a. Normal rats received 1ml/kg water orally, daily for 4 days.

Group 2: Normal rats treated with seaweeds

This group was divided into 2 groups:

a. Ethanol extract treated group: Normal rats treated with ethanol extract of seaweed (25mg/kg) orally, daily for 4 days.

b. Water extract treated group: Normal rats treated with water extract of seaweed (25mg/kg) orally, daily for 4 days.

Group 3: Diabetic control rats

a. Diabetes induced in rats by using alloxan received 1ml/kg water orally, daily for 4 days.

Group 4: Diabetic treated group

This group was divided into 2 groups:

a. Ethanol extract treated group: Diabetes induced rats treated with ethanol extract of seaweed (25mg/kg) orally, daily for 4 days.

b. Water extract treated group: Diabetes induced rats treated with water extract of seaweed (25mg/kg) orally, daily for 4 days.

Blood collection and separation of serum: After one week, 15 hours fasted animals were decapitated and blood was collected. The clotted blood was centrifuged and after that serum was collected by centrifugation at 3000 rpm for 10 minutes. Serum was stored at -20°C untill analysis. All absorbance for estimation of desired blood parameter were estimated on Microlab 300, Semi- Automated Analyzer by different Kits method (Merck & Ecoline).

Statistical Analysis

Results were presented as mean using one way analysis ($p<0.05$) on statistical computer software program, COSTAT.

RESULTS

Treatment with seaweed extract shows decreased in glucose levels in diabetic model rats when compared with untreated diabetic control rats (Fig. 1). Results of this study have shown significant increase ($p<0.05$) in triacylglycerols in alloxan induced diabetic group. Seaweed extract treated shows decreased in triglycerol levels in both diabetic and normal model rats when compared with their respective control rats (Table 1). Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT) and Alkaline Phosphatase (ALP) are released into the serum especially in hepatic damage. Data presented in this study on serum showed that ASAT, ALAT, ALP and lactate dehydrogenase (LDH) activities were significantly ($p<0.05$) altered across the groups (normal and diabetic seaweed treated rats) except ALP and LDH whose concentration was not decreases in normal rat modal treated with water and ethanol extract of seaweed (Table 2). This clearly demonstrates that consumption of these extracts may not produce harmful effects on the liver and pancreas of animal.

DISCUSSION

Diabetes mellitus (DM) is the endocrine disorder that affects about millions of people worldwide (WHO, 2016). Though a lot of medicines have been discovered including insulin, biguanides, sulfonyl urease and thiazolidinediones, but no drug relieves diabetic complications so there is still need to search for new drugs (Grover *et al*., 2000; Sharifuddin *et al.,* 2015).

Seaweeds are frequently used in the different parts of the world (Jimenez-Escrig and Sanchez-Muniz, 2000). Nowadays seaweeds are known to have several activities including anti-bacterial, anti-tumor, anti-leukemic, anti-viral, anti-hyperlipidemic activity etc. We have determined hypoglycemic activity by observing blood glucose, lipid profile and enzymatic assays in present study.

It has been reported that flavonoids of various plants have possess anti-diabetic activity (Zarzuelo *et al.,* 1996; Kim *et al.,* 2004). Some seaweed polyphenols showed hypoglycemic properties (Kang *et al.,* 2005). Results showed that ethanol and water extract of MAH (25 mg/kg) resulted in a significant reduction ($p<0.05$) in the fasting blood glucose levels of diabetic rats, when compared with its respective diabetic control group. Therefore we can say that MAH may contain some flavonoids which help to stimulate the insulin release from the pancreatic beta cells.

It is reported that diabetes caused hyperlipidemia as its complication (Nakai *et al.,* 2005). In our present study we found that water and ethanol extract of MAH, significantly reduced ($p<0.05$) the level of blood cholesterol in normal and diabetic both rat model. It may be due to the presence of fibers found in seaweed. It is also reported that in intestine fibers of seaweed may binds with cholesterol, bile salts, and inhibit the circulation of enterohepatic bile salts as reported by (Anderson, 1994).

In our present study the LDL level of both normal and diabetic rat model have significantly ($p<0.05$) reduced, it may be due to the presence of dietary fibers as Jeppesen *et al.,* (1998), confirmed that dietary fiber decreased LDL and inhibits cholesterol absorption in the intestine, which subsequently increased the excretion rate of bile salts and cholesterol. Therefore we can say that this seaweed helps to reduce LDL that may be due to the presence of fibers.

CONCLUSION

From our investigation it may concluded that the fibers of this seaweed *M. afaqhusainii* lowered the serum glucose, serum total cholesterol, serum LDL and triglyceride level, meanwhile increasing the HDL level of hyperglycemic rats. The extracts of seaweed also decreased enzymes including alanine amino transferase, lactate dehydrogenase, alkaline phosphatase and aspartate amino transferase in both normal and diabetic rat's model. These results suggested that this seaweed have potent anti-diabetic effects.

REFERENCES

Anderson, H. (1994). Effects of cabohydrates on the excretion of bile acids, cholesterol, and fat from the small bowel. *Am. J. Clin. Nutr*., 159: 785.

Bahada, H., S. Mostafalou and M. Abdollahi. (2014). Growing burden of diabetes in Pakistan & the possible role of arsenic & pesticides. *J. Diabetes Metab. Disord*., 13: 117.

Cesur, M., D. Corapcioglu, A. Gursoy, S. Gonen, M. Ozduman, R. Emral and N. Kamel. (2007). A comparison of glycemic effects of glimepiride, repaglinide, and insulin glargine in type 2 diabetes mellitus during Ramadan fasting. *Diabetes Research and Clinical Practice*, 75(2): pp. 141-147.

Grover, J.K., V. Vats and S.S. Rathi. (2000). Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. *J. Ethnopharmacol*., 73(3): 461-470.

Hira, K., V. Sultana, J. Ara, S. Ehteshamul-Haque and M. Athar. (2016). Hepatoprotective potential of three *Sargassum* species from Karachi coast against carbon tetrachloride and acetaminophen intoxication. *J. Coastal Life Med*., 4: 10-13.

Hira, K., R.M. Tariq, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2017a). Effect of seaweeds occurring at Karachi coast on mosquito larvae and liver function in rats. *Pak. J. Pharm. Sci.*, 30: 387-391.

Hira, K., V. Sultana, J. Ara and S. Ehteshamul-Haque. (2017b). Protective role of *Sargassum* species in liver and kidney dysfunctions and associated disorders in rats intoxicated with carbon tetrachloride and acetaminophen. *Pak. J. Pharm. Sci.*, 30: .721-728.

Jeppesen, J., H.D. Hein, Suadicani and F. Gyntelberg. (1998). Tryglyceride concentration and ischaemic heart diseases in the copenhagen male study. *Circulation*, 97: 1029-1036.

Jia, S.T. and W.Z. Yin. (2007). Study on hypoglycemic effect of polysaccharide from spirulina, *Acad. Periodic. of Farm Prod. Proc*., 88: 46-48.

Jimenez-Escrig, A.B. and F.J. Sanchez-Muniz. (2000). Dietary fiber from edible seaweeds, chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res*., 20: 585-598.

Joseph, B. and D. Jini. (2013). Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. *Asian Pac. J. Trop. Dis*., 3(2): 93-102.

Kang, KA. (2005). Antioxidant activity of ethanol extract of *Callophyllis japonica*. *Phytother Res*., 19: 506-510.

Kim, H.Y., B.H. Moon, H.J. Lee and D.H. Choi. (2004). Flavonoids glycosides from the leaves of *Eucommia ulmoides* with gycation inhibitory activity. *J Ethnopharmacol*., 93: 227-230.

Kook, S. Gun-Hee Kim and Kiheon Choi. (2009). The antidiabetic effect of onion and garlic in experimental diabetic rats: Meta-analysis. *Journal of Medicinal Food*, 12(3): https://doi.org/10.1089/jmf.2008.1071.

Levetan, C., P. Pozzilli, L. Jovanovic and D. Schatz. (2013). Proposal for generating new beta cells in a muted immune environment for type 1 diabetes. *Diabetes Metab. Res. Rev*., 29: 604-606.

Makkar, H.P.S., G. Tran, V. Heuzé, S. Giger-Reverdin, M. Lessire, F. Lebas and P. Ankers. (2016). Seaweeds for livestock diets: A review. *Anim. Feed Sci. Technol*., 212: 1-17.

Matheus, A.S.D.M., L.R.M. Tannus, R.A. Cobas, C.C.S. Palma, C.A. Negrato and M.D.B. Gomes. (2013). Impact of diabetes on cardiovascular disease: An update. *Int. J. Hypertension*, Vol. 2013, Article ID 653789, 15 pages http://dx.doi.org/ 10.1155/ 2013/653789

Nakai, M., Y. Fukui and S. Asami. (2005). Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *J. Agric. & Food Chem*., 53(11): 4593-4598.

Nogueira-Machao, J., F. Silva and M. Mares-Guia. (2003). Discrimination between granulocytes from type I and type II diabetics patients by calorimetry. *Thermochimica Acta*., 395: 115-120.

Nwosu, F., J. Morris, V.A. Lund, D. Stewart, H.A. Ross and G.J. McDougall. (2011). Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chem.*, 126: 1006-1012

Sarwar, N., P. Gao, S.R. Seshasai, R. Gobin, S. Kaptoge, E. Di Angelantonio, E. Ingelsson, D.A. Lawlor, E. Selvin, M. Stampfer, C.D. Stehouwer, S. Lewington, L. Pennells, A. Thompson, N. Sattar, I.R. White, K.K. Ray and J. Danesh. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *Lancet*, 375: 2215-2222.

Sharifuddin, Y., Y.X. Chin, P.E. Lim and S.M. Siew-Moi Phang. (2015). Potential bioactive compounds from seaweed for diabetes management. *Mar. Drugs*, 13: 5447-5491.

Su, C.H., M.N. Lai and L.T. Ng. (2013). Inhibitory effects of medicinal mushrooms on alpha-amylase and alpha-glucosidase-enzymes related to hyperglycemia. *Food Funct.*, 4: 644-649.

Tariq, A., J. Ara, V. Sultana, S. Ehteshamul-Haque and M. Athar. (2011). Antioxidant potential of seaweeds occurring at Karachi coast of Pakistan. *J. Appl. Bot. Food Qual.*, 84: 207-212.

Tariq, A., M. Athar, J. Ara, V. Sultana, S. Ehteshamul-Haque and M. Ahmad. (2015). Biochemical evaluation of antioxidant activity in extracts and polysaccharide fractions of seaweeds. *Global J. Environ. Sci. Manage*., 1(1): 47-62. Winter 2015 ISSN 2383 - 3572.

WHO. 2016. Global Report on Diabetes. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

Zarzuelo, A., I. Jimenez and M.J. Gamez. (1996). Effects of luteolin 5-O-betarutenoside in STZ-induced diabetic rats. *Life Sci*., 58: 2311-2316.

(Received March 2017; Accepted May 2017)