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Abstract:

Diabetes mellitus (DM) is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. The present study was designed to investigate the effect of brown algae (*Sargassum subrepandum*) powder (BAP) on DM and is complications (hyperlipidemia and immune disturbances) induced by alloxan in rats.Thirty six rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet (BD) and the second main group (30 rats) was with alloxan then classified into five sub groups as follow: group (2), fed on BD as a model control, and groups (3, 4, 5 and 6) fed on BD containing 2.5, 5.0, 7.5 and 10 % BAP, respectively. At the end of the experiment (4 weeks), treatment of rats with alloxan, model control group, induced a significant ($p \leq 0.01$) increasing in serum glucose concentration by the ratio 165.38% compared to normal control group. Dietary intervention with BAP (2.5, 5.0, 7.5 and 10%) in rats for 28 days led to significantly ($p \leq 0.05$) decreasing the levels of serum glucose which recorded 155.71, 137.08, 108.64 and 91.14% compared to the normal control group, respectively. The rate of decreasing in serum glucose was exhibited a dose- dependent increase with the levels of BAP intervention. Also, BAE was effective in protecting against DM complications including serum lipid profile (TG, TC, HDL-c, LDL-c and VLDL-c) and serum immunological parameters (Alb and TNF-α). Therefore, we recommended like of that algae powder by a concentrations up to 10% to be included in our daily diets, drinks and food supplementation.

Keywords:

Sargassum subrepandum, serum glucose, serum immunological parameters, serum lipid profile, albumin, TNF-α.

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مستخلص البحث:

يُعرَّف داء السكري بأنه حالة يتم فيها تنظيم استتباب الكربوهيدرات والتمثيل الغذائي للدهون بشكل غير صحيح بواسطة الأنسولين .صممت الدراسة الحالية لمعرفة تأثير مسحوق الطحالب البنية (*Sargassum subrepandum*) (BAP) على DM والمضاعفات (فرط شحميات الدم والاضطرابات المناعية) التي يسببها الألوكسان في الجرذان ، وتم تقسيم ستة وثلاثون فأرًا إلى مجموعتين رئيسيتين ، المجموعة الأولى)المجموعة 1 ، 6 جرذان) لا تزال تتغذى على النظام الغذائي الأساسي (BD) والمجموعة الرئيسية الثانية (30 جرذًا) كانت مع الألوكسان ثم صنفت إلى خمس مجموعات فرعية على النحو التالي: المجموعة (2) ، تغذت على BD كعنصر تحكم نموذجي ، و مجموعات (3 و 4 و 5 و 6) تغذى على BD تحتوي على 2.5 و 5.0 و 7.5 و 10٪ من BAP على التوالي .في نهاية التجربة (4 أسابيع) أدت معاملة الجرذان بألوكسان ، مجموعة التحكم النموذجية إلى زيادة معنوية ($p\leq0.01$) في تركيز الجلوكوز في الدم بنسبة 165.38٪ مقارنة بمجموعة التحكم العادية .أدى التدخل الغذائي مع BAP (2.5 و 5.0 و 7.5 و 10٪) في الجرذان لمدة 28 يومًا إلى انخفاض معنوي ($p\leq0.05$) في مستويات الجلوكوز في الدم التي سجلت 155.71 و 137.08 و 108.64 و 91.14٪ مقارنة بمجموعة التحكم العادية ، على التوالي .أظهر معدل النقص في الجلوكوز في الدم زيادة تعتمد على الجرعة مع مستويات تدخل BAP. أيضًا ، كان BAE فعالًا في الحماية من مضاعفات DM بما في ذلك تحليل الدهون في الدم (TG ، TC، HDL-c، LDL-c، VLDL-cوالمعلمات المناعية في الدم Alb ، α -TNF.لذلك ، أوصينا بمثل مسحوق الطحالب هذا بتركيزات تصل إلى 10٪ ليتم تضمينه في وجباتنا الغذائية اليومية والمشروبات والمكملات الغذائية.

الكلمات المفتاحية:

***Sargassum subrepandum*، الجلوكوز في الدم ، المقاييس المناعية في الدم ، صورة الدهون في الدم ، الألبومين ، معامل النخر الورمى- الفا..**

Introduction

Diabetes mellitus (DM) is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. These results lead to elevate fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia (WHO, 1999 and Tiwari and Madhusudana, 2002). DM is widely distributed all over the world including Egypt, and nearly one of each 10 person is diabetic. About 537 million people worldwide have diabetes and the majority living in low-and middle-income countries (IDF, 2021). Also, in 2021, global statistics have shown that diabetes is one of the main causes of death in the world, as one person will die from this disease every five seconds (IDF, 2021 and WHO, 2021). In Egypt, the number of people living with diabetes is estimated at 10.93 million in 2020, and this figure is predicted to rise to 13.74 million by 2030 (IDF, 2021). Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes.

Reports from different international organizations indicate that DM is one of the major killers of our time, with people in Southeast Asia and Western Pacific as well as Middle East being most at risks (Tiwari A, Madhusudana, 2002). DM is one of the world's most common chronic diseases as common, morbid and costly disease which changing lifestyles drive to reduced physical activity and increased obesity with its complications (Wild et al., 2004). It is the leading cause of new blindness, amputation, cardiovascular symptoms, oxidative stress and end-stage renal diseases and it also contributes to a host of other conditions (Lu et al., 2014). In 2017, data for the total medical cost of diabetes in Egypt was calculated to be (EGP 25.2 billion) equivalent to (USD 3.5 billion), using the exchange rate of *EGP 1 = USD* 0.13976 (Assaad-Khalil et al., 2017). Thus, there is a great interest in novel approaches to indirect DM management. However, despite the increasing number of drugs available for DM treatment, significant improvements in the control of DM have not been observed (Saydah et al., 2004 and Wang et al., 2015). Guidance suggests that DM complications can be markedly attenuated with appropriate control of hyperglycemia and with successful treatment of hyperlipidemia and enhancement of immunodeficiency (Mohamed, 2023). The previous studies have confirmed that the treatment/intervention with natural antioxidants can reduce diabetic complications (Sayed Ahmed et al., 2016; El-Nassag et al., 2019; Elhassaneen et al., $2021_{a\text{-}c}$). Also, the continuous efforts to discover new antioxidants as useful drug candidates to combat diabetic complications are on-going.

Brown algae (Family, *Phaeophyceae*) is a well-known Egyptian in traditional medicine which has been clinically used in Egypt, Japan and other countries for more than several centuries. Algae of the genus *Sargassum* have been shown to be a rich

source of bioactive compounds including polyphenols, polysaccharides, carotenoids, anthocyanins, sterols, alginates, terpenoids, nucleotides, , steroids, and other bioactive ingredients (Abd Elalal et al., 2021; Elhassaneen et al., 2021-d; Fayez, 2021; Abdelrahmam, 2022).Other components include peptides, free mannitol, minerals , vitamins, fatty compounds, and various pigments (Chapman and Chapman, 1980, Helen, 2003 and El-Gamal, 2020). Many are active against current major chronic diseases such as anti-obese and carcinogenic and a range of other biological activities (El-Gamal, 2020; Elhassaneen et al., 2020-a; Mohamed, 2020; Abd Elalal et al., 2021; Fayez, 2021; Abdelrahmam, 2022; Elhassaneen et al., 2022). However, to the date, few detailed studies described the effects of brown algae powder on blood glucose and lipid compositions in alloxan induced diabetic rats. According to our knowledge, there are few studies in the literature evaluating the feasibility of using brown algae as a potential antidiabetic agent. Also, the co-existing of the multi-active compounds in brown algae powder might provide a stronger synergistic or positively effect on improving the diabetic status than the consumption of the single active compound. Therefore, in this study, alloxan-induced-diabetic rats are used to investigate the changes in the hyperglycemia and hyperlipidemia as well as immune disturbances after brown algae (*Sargassum subrepandum*) treatment.

Materials and Methods

Material

Brown algae

Brown algae (*Sargassum subrepandum*) samples were obtained and toxonomic confirmation achieved in Faculty of Agricultures, Alexandria University, Alexandria, Egypt.

Chemicals and kits

Alloxan was purchased from Sigma Chemical Co., St. Louis, MO. Casein was purchased from Morgan Company for Chemicals. Cairo, Egypt. Vitamins and salts mixtures, organic solvents, buffers and other chemicals were obtained in analytical grade from El-Ghomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Kit's assays for glucose, was purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. Triglycerides (TG) and total cholesterol (TC), were purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. HDL and LDL/VLDL cholesterol assay provided by Cell Biolabs, Inc., San Diego, CA, USA.

METHODS

Preparation of brown algae powder (BAP)

BAP was prepared according to the method of Elhassaneen et al, (2022) and kept in suitable closed bottles as well as stored at room temperature until use.

Biological Experiments

Ethical approval

The biological experiments of the present study were approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 19- SREC-02-2022).

Animals

Animals used in this study, adult male albino (*Sprague Dawley*) rats, (150± 7.9g per each) were purchased from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

Basal diet (Standard diet)

The basal diet (BD), and salt and vitamin mixtures were prepared according to Reeves *et al.,* (1993).

Induction of diabetic mellitus

Diabetes was induced in normal healthy rats (30 rats) by subcutaneous injection with freshly prepared alloxan monohydrate in saline at a dose level of 150 mg/ kg body weight (Lazarow and Palay, 1954). Immediately after injection animals were received 5% glucose solution over night to overcome drug induced hypoglycemia (Wohaieb and Godin, 1987and Kakkar *et al.*,1998). After one week fast blood glucose (FBG) was analyzed using a specific by a drop of blood was obtained from tail vein and subjected to a strip of haemogluco test. All rats with FBG >200 mg/dl were considered to be diabetics and included in the study.

Experimental design

Biological experiments were achieved in accordance with the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences Rules (NRC, 1996). Rats (n=36) were housed individually in wire cages in a room maintained at 25 ± 1.8 ^{0}C and kept under normal healthy conditions. All rats were fed a basal diet (BD) for one week before beginning the experiment for acclimation. Then, the rats were divided into two main groups, the first group, normal control, (Group 1, 6 rats) still fed on BD and the other main group (30 rats) was used for diabetes induction and classified into five sub groups as follow: group (2), model control, fed on BD only as a positive control (rats with diabetes) and groups (3-6) fed on BD containing 2.5, 5.0, 7.5 and 10.0% BAP, respectively. BAP concentrations were selected for present experiments based on many of the results of previous

studies (**El-Gamal, 2020 and Elhassaneen et al., 2020-a).** For 28 days, each of the above groups was housed in a single cage. The diet consumed was recorded every day and body weight was recorded every week during the experimental period (28 days). The body weight gain (BWG, %), food intake (FI) and food efficiency ratio (FER) were determined according to Chapman *et al*., (1959).

Blood sampling

After 28 days, end of the experiment, after 12 hours of fasting, rats were anesthetized under the influence of ether and blood samples were collected using the abdominal aorta. Blood samples were taken in a dry clean glass centrifuge tubes and left to clot in water bath (37°C) for 28 minutes, then centrifuged for 10 minutes at 3000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen at -20°C till analysis according to the method described by **Schermer (1967).**

Hematological Analysis

Serum glucose

Serum glucose was determined by the colorimetric method explained by Tietz, (1976).

Serum lipid profile,

Triglycerides (TGs), total cholesterol (TC), HDL-Cholesterol, and LDL-cholesterol and VLDL-cholesterol were determined in serum according to the methods of Fossati, (1982), Richmod (1973), Lopes-Virella et al., (1977) and Ahmadi et al., 2008, respectively.

Albumin

Albumin was determined in plasma using the method explained by Doumas et al., (1971).

TNF-α assay

TNF-α was determined by a sandwich enzyme-linked immeunosorbent assay (ELISA), utilizing two monoclonal antibodies directed against separate antigenic determinants on rat TNF- α according to the method described by Petrovas et al., (1999).

Statistical Analysis

All measurements were carried out put in triplicates and expressed as mean± standard deviation (SD). Statistical analysis was performed with the Student *t*-test and MINITAB-12 computer program (Minitab Inc., State College, PA).

Results and Discussion

Effect of BAP intervention on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of diabetic rats induced by alloxan

The effect of BAP intervention on BWG, FI and FER of diabetic rats induced by alloxan were shown in Tables (1 and 2). Such data indicated that the model control group, alloxan-treated rats, exhibited significantly ($p \leq 0.05$) decreased in BWG (-34.07), FI (-27.87) and FER (-23.75) compared to the normal group. The feeding intervention with BAP (2.5, 5.0, 7.5 and 10%) in feeding rats for 28 days significantly ($p \leq 0.05$) increases the levels of BWG, FI and FER by different rates. The rate of increasing in these biological parameters exhibited a dose-dependent increase with the levels of BAP intervention. At the end of the experiments, 28 days, the values of BWG, FI and FER were also recorded close to the values of the normal control group. Such data are in agreement with that reviewed by several authors (**El-Gamal, 2020;** Mohamed, 2020; **Fayez, 2021;** Elsemelawy **et al., 2021**; Elhassaneen et al., 2022**).** All of these studies indicated that the increasing in BWG, FI and FER as the result of BAP intervention could be attributed to its high nutrients and bioactive constituent's content, and their different biological activates.With the same context, **Tahoon, (2019), Elhassaneen *et al.*, 2021** a and b reported that injection of rats by CCl_4 inducing diabetic effects beside liver disorders which led to decrease the BWG, FI and FER. Such biological parameters disorders were improved by intenvension with plant parts contains bioactive constituents such as found in BAP. Other studies reported that liver rat's disorders induced by diabetes reveal significant decreasing of the body weight and FI (**Hamzawy *et al.*, 2013; Abd El-Rahman, 2021; Elhassaneen et al., 2021 c and d). Also,** numerous studies have shown that diabetes and liver disease can result in symptoms of malnutrition, which constitute feed intake, poor digestion, malabsorption, and abnormalities in metabolism and storage of macro- and micronutrients **(Morresion and Hark, 1999; Elhassaneen *et al.*, 2014; Sayed Ahmed *et al.*, 2016; Aly *et al.*, 2017; Abd El-Rahman, 2021).**

Table 1. Effect of BAP intervention on BWG, FI and FER of diabetic rats induced by alloxan

Group	BWG (%)	FI (g/day/rat)	FER
Normal control	0.91 ± 0.06^{a}	10.98 ± 0.55^{a}	0.080 ± 0.005^{a}
Model control	0.60 ± 0.02^{c}	7.92 ± 0.49^{b}	0.061 ± 0.020^{c}
BAP intervention (2.5%)	0.65 ± 0.05^{bc}	8.25 ± 0.60^{b}	0.065 ± 0.008^{bc}
BAP intervention (5.0%)	0.71 ± 0.03^{b}	8.91 ± 0.71^{b}	0.068 ± 0.007^{b}
BAP intervention (7.5%)	0.79 ± 0.02^{ab}	9.74 ± 0.73^{ab}	0.073 ± 0.006^{ab}
BAP intervention (10%)	0.82 ± 0.05^{a}	10.14 ± 0.76^{a}	0.077 ± 0.009^{a}

Results are expressed as means ±SD (n = 6). Means with different superscript letters on the same column show significant differences at $P \leq 0.05$. Normal control: normal rats without intervention; Model control: alloxan induced diabetic rats without intervention; BAP intervention: alloxan induced diabetic rats with BAP intervention. BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio.

Table 2. Effect of BAP intervention on BWG, FI and FER of diabetic rats induced by alloxan

Group	BWG (% of change)	FI (% of change)	FER (% of change)
Normal control	-----	-----	-----
Model control	-34.07	-27.87	-23.75
BAP intervention (2.5%)	-28.57	-24.86	-18.75
BAP intervention (5.0%)	-21.98	-18.85	-15.00
BAP intervention (7.5%)	-13.19	-11.29	-8.75
BAP intervention (10%)	-9.89	-7.65	-3.75

Results are expressed as means (n = 6). Normal control: normal rats without intervention; Model control: alloxan induced diabetic rats without intervention; BAP intervention: alloxan induced diabetic rats with BAP intervention. BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio

Effect of BAP intervention on serum glucose and insulin of diabetic rats induced by alloxan

Effect of BAP intervention on serum glucose of diabetic rats induced by alloxan was in Table (3). From such data it could be noticed that treatment of rats with alloxan, model control group, induced a significant ($p \leq 0.01$) increasing in serum glucose concentration by the ratio 165.38% compared to normal control group. Dietary intervention with BAP (2.5, 5.0, 7.5 and 10%) in rats for 28 days led to significantly ($p \leq 0.05$) decreasing the levels of serum glucose which recorded 155.71, 137.08, 108.64 and 91.14% compared to the normal control group, respectively. The rate of decreasing in serum glucose was exhibited a dose-dependent increase with the levels of BAP intervention. Alloxan is widely used as inducer of diabetes in experimental animals by destroying the insulin-secreting cells of Langerhans islet in pancreas **(Mathe, 1995; Elhassaneen *et al.*, 2021 c, d and e).** The presented data show that serum glucose levels were significantly increased in diabetic group compared to normal rats. The chronic hyperglycemia could arise from a defect in insulin secretion as in case of insulin dependent diabetes mellitus **(Kandeil *et al.*, 2007).** Aso, serum glucose levels decreased in diabetic group intervention BAP in comparison with the diabetic, model group. Such hypoglycemic effect of BAP in alloxan-induced diabetic rats may be related to the diverse bioactive constituents found in BAP. For example, several studies reported that brown algae are a rich source of different bioactive constituents including polyphenols, carotenoids, polysaccharides, terpenoids and flavonoids, and vitamins (A, B and E). These compounds are known for their vital biological properties including antioxidant and scavenging activities, inhibition of lipid oxidation, improve glucose response and alleviating insulin resistance associated with type 2 diabetes **(Lenzen, 2008; Elmaadawy et al., 2016; Aly et al., 2017; Elbasouny et al., 2019; Elhassaneen et al., 2012; 2021 c,d and e).**

Table 3. Effect of BAP intervention on serum glucose and insulin of diabetic rats induced by alloxan

Group	Serum glucose conc. (mg/dL)	Serum glucose conc. (% of change)
Normal control	97.60 ± 6.11 [e]	-----
Model control	259.01 ± 12.88 [a]	165.38
BAP intervention (2.5%)	249.57 ± 12.78 [a]	155.71
BAP intervention (5.0%)	231.39 ± 8.29 [bc]	137.08
BAP intervention (7.5%)	203.64 ± 10.09 [c]	108.64
BAP intervention (10%)	186.56 ± 8.59 [d]	91.14

Results are expressed as means ±SD (n = 6). Means with different superscript letters on the same column show significant differences at $P \leq 0.05$. Normal control: normal rats without intervention; Model control: alloxan induced diabetic rats without intervention; BAP intervention: alloxan induced diabetic rats with BAP intervention

Effect of BAP intervention on serum lipid profile of diabetic rats induced by alloxan

The effect of BAP intervention on serum lipid profile of diabetic rats induced by alloxan was shown in Tables (4 and 5) and Tables (4 and 5). From such data it could be noticed that alloxan induced a significant increased ($p \leq 0.05$) in TG (223.73%), TC (76.78%), LDL (594.93%) and VLDL-c (223.73%) while significant decreased ($p \leq 0.05$) in HDL (-47.93%) compared to normal control group. Intervention with BAP in rat diets by concentrations of 2.5, 5.0, 7.5 and 10% leads to increase in TG, TC, LDL-c and VLDL-c by the ratio of 205.08, 188.14, 118.64 and 64.41%;75.28, 72.66, 43.45 and 21.35%; 578.64, 516.69, 312.27 and 179.38%; and 205.08, 188.14, 118.64 and 64.41%, respectively. The rate of increasing in all determined serum lipid profile parameters were exhibited a dose-dependent decrease with the levels of BAP intervention. The opposite direction was observed for the HDL levels.

In general, cardiovascular diseases (CVDs) represent one of the most complications induced by DM. It is a major health problem in both industrial and developing countries including Egypt. They are the leading cause of death globally, taking an estimated 17.9 million lives each year (https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1). CVDs are a group of disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. Several studies have now shown that blood elevated concentrations of TC or LDL-c in the blood are powerful risk factors for CVDs, whereas high concentrations of HDL-c or a low LDL-c (reviewed in Bedawy, 2008, Aly et al., 2017). The composition of the human diet plays an important role in the management of lipid and lipoprotein concentrations in the blood. For example, reduction in saturated fat and cholesterol intake has traditionally been the first goal of dietary therapy in lowering the risk for CVDs. In recent years,

however, the possible hypocholesrerolemic effects of several dietary components, such as found in BAP including, polyphenols, flavonoids, alkaloids, carotenoids, phytosterols and organosulfur compounds etc., have attracted much interest. Such compounds exerts their beneficial effects on cardiovascular health by antioxidant, scavenging and anti-inflammatory activities (Kuhlmann et al., 1998; Bedawy, 2008; Aly, 2017). LDL oxidation and endothelial cell damage is believed to be involved in the early development of atherosclerosis (Kaneko et al., 1994). Researchers found that presence of polyphenolics and carotenoids significantly reduced LDL oxidation in vitro from various oxidases including 15-lipoxygenase, copper-ion and linoleic acid hydroperoxide (Kaneko et al., 1994 and Aly et al., 2017).

Table 4. Effect of BAP intervention on serum lipid profile of diabetic rats induced by alloxan

Group	TG (mmol/l)	HDL-c (mmol/l)	TC (mmol/l)	LDL-c (mmol/l)	VLDL-c (mmol/l)
Normal control	0.59 ± 0.08^{d}	2.17 ± 0.03^{a}	2.78 ± 0.30^{c}	0.49 ± 0.06^{d}	0.12 ± 0.08^{d}
Model control	1.91 ± 0.21^{a}	1.13 ± 0.04^{c}	4.91 ± 0.93^{a}	3.40 ± 0.14^{a}	0.38 ± 0.21^{a}
BAP intervention (2.5%)	1.80 ± 0.17^{a}	1.19 ± 0.14^{c}	4.87 ± 0.48^{a}	3.32 ± 0.19^{b}	0.36 ± 0.17^{a}
BAP intervention (5.0%)	1.70 ± 0.20^{ab}	1.44 ± 0.08^{bc}	4.79 ± 0.29^{a}	3.01 ± 0.41^{ab}	0.34 ± 0.20^{a}
BAP intervention (7.5%)	1.29 ± 0.09^{b}	1.71 ± 0.22^{b}	3.98 ± 0.37^{ab}	2.02 ± 0.17^{b}	0.26 ± 0.09^{b}
BAP intervention (10%)	0.97 ± 0.12^{c}	1.81 ± 0.25^{b}	3.37 ± 0.19^{b}	1.37 ± 0.09^{c}	0.19 ± 0.12^{c}

Results are expressed as means ±SD (n = 6). Means with different superscript letters on the same column show significant differences at $P \leq 0.05$. Normal control: normal rats without intervention; Model control: alloxan induced diabetic rats without intervention; BAP intervention: alloxan induced diabetic rats with BAP intervention. TG, triglycerides; HDL-c, high density lipoprotein-cholesterol; TC, total cholesterol; LDL-c, low density lipoprotein-cholesterol; VLDL-c, very low density lipoprotein-cholesterol

Table 5. Effect of BAP intervention on serum lipid profile of diabetic rats induced by alloxan

Group	TG (% of change)	HDL -c (% of change)	TC (% of change)	LDL-c (% of change)	VLDL-c (% of change)
Normal control	-----	-----	-----	-----	-----
Model control	223.73	-47.93	76.78	594.93	223.73
BAP intervention (2.5%)	205.08	-45.16	75.28	578.64	205.08
BAP intervention (5.0%)	188.14	-33.64	72.66	516.69	188.14
BAP intervention (7.5%)	118.64	-21.20	43.45	312.27	118.64
BAP intervention (10%)	64.41	-16.59	21.35	179.38	64.41

Results are expressed as means (n = 6). Normal control: normal rats without intervention; Model control: alloxan induced diabetic rats without intervention; BAP intervention: alloxan induced diabetic rats with BAP intervention. TG, triglycerides; HDL-c, high density lipoprotein-cholesterol; TC, total cholesterol; LDL-c, low density lipoprotein-cholesterol; VLDL-c, very low density lipoprotein-cholesterol

Effect of BAP intervention on immunological markers of diabetic rats induced by alloxan

The effect of BAP intervention on immunological markers of diabetic rats induced by alloxan was shown in Tables (6 and 7) and Figure (3 and 4). Such data indicated that treatment of rats with alloxan, model control group, induced a significant ($p \leq 0.01$) decreasing in serum albumin level by the ratio of -28.77% compared to normal control group. Dietary intervention with BAP (2.5, 5.0, 7.5 and 10%) in rats for 28 days led to significantly ($p \leq 0.05$) increasing the levels of serum albumin which recorded -16.75, -15.09, -8.25 and -3.77%, compared to the normal control group, respectively. The opposite direction was recorded for the TNF-α level. Treatment of rats with alloxan caused a significant ($p \leq 0.01$) increasing in serum TNF-α level by the ratio 78.99% compared to the normal control group. Dietary intervention with BAP (2.5, 5.0, 7.5 and 10%) in rats for 28 days led to significantly ($p \leq 0.05$) decreasing the levels of serum TNF-α which recorded 77.31, 68.91, 45.13 and 35.29% compared to the normal control group, respectively. The rate of increasing in serum albumin and decreasing in serum TNF-α were exhibited a dose-dependent increase with the levels of GLE intervention. In similar studies, Kashap et al., (2017) and Yongjun *et al.,* (2018) reported that increasing in brown algae level in diets leads to increase in blood albumin level of experimental animals. In general, many authors reported that hypoalbuminaemia is most frequent in the presence of liver diseases. For example, Abd El-Rahman, (2013) and Abd El-Fatah, (2013) showed that CCl_4 induced significant decrease in the serum albumin content. In this study, BAE significantly ($p \leq 0.05$) increased serum albumin levels which demonstrating that it can prevent or repair the hepatocytes damage. Such role of BAP in amelioration the hypoalbuminaemia represented a high degree of importance because human serum albumin is the main protein of blood plasma and makes up around 50%. The imported vital role of albumin is being a transporting protein, bind to various ligands and carry them around including water, bilirubin, fatty acids, hormones, different pharmaceuticals and cations to regulate the oncotic pressure of blood (Champe and Harvey, 1994; Farrugia, 2010). For TNF-α, Yeong-In *et al.*, (2016) found that treatment with brown algae extract down-regulated all hyper-inflammatory responses in macrophages including TNF-α. Also, Salman (2016) found that the intervention of *Ganoderma lucidum* extract, have almost bioactive constiuents such found in brown algae, attenuated the CCl_4-induced alterations in TNF-α levels. Furthermore, polysaccharides and alkaloids, mainly bioactive constituents found in brown algae, have shown good immunomodulatory properties associated with anti-tumor effects and suggested as anti-neoplastic agent (de Souza, Marques et al., 2007 and Dias et al., 2008; Elhassaneen et al., 2022). The attenuate effect of BAP in serum TNF-α levels proved that it can prevent tissue damage. Such role of BAP in suppression the TNF-α could be represented a high degree of importance because it is a pro-inflammatory cytokine which plays an important role in initiating the tissue inflammatory reaction (Kim *et al.,* 2003). With that context, treatment of patients groups with brown algae extracts decreased the expression of

tumor markers, and increased natural killer (NK) cells activities, type of white blood cell that can kill tumor cells or cells infected with a virus, compared to control groups (reviewed in Shilpi and Nissreen, 2011).

In conclusion, brown algae powder (BAE) was effective in protecting against DM and its complications. These results supported our hypothesis that BAP contains several classes of bioactive constituents with their biological effects that are able to improve blood glucose levels, serum lipid profile (TG, TC, HDL-c, LDL-c and VLDL-c) and serum immunological parameters (Alb and TNF-α). Therefore, we recommended like of that algae powder by a concentrations up to 10% to be included in our daily diets, drinks and food supplementation.

Table 6. Effect of BAP intervention on immunological markers of diabetic rats induced by alloxan

Group	Alb (g/dl)	TNF-α (ng/L)
Normal control	4.24 ± 0.75^{a}	1.19 ± 0.10^{c}
Model control	3.02 ± 0.98^{c}	2.13 ± 0.12^{a}
BAP intervention (2.5%)	3.53 ± 0.49^{bc}	2.11 ± 0.18^{a}
BAP intervention (5.0%)	3.60 ± 0.43^{b}	2.01 ± 0.12^{a}
BAP intervention (7.5%)	3.89 ± 0.27^{ab}	1.73 ± 0.08^{b}
BAP intervention (10%)	4.08 ± 0.51^{a}	1.61 ± 0.14^{b}

Results are expressed as means ± SD (n= 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$). Normal control: healthy rats without intervention; Model control: STZ induced diabetic rats without intervention; GLE intervention: STZ induced diabetic rats with GLE intervention. Alb, albumin; TNF-α, Tumor necrosis factor-α)

Table 7. Effect of BAP intervention on immunological markers of diabetic rats induced by alloxan

Group	Alb (% of change)	TNF-α (% of change)
Normal control	-----	-----
Model control	-28.77	78.99
BAP intervention (2.5%)	-16.75	77.31
BAP intervention (5.0%)	-15.09	68.91
BAP intervention (7.5%)	-8.25	45.13
BAP intervention (10%)	-3.77	35.29

Results are expressed as means (n= 6). Normal control: healthy rats without intervention; Model control: STZ induced diabetic rats without intervention; GLE intervention: STZ induced diabetic rats with GLE intervention. Alb, albumin; TNF-α, Tumor necrosis factor

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Conflict of Interests

Authors declared no competing of interest whatsoever.

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