

Possible Mechanisms Underlying the Therapeutic Effects of Brown Algae (*Sargassum subrepandum*) for Oxidative Stress in Diabetic Rats

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Abstract Diabetes is a metabolic disease, involving inappropriately elevated blood glucose levels which are called hyperglycemia. Besides that, many other factors play a great role in the pathogenesis of diabetes such as oxidative stress leading to a high risk of complications. The present study was designed to investigate the possible mechanisms underlying the therapeutic effects of brown algae (*Sargassum subrepandum*) powder (BAP) for oxidative stress in diabetic 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 injected 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. Treatment of rats with alloxan, model control group, induced a significant ($p \leq 0.01$) increasing in serum glucose concentration compared to normal control group. Dietary intervention with BAP ranged 2.5 to 10% in rats for 28 days led to significantly ($p \leq 0.05$) decreasing the levels of serum glucose 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. The same behavior was recorded for the biomarkers of oxidative stress levels in plasma including malonaldehyde (MDA), nitric oxide (NO) and reactive oxygen species (ROS). On contrary, significant ($P \leq 0.05$) improvement in different antioxidant defense systems in both serum (glutathione fractions) and RBC's (antioxidant enzymes such glutathione peroxidase, GSH-Px, glutathione reductase, GSH-Rd, superoxide dismutase, SOD and catalases, CAT) were recorded. Data of the present study has demonstrated the efficiency of BAP to partially attenuate hyperglycemia and diabetes-associated oxidative stress in diabetic rats. Therefore, we recommended BAP by concentrations up to 10% to be included in our daily diets, drinks and food supplementation.

Keywords: *sargassum subrepandum*, blood glucose, liver functions, glutathione fractions, antioxidant enzymes

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1. Introduction

Oxidative stress is defined as a phenomenon that results from an imbalance between the production and accumulation of reactive/free radical's species such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and others in cells and tissues and the ability of the biological system to detoxify these reactive products [1,2]. Free radical species can play many physiological roles and are typically synthesized as by-products of oxygen from both endogenous and exogenous sources. The endogenous production includes Immune cell activation, inflammation, ischemia, infection, cancer, excessive exercise, mental stress, and aging [3,4,5,6]. Environmental pollutants, heavy metals and ionizing

radiations, xenobiotics/certain drugs, chemical solvents, cigarette smoke, alcohol and cooking foods (smoked, grilling and deep-fat frying) are all responsible for endogenous free radical production [4] [7-10]. When these exogenous compounds penetrate the body, they are degraded or metabolized, and free radicals are generated as by-products which lead to an imbalance that causes damage to cells and tissues in what is known as oxidative stress. Oxidative stress of cells can show harmful effects on important cellular structures through the production of peroxides (superoxide radicals, $O_2^{\cdot-}$, hydrogen peroxide, H_2O_2 , hydroxyl radicals, $\cdot OH$ and singlet oxygen, $1O_2$) and free radicals that damage all components of the cell, including proteins, lipids, and nucleic acids [11]. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA [12]. Also, reactive oxidative species act as cellular messengers in

redox signaling and can cause disruptions in normal mechanisms of cellular signaling [6].

In humans, a large body of evidences shows that oxidative stress is thought to be involved in the development of in several diseases including cancer, atherosclerosis, metabolic disorders, cardiovascular diseases, chronic fatigue syndrome, rheumatoid arthritis and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease [2,13,14]. Also, infection by *Helicobacter pylori* which increases the production of reactive oxygen and nitrogen species in human stomach is also thought to be important in the development of gastric cancer [12]. Regarding the diabetes, associations between diabetes and markers of oxidative stress and the susceptibility of lipid to oxidative modification have been observed in both animals and humans [15,16]. Also, Giacco and Brownlee [17] reviewed that oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. Furthermore, Saydah et al., reported that type 2 diabetes increases mitochondrial ROS production from free fatty acids leads to inactivation of the anti-atherosclerosis enzymes and causes the atherosclerosis and cardiomyopathy [18].

For decades, many therapeutic strategies have been proposed to improve the complications of diabetes, which include oxidative stress, including the use of plant parts. This therapeutic approach has been used to overcome many of the problems associated with the use of pharmaceuticals (synthetic drugs), which are undesirable side effects, especially with prolonged use, in addition to the high cost that is usually out of the reach of most low-income people like those who live in primitive and rural areas (<https://londondiabetes.com/news-and-events/common-side-effects-of-diabetes-medication/>). Therefore, this therapeutic strategy was used to overcome these previous problems, especially after our previous studies demonstrated that plant parts are an important and sustainable resource for many bioactive compounds, which represent an interesting and mostly untapped source for the development of new drugs for diabetes [19,20,21].

Brown algae, Family, *Phaeophyceae*, belong to the group of seaweeds, which is a large group of eukaryotic organisms, most prominently characterized by the presence of chloroplasts surrounded by four membranes. Most brown algae contain the pigment fucoxanthin responsible for the greenish-brown color that gives it its name and they reproduce by means of spores and parenchyma that resemble other cells [22]. They play an important role in marine environments, both as food and as habitats. For example, *Sargassum* creates unique floating mats of seaweed in the semitropical and tropical waters/countries (including Egypt) that serve as the habitats for many species. Member of *Sargassum* genus (*Sargassum subrepandum*) represent valuable source of a several nutrients including proteins, lipids, minerals, fibers, minerals (K, Na, Ca, Mg, Fe, Se and Zn), vitamins (A, B members, C and E), and essential fatty and amino acids [23,24]. This algae also acquires special importance as a result of containing biologically active compounds, the most important of which are polysaccharides, alginate, polyphenols, alkaloids, carotenoids, polyphenols, fatty compounds, various pigments etc., [25-29]. And due to

the high nutritional value and vital compounds that these marine organisms contain, they have been used in many therapeutic applications, which include treatments for cancer, obesity, and cardiovascular and bone diseases [27,28] [30,31,32]. Despite these previous studies, what was done on the relationship of brown algae to diabetes is still in dearth and not fully understood. Therefore, one of the aims of the present study is to investigate the oxidative stress and antioxidant defense status in diabetes, which represents one of the leading preventable causes of death worldwide. Also, the effect of feeding intervention brown algae (*Sargassum subrepandum*) on that status in diabetes rats will be in the scope of this investigation.

2. Materials and Methods

2.1. Materials

2.1.1. Brown Algae

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

2.1.2. Chemicals

Alloxan, sulphanilamide, naphthylethylene diamine dihydrochloride, 2- (4-iodophenyl)-3 (4-nitrophenol) 5-phenyltetrazolium chloride (INTH2), glutathione and thiobarbituric acid (TBA) were 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.

2.1.3. Kits

Kit's assays for glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA) were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. GSH and GSSG were assayed by the kits provided by MyBioSource, Inc., San Diego, CA, USA. SOD was assayed by the kits purchased from Creative BioLab, NY, USA.

2.1.4. Equipment's

Absorbance (Abs) and fluorescence (FL) for different assays were measured using Labo-med. Inc., spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively.

2.2. Methods

2.2.1. Preparation of Brown Algae Powder (BAP)

BAP was prepared such as mentioned in our previous study [33]. In brief, brown algae were cleaned up from epiphytes and non-living matrix in running water, and rinsed many times in distilled water. The samples were then spread on string nets and allowed to dry in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 50 °C for 3 hours i.e. the

moisture in the final product about 9%. The dried algae were ground into a fine powder by high grinder speed (Moulinex Egypt, ElAraby Co., Benha, Egypt). The material that passed through an 80 mesh sieve was retained, kept in suitable closed bottles, stored at room temperature until use.

2.2.2. Biological Experiments

2.2.2.1. 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. 18- SREC- 07-2022).

2.2.2.2. Animals

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

2.2.2.3. Basal Diet (Standard Diet)

The basal diet (BD) was prepared according to Reeves et al., [34].

2.2.2.4. Induction of Diabetic Mellitus (DM)

DM 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 [35]. Immediately after injection animals were received 5% glucose solution over night to overcome drug induced hypoglycemia [36,37]. 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.

2.2.2.5. Experimental Design

Biological experiments were achieved in accordance with the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences Rules.[38] Rats (n=36) were housed individually in wire cages in a room maintained at 25 ± 1.8 OC 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 [24]. For 28 days, each of the above groups was housed in a single cage.

2.2.2.6. Biological Evaluation

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., using the following equations: $BWG (\%) = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$, $FER = \frac{\text{Grams gain in body weight (g/28 day)}}{\text{Grams feed intake (g/28 day)}}$. [39]

2.2.2.7. 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., [40]. The erythrocyte residue was washed with three consecutive portions of NaCl solution (0.9%) and the blood was lysed with deionized water for 30 min. Haemolysate was then centrifuged at 30,000 rpm for 30 minutes. The supernatant fractions were transferred to a clean test tube and analyzed for antioxidant enzymes [41].

2.2.3. Hematological Analysis

2.2.3.1. Serum Glucose

Serum glucose was determined by the colorimetric method explained by Tietz,[42].

2.2.3.2. Liver Functions

Alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of Tietz, [42] while alkaline Phosphatase (ALP) activity was determined using modified kinetic method of Vassault et al., [43].

2.2.3.3. Glutathione Fractions

GSH and GSSG were measured by colorimetrically methods in serum samples such as described by Davies et al., [44].

2.2.3.4. Antioxidant Enzymes

GSH-Px and CAT activities were determined as described by Splittergerber and Tappel, and Aebi, respectively [45,46]. Superoxide dismutase (SOD) activity was measured by colorimetric assay according to the method of Mett and Müller, [47]. Activities of SOD and GSH-Px enzymes were expressed in international unit per milliliter erythrocyte sediment and one unit of SOD was expressed as the enzyme protein amount causing 50% inhibition in 2- (4-iodophenyl)-3 (4-nitrophenol) 5-phenyltetrazolium chloride (INTH2) reduction rate. GSH-Rd activity was determined according to the method recommended by the International Committee for Standardization in Haematology [48].

2.2.3.5. Serum Oxidant Status

Malonaldehyde (MDA) content was measured using the colorimetric method described by Buege and Aust, based on the reaction of thiobarbituric acid (TBA) with MDA, one of the aldehyde products of lipid peroxidation [49]. Reactive oxygen species (ROS) was determined by a

colorimetric method described by Erel, [50]. Nitric oxide (NO) estimation was done as the sum of NO₂ and NO₃ such as mentioned by Miranda et al., [51]. The levels of NO were estimated by using Classical Griess Reaction (1% sulphanilamide, 1% naphthylethylene diamine dihydrochloride and 2.5% phosphoric acid). Serum samples were added to Griess reagent, transferred to the spectrophotometer and their Abs were recorded. The standard graph was plotted and Abs of rats sample were taken and correlated over the standard curve.

2.3. Statistical Analysis

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

3. Results and Discussion

3.1. Effect of BAP Intervention on Body Weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER) of Diabetic Rats Induced by Alloxan

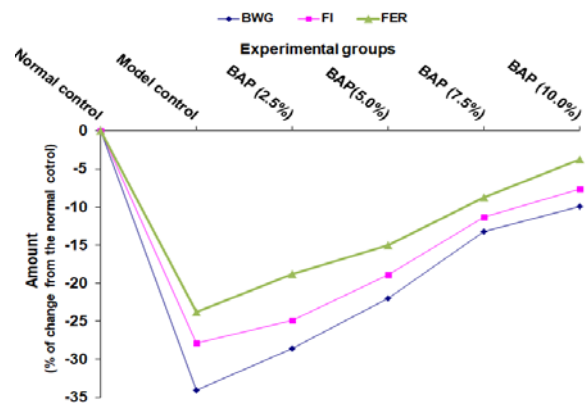
Table 1. Effect of BAP intervention on BWG, FI and FER of diabetic rats induced by alloxan

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

Results are expressed as means $\pm$ 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

The effect of BAP intervention on BWG, FI and FER of diabetic rats induced by alloxan were shown in Table 1 and Figure 1. 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 author [24,28,33,52,53]. 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, Elhassaneen et al., and Tahoon reported that injection of rats by CCl₄ inducing diabetic effects beside liver disorders which led to decrease the BWG, FI and FER [27,54]. Such biological parameters disorders were improved by intervention 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 [55,56]. 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 [57,58,59].



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

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

Table 2. Effect of BAP intervention on liver functions of diabetic rats induced by alloxan

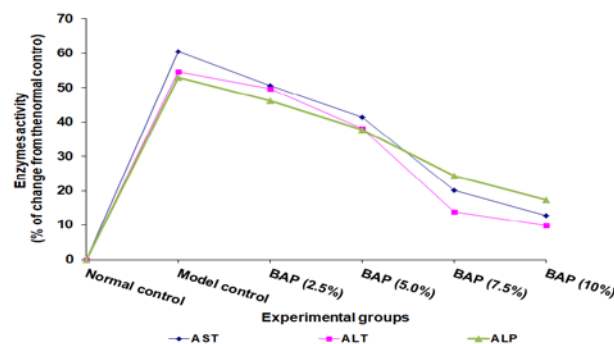
Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal control	41.67 $\pm$ 3.21 ^c	26.45 $\pm$ 1.99 ^b	105.60 $\pm$ 6.67 ^d
Model control	66.89 $\pm$ 3.98 ^a	40.87 $\pm$ 5.17 ^a	161.43 $\pm$ 10.42 ^a
BAP intervention (2.5%)	62.76 $\pm$ 4.05 ^a	39.58 $\pm$ 2.56 ^a	154.32 $\pm$ 7.08 ^a
BAP intervention (5.0%)	58.90 $\pm$ 2.67 ^{ab}	36.50 $\pm$ 2.57 ^a	145.38 $\pm$ 9.31 ^{bc}
BAP intervention (7.5%)	50.08 $\pm$ 4.71 ^b	30.12 $\pm$ 3.11 ^{ab}	131.22 $\pm$ 10.63 ^c
BAP intervention (10%)	47.00 $\pm$ 3.99 ^b	29.05 $\pm$ 2.91 ^b	123.84 $\pm$ 7.49 ^{cd}

Results are expressed as means $\pm$ 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. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase

3.2. Effect of BAP Intervention on Liver Functions of Diabetic Rats Induced by Alloxan

The effect of BAP intervention on liver functions of diabetic rats induced by STZ was shown in Table 2 and Figure 2. From such data it could be noticed that alloxan, model control group, induced a significant ($p \leq 0.05$) increasing in AST, ALT and ALP with the ratio of 60.52, 54.55 and 52.87% compared to normal control group, respectively. Feeding intervention with BAP (2.5, 5.0, 7.5 and 10%) in rats for 28 days led to significantly ($p \leq 0.05$) decreasing in the levels of those enzymes activities which recorded 50.61, 41.35, 20.18 and 12.78% (for AST), 49.67, 38.02, 13.90 and 9.85% (for ALT), and 46.14, 37.67, 24.26 and 17.27% (for ALP) compared to the normal control group, respectively. So it was clear that the rate of decreasing in serum liver enzymes activities were exhibited a dose- dependent increase with the levels of BAP intervention. Also, at the end of the experiments, 28 days, the values of AST, ALT and ALP were also recorded close to the values of the normal control group.

In general, aminotransferases (AST and ALT) plus ALP are normally intracellular enzymes and their presence of elevated levels in plasma indicates damage to cells rich in these enzymes. Several studies indicated that liver and pancreas disease process can cause cell lysis resulting in release of intracellular enzymes into the blood [60-62]. Data of the present study indicated that BAP enhance the liver functions in diabetic rats induced by alloxan through the activity of hepatic minotransferases plus ALP. Such data are accordance with that reported by Fitton, who found that eat of brown algae is thought to ameliorate some inflammatory disorders including liver diseases [63]. With the same context, a strong association between the increasing of the consuming of brown algae and diseases prevention in human was reported by Hamza et al., [64]. Such relationship has been explained by the brown algae content of bioactive compounds including alkaloids, phenolics, flavonoids, coumarins, and steroids. Other study was carried out by Sayd-Ahmed et al., indicate that extracts including the same bioactive constituents exhibit protective activities against liver injury induced by carbon tetrachloride [61]. Furthermore, data of the present study go in line with what Shannon et al., decided, the hepatoprotective effect of brown algae due to its high antioxidant activity which increasing the antioxidative defense system in hepatic cells [65]. This action include reducing malonaldehyde (MDA) levels, increasing antioxidant enzymes (glutathione peroxidase GSH-Px, superoxide dismutase, SOD and catalase, CAT) activity, and hepatic glutathione fractions (GSH and GSSG) levels. It comes in this context the study of Iwai, who reported that brown algae extract has been shown to markedly reduce lipid peroxidation levels in serum, red blood cells and liver of diabetic mice [66]. Also, brown algae are a rich source of different bioactive constituents including polyphenols, polysaccharides, carotenoids, terpenoids and flavonoids, and vitamins (A, B and E) [25,28,29]. Such of theses bioactive constituents could be enhanced the liver serum enzymes activity through many proposed process including improve the antioxidant capacity of the liver, block the hepatocellular uptake of bile acids, diminish the bilirubin concentration, scavenge the free radicals, and inhibit the lipid peroxidation [25,59,61,67,68].



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. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase

Figure 2. Effect of BAP intervention on liver functions of diabetic rats induced by alloxan.

3.3. Effect of BAP Intervention on Serum Glucose and Insulin of Diabetic Rats Induced by Alloxan

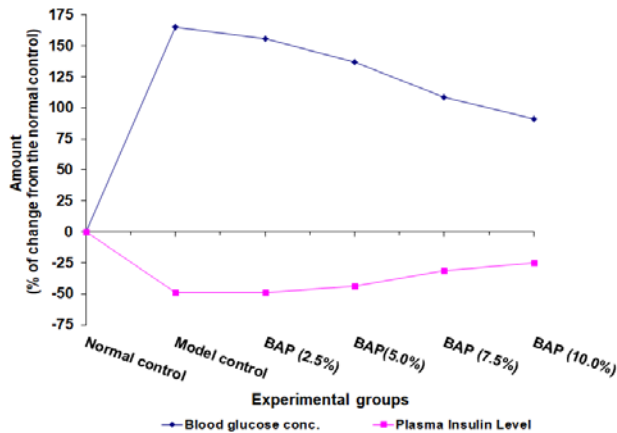
Effect of BAP intervention on serum glucose and insulin of diabetic rats induced by alloxan were in Table 3 and Figure 3. From such data it could be noticed that treatment of rats with alloxan, model control group, induced a significant ($p \leq 0.01$) increasing in serum glucose concentration by the 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 opposite direction was recorded for the insulin level. Treatment of rats with alloxan caused a significant ($p \leq 0.01$) decreasing in serum insulin level by the ratio -48.80% compared to the normal control group. Dietary intervention with with BAP (5.0, 7.5 and 10%) in rats for 28 days led to significantly ($p \leq 0.05$) increasing the levels of serum insulin which recorded -43.98, -31.24 and -24.71% compared to the normal control group, respectively, while the levels of 2.5% have no effect. The rate of decreasing in serum glucose and increasing in serum insulin were exhibited a dose- dependent increase with the levels of BAP intervention.

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

Group	Serum glucose conc. (mg/dL)	Insulin (μ IU/mL)
Normal control	97.60 $\pm$ 6.11 ^e	12.87 $\pm$ 0.98 ^a
Model control	259.01 $\pm$ 12.88 ^a	6.59 $\pm$ 0.64 ^c
BAP intervention (2.5%)	249.57 $\pm$ 12.78 ^a	6.59 $\pm$ 0.53 ^c
BAP intervention (5.0%)	231.39 $\pm$ 8.29 ^{bc}	7.21 $\pm$ 0.29 ^c
BAP intervention (7.5%)	203.64 $\pm$ 10.09 ^c	8.85 $\pm$ 0.44 ^{bc}
BAP intervention (10%)	186.56 $\pm$ 8.59 ^d	9.69 $\pm$ 0.89 ^b

Results are expressed as means $\pm$ 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.



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.

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

Alloxan is widely used as inducer of diabetes in experimental animals by destroying the insulin-secreting cells of Langerhans islet in pancreas [69]. 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 [70]. Such as explained by Lenzen, in experimental diabetes that may represent a model of type 2 diabetes, alloxan has two distinct pathological effects as follow: 1) selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the beta-cell glucose sensor, and 2) causes a state of insulin-dependent diabetes through its ability to induce ROS formation, leading to selective necrosis of beta cells [71]. Also, Gunnarsson and Hellerström, [72] reported that inhibition of glucokinase reduces glucose oxidation and ATP generation, thereby suppressing the ATP signal that triggers insulin secretion. Inhibition of glucokinase is achieved within 1 min of exposure to alloxan. Perhaps this theory is in the line with the data of the present study, which are serum glucose levels decreased while serum insulin levels increased 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 [59,71,73,74]. Also, Tiwari and Madhusudana, reviewed that bioactive constituents determined in brown algae such polyphenolics, apart from

their much-cited antioxidant activities, also have been reported to inhibit alpha-amylase and sucrase, and have been shown to be the principle substance for suppressing postprandial hyperglycemia [75].

3.4. Effect of BAP Intervention on Serum Glutathione Fractions of Diabetic Rats Induced by Alloxan

The effect of BAP intervention on serum glutathione fractions, biological antioxidant macromolecules, of diabetic rats induced by alloxan in was shown in Table 4 and Figure (4). Such data indicated that treatment of animals with alloxan, model control group, caused a significant ($p \leq 0.05$) decreasing in GSH and GSSG by the ratio of -37.00 and -25.34% when compared to normal control group, respectively. Intervention of the rat diets with BAP at levels 2.5 to 10% prevented the decreasing of the mean serum GSH and GSSG levels. The preventative rates were elevated with the increasing of BAP concentration. Data of the present study are in accordance with that reported by several authors [24,33]. Others found that brown intervention with brown algae extracts leads to increase the hepatic glutathione and total antioxidant capacity in diabetic animals.

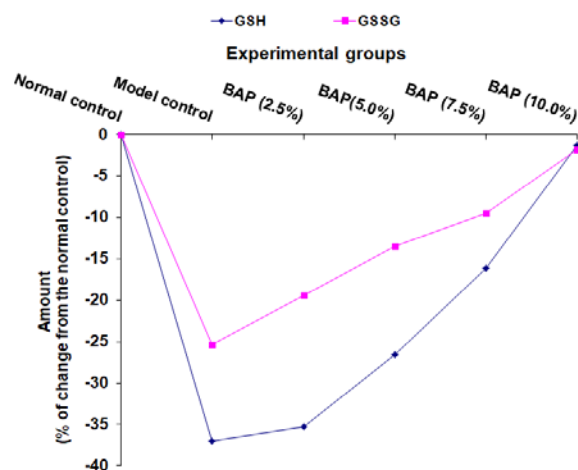
Table 4. Effect of BAP intervention on serum glutathione fractions of diabetic rats induced by alloxan

Group	GSH ($\mu\text{mol/L}$)	GSSG ($\mu\text{mol/L}$)
Normal control	9.41 ± 1.15^a	0.734 ± 0.033^a
Model control	5.93 ± 0.76^c	0.548 ± 0.057^b
BAP intervention (2.5%)	6.09 ± 1.02^{bc}	0.592 ± 0.024^b
BAP intervention (5.0%)	6.91 ± 0.98^b	0.635 ± 0.030^{ab}
BAP intervention (7.5%)	7.89 ± 0.91^b	0.665 ± 0.047^a
BAP intervention (10%)	9.28 ± 1.03^a	0.721 ± 0.039^a

Results are expressed as means ($n = 6$). 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. GSH, reduced glutathione; GSSG, oxidized glutathione

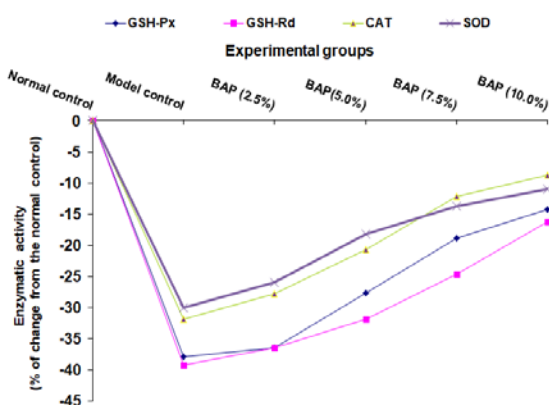
In general, GSH is a tripeptide-thiol that has received great attention due to its various vital intracellular functions [76]. Among of these functions are the roles related to the detoxification process. GSH acts as a key conjugate of electrophilic intermediates in phase II metabolism/biotransformation, via glutathione-S-transferase activities. Also, it is representing as an important biological antioxidant via serve as a nonenzymatic scavenger of free radicals [77]. So, several studies reported that a fall in GSH fractions is accompanied by a concomitant increase in the liver lipid peroxidation [7,58,78,79]. Also, these studies with others reported that secretion of GSH from liver to blood might be blocked by different diseases including diabetes because of intracellular structural failure, increasing of the lipid peroxidation and/or energy depletion suggested by the marked decrease in liver glycogen content [8,33]. High content of different bioactive compounds including polyphenols, polysaccharides, alkaloids, carotenoids, terpenes and flavonoids were determined in BAP

and exhibited different biological roles such antioxidant, scavenging and anticarcinogenic activities [25,80-82]. Therefore, intervention of the diabetic rat diets with BAP leads to reduce the lipid peroxidation process subsequently elevates the glutathione fractions content in liver. Such observation was confirmed by several authors [33,78,79].



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. GSH, reduced glutathione; GSSG, oxidized glutathione

Figure 4. Effect of BAP intervention on serum glutathione fractions of diabetic rats induced by alloxan.



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. GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; CAT, catalase; SOD, superoxide dismutase

Figure 5. Effect of BAP intervention on erythrocytes antioxidant enzymes activities of diabetic rats induced by alloxan.

3.5. Effect of BAP Intervention on Erythrocytes Antioxidant Enzymes Activities of Diabetic Rats Induced by Alloxan

The effect of BAP intervention on erythrocytes antioxidant enzymes activities of diabetic rats induced by alloxan were shown in Table 5 and Figure 5. From such data it could be noticed that that injection of alloxan, model control group, induced a significant ($p \leq 0.05$) decreasing in erythrocytes antioxidant enzymes activities

(GSH-Px, GSH-Rd, CAT and SOD) levels by the ratio -37.87, -39.18, -31.82 and -29.97% compared to the normal control group, respectively. Intervention with BAP in rat diets by concentrations of 2.5, 5.0, 7.5 and 10% leads to increase all of these values by different rates. The rate of increasing in all determined erythrocytes antioxidant enzymes activities were exhibited a dose-dependent increase with the levels of BAP intervention.

It is generally known that the antioxidant defense systems of the organism depend to a large extent on antioxidant enzymes, including GSH-Px, GSH-Rd, CAT and SOD, which have the ability to search for and deal with free radicals to prevent their damage. SODs are responsible for the reduction of O_2^- to H_2O_2 and a group of enzymes will act to remove H_2O_2 including GSH-Px and CAT [83]. Also, GSH reduces Se and the reduced form of the enzyme and then reacts with H_2O_2 . There must therefore be a mechanism for the return of GSSG to GSH and this is accomplished by the GSH-Rd enzyme catalyzing the following reaction: $GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$. Mammalian RBCs operate the pentose phosphate pathway to provide NADPH for GSH reduction. GSH-Rd can also catalyze the reduction of decidedly mixed disulfides such as those between GSH and coenzyme A [84]. Several studies reported that antioxidant enzymes systems are active in liver cells [7] [85-87]. Decreasing in the activity of the antioxidant enzymes result in increased ROS production and cell organelles dysfunction including mitochondria, lysosomes and cell wall membrane [7] [78,85] [87-89]. The selected BAP in the present study interventions is rich in bioactive compounds such polyphenolics, carotenoids, polysaccharides, alkaloids and flavonoids, which exhibited antioxidant defense in different biological systems [25,27,58]. Such antioxidant defense systems including antioxidants enzymes play important roles in the manipulation of the diabetes and its complications through ROS scavenging processes in RBC's.

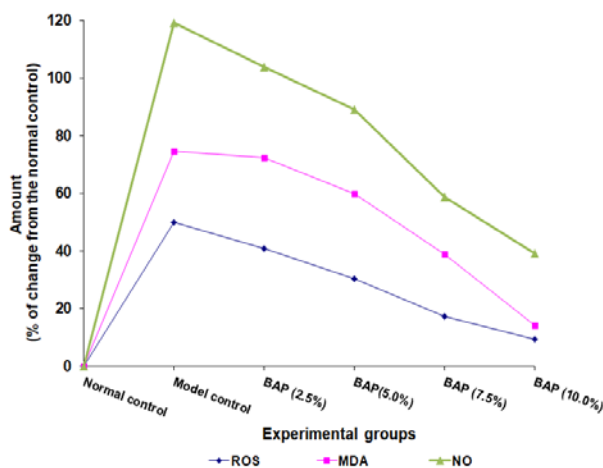
Table 5. Effect of BAP intervention on erythrocytes antioxidant enzymes activities of diabetic rats induced by alloxan

Group	GSH-Px (U/g Hb)	GSH-Rd (U/g Hb)	CAT (U/g Hb)	SOD (U/g Hb)
Normal control	31.45 ± 2.22 ^a	14.65 ± 1.07 ^a	191.16 ± 7.02 ^a	7.04 ± 1.40 ^a
Model control	19.54 ± 1.91 ^c	8.91 ± 1.09 ^b	130.33 ± 6.78 ^d	4.93 ± 0.93 ^{bc}
BAP intervention (2.5%)	19.98 ± 2.31 ^c	9.31 ± 1.48 ^b	138.11 ± 3.79 ^d	5.21 ± 1.22 ^b
BAP intervention (5.0%)	22.76 ± 1.89 ^{bc}	9.98 ± 1.55 ^b	151.70 ± 7.81 ^c	5.76 ± 1.59 ^b
BAP intervention (7.5%)	25.54 ± 2.66 ^b	11.05 ± 1.17 ^{ab}	167.84 ± 5.90 ^b	6.07 ± 1.12 ^{ab}
BAP intervention (10%)	26.98 ± 1.70 ^b	12.28 ± 2.27 ^a	174.54 ± 8.16 ^b	6.27 ± 1.25 ^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. GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; CAT, catalase; SOD, superoxide dismutase

3.6. Effect of BAP Intervention on Serum Oxidants of Diabetic Rats Induced by Alloxan

Data in Table 6 and Figure 6 were shown the effect of BAP intervention on serum oxidants of diabetic rats induced by alloxan. From such data it could be noticed that injection of alloxan, model control group, induced a significant ($p \leq 0.05$) increasing in serum MDA, NO and ROS levels by the ratio 74.67, 119.05 and 49.92% compared to normal control group, respectively. Feeding intervention of the rat with BAP by concentrations of 2.5, 5.0, 7.5 and 10% leads to decrease these values by the rate of 72.22, 59.85, 38.86 and 14.11%; 103.78, 89.18, 58.82 and 39.05%; and 40.75, 30.40, 17.20 and 9.44% for MDA, NO and ROS, respectively. The rate of decreasing in serum MDA, NO and ROS were exhibited a dose-dependent increase with the levels of BAP intervention. The present data are in accordance with observed by several studies [24,28,33,52,56].



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. MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species

Figure 6. Effect of BAP intervention on serum oxidants of diabetic rats induced by alloxan

Table 6. Effect of BAP intervention on serum oxidants of diabetic rats induced by alloxan

Group	MDA (nmol/mL)	NO (nmol/L)	ROS (U/mL)
Normal control	5.71 ± 0.59^d	44.89 ± 2.78^e	61.42 ± 4.21^c
Model control	9.98 ± 1.32^a	98.33 ± 4.04^a	92.08 ± 6.09^a
BAP intervention (2.5%)	9.84 ± 1.50^a	91.47 ± 4.23^a	86.45 ± 7.11^a
BAP intervention (5.0%)	9.13 ± 0.88^a	84.92 ± 3.78^b	80.09 ± 8.20^{ab}
BAP intervention (7.5%)	7.93 ± 0.73^b	71.29 ± 5.22^c	71.98 ± 5.90^b
BAP intervention (10%)	6.52 ± 0.56^c	62.42 ± 3.07^d	67.22 ± 6.21^{bc}

Results are expressed as means $\pm$ 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. MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species

In similar studies, clinical evidence for diabetes-associated oxidative stress has been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes [19,27,58]. For example, lipid peroxidation markers such as MDA, the major products of the oxidation of polyunsaturated fatty acids, lipid hydroperoxides, and conjugated dienes are found to be increased in plasma from diabetic subjects in survey clinical studies [27,78,90]. Official reports indicated that MDA is a mutagenic and/or carcinogenic (Shamberger et al., 1974). For NO, it is playing an important role in the communication among liver cells and regulates the important liver functions. It is formed by catalyzing the conversion of L-arginine to citrulline and nitric oxide (NO) by nitric oxide synthase. NO reacts with oxygen and water to form nitrite (NO₂) and nitrate (NO₃) and with hemoglobin to form iron nitrosyl adducts and/or nitrate in blood, with superoxide anions to produce nitrate, and with protein amino groups and thiols to form nitrosylated species [92,93]. Studies have shown that excess production of NO causes pathogenesis and tissue destruction for a large number of immune and inflammatory diseases, including diabetes [59,94,95]. Several studies found that BAP selected in the present study intervention is rich in bioactive constituents including polyphenolics, carotenoids, alkaloids, flavonoids, polysaccharides and vitamins which exhibited different activities in different biological systems [16,25,28,56]. Such antioxidant properties are important in the manipulation of diabetes and its complications through ROS scavenging processes and inhibiting of the lipid oxidation in plasma. With the same context, Liu et al., investigated the effects of bioactive constituents of brown algae on hepatocytes lipid peroxidation induced by ferric nitrilotriacetate in vitro, and showed that significantly decreased intracellular ROS and DNA damage, and markedly decreased the level of MDA and protein carbonyl contents [96]. Also, previous studies proved that the different plant parts are rich by different classes of bioactive constituents such as found in BAP, have the ability to decrease the oxidant status level i.e. the level of MDA, NO and ROS in different biological fluids and organs [2,9] [97-99]

Table 7. Correlation between oxidative stress and antioxidant defense systems parameters in diabetes rat's intervention with BAP

Parameters	r^{2*}	Parameters	r^2
MDA/GSH	- 0.9251**	NO/GSH	- 0.9061**
MDA/GSSG	- 0.8887*	NO/GSSG	- 0.8390*
MDA/GSH-Px	- 0.9343**	NO/GSH-Px	- 0.9207**
MDA/GSH-Rd	- 0.9005*	NO/GSH-Rd	- 0.8714*
MDA/CAT	- 0.8584*	NO/CAT	- 0.8879*
MDA/SOD	- 0.8942*	NO/SOD	- 0.9132**
MDA/ROS	0.9707**	NO/ROS	0.9864**

* $P \leq 0.05$, ** $P \leq 0.01$

3.7. Correlation Studies

Correlation analysis between oxidative stress (MDA, NO, ROS) and antioxidant defense systems (GSH fractions and antioxidant enzymes) parameters in DM rats intervention with BAP was shown in Table 7. When all treatments were included in the statistical analysis important significant

differences were found between oxidative stress and antioxidant defense systems parameters. There was a strong negative significant ($p \leq 0.05$) relationship between glutathione fractions concentration in plasma [GSH ($r^2 = -0.9251$) and GSSG ($r^2 = -0.8887$)] and antioxidant enzymes in RBC's [GSH-Px ($r^2 = -0.9343$), GSH-Rd ($r^2 = -0.9005$), CAT ($r^2 = -0.8584$) and SOD ($r^2 = -0.8942$)], and MDA concentration in plasma. On the other side, strongly positive correlation ($r^2 = 0.9707$) between ROS and MDA was observed. The same behavior i.e. correlations/relationships were observed between all of those parameters and NO concentrations in plasma. These correlations confirm that if there were no change in the antioxidant defense systems parameters of DM rats, it would be difficult to observe high concentrations of MDA, NO and ROS. In a similar study, Bohm et al., in some model systems noticed a

combination of antioxidant vitamins (such as determined in BAP), α -tocopherol and β -carotene interacts synergistically to inhibit lipid peroxidation and subsequently increased MDA [100]. Also, Shalaby, reported that high levels of lipid peroxidation i.e. MDA in the plasma of diabetic rats were associated with rather low levels of antioxidant vitamins and enzymes [101]. Furthermore, Elhassaneen et al., reported that some important differences were found between plasma MDA and both GSH fractions and antioxidant enzymes in diabetic rats feeding powders/extracts of husks (*Plantago ovata*), vinca (*Catharanthus roseus*) and olive and mango leaves [21]. Such studied plant parts often contain several bioactive compounds that are found in the algae powder under study, BAP. As shown in Figure 7, brown algae supplementation can overcome the alloxan-induced diabetic complications through many intracellular pathways.

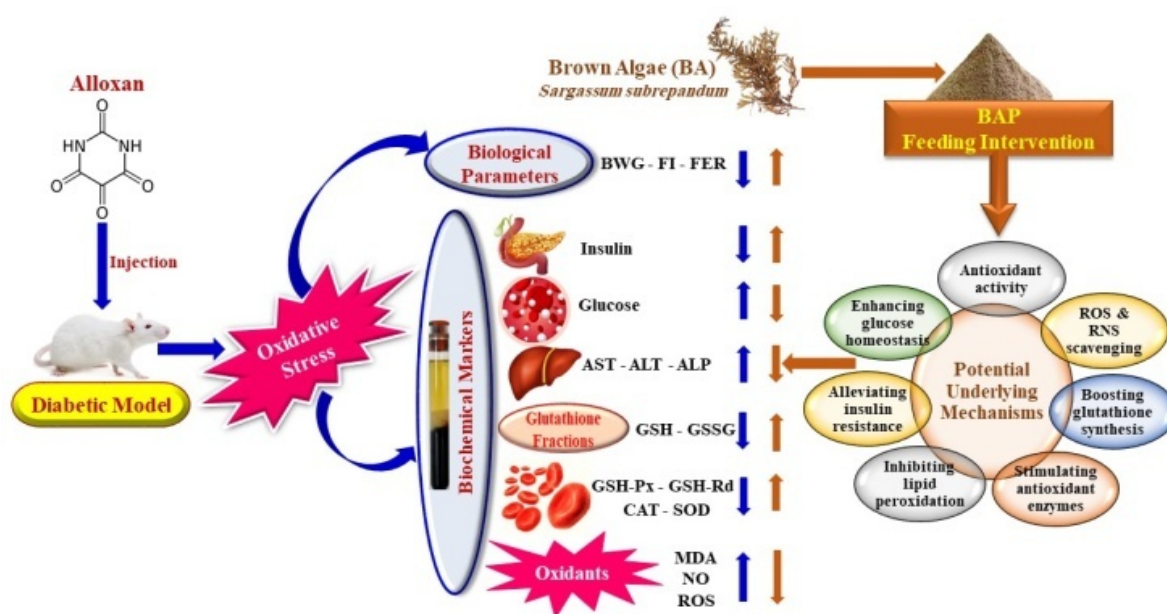


Figure 7. Graphical summary showing the effect of brown algae powder (BAP) on alloxan-induced diabetic complications

4. Conclusion

DM is a metabolic disease, involving inappropriately elevated blood glucose levels which are called hyperglycemia. Besides that, many other factors play a great role in the pathogenesis of diabetes such as oxidative stress leading to a high risk of complications. Data of the current study has demonstrated that efficiency of brown algae powder (BAP) to partially attenuate hyperglycemia and diabetes-associated oxidative stress in diabetic rats induced by alloxan. Such BAP exhibited antioxidant activities against oxidants/ROS formation as the DM development through different mechanisms including improvement of liver functions, increasing the glutathione fractions synthesis, stimulating antioxidant enzymes defense system in RBC's, and inhibiting the lipid oxidation in plasma. These observations provide a basis for the application of BAP for the prevention and/or treatment of type-2 DM and its complications. Therefore,

we recommended BAP by concentrations up to 10% to be included in our daily diets, drinks and food supplementation.

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Author's Contribution

All authors contributed equally to the activities/components of the current study, which include

collecting the information (Review of literatures), designing the experiments, conducting the biological experiments and hematological laboratory analyses, preparing results and statistically analyzing, adding figures, and writing the first draft of the manuscript. Professor Yousif also restructured the first draft of the manuscript, reviewing it in terms of intellectual content and coordinated the study. All authors edited and reviewed the various versions of the manuscript and approved the final version for submission for publication.

Conflict of Interests

Authors declared no competing of interest whatsoever.

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