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Original Article

Spirulina platensis extract in alloxan-induced diabetic rabbits: a combined experimental study of antidiabetic activity *in vivo* and anticancer ability *in vitro*

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Abstract

The present study was designed to study the efficacy of *Spirulina platensis* algal extract on hepatocellular carcinoma cell lines (HAMC Cell line), as well as its antidiabetic effect on alloxan-induced diabetic male rabbits. The study included the treatment of 24 male rabbits for four periods (7, 14, 21, 28) of days. The experiment included four groups of rabbits: the negative group, the positive group, the first treatment group and the second treatment group. Each group contained six rabbits. The negative (intact) and positive (diabetic induced), control groups were injected with 1ml of the normal saline and the first treatment group (diabetic induced) was injected with a concentration of 50 mg/kg of the algal extract. In contrast, the second treatment group (diabetic induced) was injected with a concentration of 100 mg/kg of the algal extract. The treatment results with algal extract showed a significant decrease in blood glucose levels in the first and second treatment groups at a level of probability ($P<0.05$) compared with the positive control group for all periods. The results also showed that the algal extract had efficacy against cancer, as the concentration of 17.2 µg caused the highest HAMC cell line inhibition rate at 62.23%. The current study concluded that *Spirulina platensis* had therapeutic effects in alloxan-induced diabetic rabbits as well as high potential inhibition of cancer cells.

Keywords: *Spirulina platensis*, rabbits, diabetes, alloxan, HAMC cell line.

Introduction

Diabetes is a chronic metabolic disease characterized by a rise in the glucose level, i.e., more than the normal level, resulting from a complete or relative deficiency of the hormone insulin or because of factors against insulin action [1]. It is a chronic condition caused by various factors, including hereditary, viral, environmental, or functional [2]. Global estimations indicate that the proportion of adults with diabetes will increase to 69% in 2030 [3]. Many health problems result from taking the chemical drugs used to treat diabetes, and their prolonged use may cause side effects that negatively affect the body's organ functions [4]. In this regard, many studies were conducted to find natural treatment sources. Researchers have turned their attention to alternative treatments from natural products such as plants, herbs and algae, which can be more effective in decreasing glucose levels and reducing side effects compared with chemical drugs [5]. Algae, including cyanobacteria, have gained wide attention in recent years in this field due to the biologically active compounds that they contain [6].

Spirulina algae is one of the most important algae belonging to the cyanophytes/cyanobacteria group from the eukaryotic algae. *S. platensis* contains a high content of carotenoids, especially β-carotene and vitamins, including vitamins B3, B9, B12, B1, B2, B6, C, D, E and Folic acid [7]. Algae produce many bioactive compounds within their secondary metabolism. Moreover,

secondary bioactive compounds in algae have an effective role as anticancer, antioxidants, and antihyperlipidemic and enhance the body's immunity [8–10]. *Spirulina* fusiformis significantly reduces high blood glucose levels at a dose of 400 mg/kg and has an antidiabetic effect in rats-induced diabetes [11]. The treatment of laboratory rats with an alcoholic extract of *Caulerpa lentillifera* showed a high ability to regulate hyperglycemia metabolism [12].

The study of Ghwenm *et al.* (2020) confirmed the efficiency of *Chlorella vulgaris* in reducing the glucose level in diabetic-induced mice after injecting them with concentrations of 40 and 80 mg/kg of the algal ethanolic extract for a month [13]. Sun *et al.* (2010) found the effective role of *Chlorella protothecoides* and *Nitzschia laevis* in reducing blood glucose among 20 selected green algae [14]. Roy *et al.* (2007) mentioned the possibility of inhibiting liver cancer in laboratory rats through the use of the substance phycocyanin that was extracted from *S. platensis*, as well as the polysaccharides which were extracted from the algae [15], also considered an active agent against stomach cancer [16]. Laungsuwon & Chulalaksananukul's study (2013) showed that the algal extracts of *Cladophora glomerata* and *Microspora floccose* were high efficiency against cancer diseases due to their containing a high percentage of chemically active compounds in killing cancer cells [17]. Many sulfated polysaccharides were extracted and diagnosed from *Bryopsis* sp. which was used as an antibiotic in lung cancer treatment [18].

Finally, in the light of the medicinal importance of algae and their widespread usage in medicinal and pharmaceutical products, and their contribution to the studies which deal with the effect of some species of algae as anticancer and hyperglycemia, the current project aimed to know the effect of *S. platensis* algal extract on blood glucose level in diabetic-induced male rabbits and its inhibitory effect on HAMC cell lines.

Material and methods

Ethics declaration

The College of Education for Pure Science, University of Basrah ethics committee reviewed and approved this study (No. 2020/4402). Animals were cared for in accordance with the Guide to the Care and Use of Experimental Animals by Canadian Council on Animal Care, Vol. 1, (2nd Ed.), (1993) and its revision version (2020).

Experiment animals

A total number of 24 male rabbits (1250–1400 g) were used in the animal house of the Dep. Biology/Col. Education for pure science/Uni. Basrah and the animal house were maintained at a temperature of 20°C and humidity of 40% and provided on a 14:00–10:00-hour light: dark cycle. Rabbits were fed with food and water ad libitum. The animals were left fasting for at least 18:00 hours before the experiments were carried out. Male laboratory rabbits were randomly divided into four treatment categories and each category consisted of six rabbits. The negative control group included intact animals that were not induced by diabetes and were orally administered with 1ml of normal saline. The positive control group represented alloxan-induced diabetic animals and were orally administered with 1ml of normal saline. Whereas the first and second treatment groups contained rabbits induced by diabetes and were orally administered at doses of 50 and 100 mg/kg of algae extract, respectively.

Induction of diabetes in laboratory animals

Diabetes was induced in laboratory rabbits after the food was withheld for 12 hours and then injected with the Alloxan (150 mg/kg for b.w) in the intraperitoneal cavity (IP) and the volume (0.1 ml) for each animal for three days. Drinking water was replaced with w/v (20%) of glucose to reduce hypoglycemia's shock due to alloxan treatment. After the first week of injection, the glucose level in the serum was measured to confirm the occurrence of diabetes [19].

Preparing doses

Two concentrations of *S. platensis* extract were prepared. The highest dose represented 1/50 LD50, and it measured 100 mg/kg b.w., while the lowest dose represented 1/100 LD50 and it measured 50 mg/kg b.w., depending on the LD50 value of 5,000 mg/kg [20]. Moreover, laboratory rabbits were orally treated for 28 days, within one dose per day.

Collection blood samples

Blood samples were drawn weekly for a month directly from the heart using a 3-ml medical syringe. Blood samples were placed in container tubes on Heparin Lithium/Gel and left for 10–15 minutes to coagulate, then were placed in a centrifuge at 3,500 rpm for ten minutes

Table 1: Impact of S. *platensis* extract treatment on serum glucose of alloxan-diabetic rabbits for 7 and 14 days (mean±standard deviation).

Treatments / Period	7 days Glucose mg/dl	14 days Glucose mg/dl
Negative control (normal saline)	110.65^{c}±14.16	115.78^{c}±10.56
Positive control group (alloxan)	414.45^{a}±34.86	404.58^{a}±45.72
The first treatment group (50 mg/kg)	211.53^{b}±22.35	176.34^{b}±29.24
The first treatment group (100 mg/kg)	195.69^{b}±17.60	161.88^{b}±21.02

Note: $^{a, b, c}$ – Different letters indicate the significance differences at (P≤0.05).

Table 2: Impact of S. *platensis* extract treatment on serum glucose of alloxan-diabetic rabbits for 21 and 28 days (mean±standard deviation).

Treatments / Period	21 days Glucose mg/dl	28 days Glucose mg/dl
Negative control (normal saline)	108.25^{c}±8.38	112.56^{b}±8.87
Positive control group (alloxan)	393.86^{a}±12.50	378.88^{a}±19.94
The first treatment group (50 mg/kg)	134.02^{b}±14.09	119.03^{b}±16.84
The first treatment group (100 mg/kg)	140.32^{b}±16.75	124.51^{b}±14.57

Note: $^{a, b, c}$ – Different letters indicate the significance differences at (P≤0.05).

to obtain the serum, then pulled the serum separated from the coagulated blood and saved in special tubes at -10°C until serum glucose tests were performed [21].

Determination of serum glucose in rabbits

The serum glucose concentration was estimated according to the enzymatic method Tietz, (1999) by the kit supplied by the British company Randox [22]. The absorbance of the sample is examined at a wavelength of 500 nm.

Estimating cancer cell inhibition

All solutions, buffers and tissue culture media were prepared according to Freshney (2010) [23] by using the hepatocellular carcinoma cell lines (HAMC), which were obtained from the Iraqi Center for Cancer Research and Medical Genetics in Baghdad (ICCMGR).

Statistical analysis

Data were analyzed using SPSS V.20 program. Differences between groups were calculated for statistical significance using One-way ANOVA. The data were analyzed at a probability level (P≤0.05).

Results

The treatment of rabbits with two doses of algal extract (50&100 mg/kg) for 4 weeks caused a significant reduction in serum glucose level at (P≤0.05) when compared with the control group. Also, the glucose level in both groups treated with two doses of the algal extract was not significantly affected compared with the negative control group for a period of 28 days (Table 1 and Table 2).

The current results showed the inhibitory efficacy of the algal extract in the hepatocellular carcinoma cell lines in all concentrations, and the concentration of 17.2 µg/ml was the most effective in killing cancer cells among the used concentrations in measuring efficacy against cancer Table (3).

Table 3: Effectiveness of the S. *platensis* extract on HAMC cell line.

Concentrations of algal extract (µg/ml)	Viability %	Inhibition %
6.4 µg/ml	44.56	55.43
10 µg/ml	45.27	54.72
17.2 µg/ml	37.76	62.23

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Discussion

The induction of diabetes by using the alloxan and at a dose of 150 mg/kg in male rabbits had a distinct role in raising the level of blood glucose by causing damage to the pancreas function, as the high level can infer in the blood serum to 400 mg/100 dl. This finding is consistent with the results of the researcher [24].

Treatment of male rabbits with the *S. platensis* ethanolic extract caused a significant decrease in the glucose level in the first and second treatment groups compared to the positive control group. This finding was consistent with a study by Mridha *et al.* (2010) [25]. The algal extract possesses many effective chemical compounds that have the ability to dissolve in water, such as saponins, glycosides, carbohydrates and phenols. These compounds have a significant effect in reducing the level of glucose in the blood, especially in animals with hyperglycemia, because these compounds possess the ability to stimulate the body tissues to uptake glucose from the blood, so the tissue's consumption of glucose increases [26].

In addition, terpenoids are considered antioxidants, as they prevent the oxidation of the hormone insulin via free radicals and prevent the formation of lipid peroxides that disturb the hormone insulin [27]. Moreover, they diminish glucose absorption in the intestine while stimulating the pancreas secretion of the hormone insulin and facilitating the entry of glucose into the cells of surrounding tissues and muscles [27]. As a result, these active compounds depend on a combination of antidiabetic mechanisms and measures, which include preventing diabetic nephropathy, stimulating insulin secretion from beta cells, inhibiting insulin catabolism, preventing oxidative processes and increasing glycogenesis and glycolysis [28–32].

The active compounds of *Spirulina platensis*, such as ascorbic acid, 9, 12-octadecadienoic acid – gamolenic acid – and hexadecanoic acid, have an important protective role against free radicals generation. These free radicals cause damage to the DNA, lipids and proteins, so these active compounds reduce the effect of free radicals from oxidation of the insulin hormone [10, 33]. The decrease in glucose may also be due to the presence of carotenoids and phycocyanin pigments that have a similar function to the action of ascorbic acid. They also have an antioxidant effect in removing free radicals and thus prevent pancreatic disorders by inhibiting apoptosis (programmed cell death) and suppressing free radicals production to alleviate Oxidative stress. In turn, these pigments reduce damage in the pancreas cells caused by alloxan treatment. In addition to their ability to reduce cell resistance to insulin and increase insulin sensitivity, they improve the function of beta cells and renew their construction, and more importantly, their ability to activate insulin receptors [7, 33–35]. Epidemiological and clinical algae studies showed an inverse correlation between antioxidant properties and the incidence of chronic diseases such as cardiovascular diseases associated with hyperlipidemia and diabetes [36].

Moreover, the composition of the algal ethanolic extract contains turbines and steroids that stimulate β cells in the pancreas to secrete insulin hormone, and these effective chemical compounds have pharmacological effects, as they are used as antithyroid, anticancer and antihypercholesterolemic. Interestingly, these compounds do not cause damage to the heart and liver functions [37]. In this context, the compound 9, 12, 15-Octadecatrienoic acid (Z, Z, Z) identified within the algal extract may increase the sensitivity of cells to the insulin hormone [10, 38].

The results of the current study showed the ability of the algal extract to inhibit the hepatic cell line (HAMC), as the highest rate of killing the cancer cells was 62.21% at a concentration of 17.2 µg/ml. Perhaps the reason for this is that algal extract possesses many active compounds that act as antioxidants and play a role in suppressing cancer development in its last stages. The most active water-soluble antioxidants isolated from algae include phenols, vitamins and carotenoids [39]. In fact, oxidative stress and free radicals generation promote the process of carcinogenesis, while antioxidants, including Linolenic acid, cause scavenging reactive oxygen species (ROS), thereby preventing the emergence of cancer cells [20–40].

Some studies have confirmed the algal extract's protective role by inhibiting proliferation and stimulating apoptosis in treating cancer patients [41]. Cancer cells operate under oxidative stress, and their vitality can be impaired by scavenging free radicals via various antioxidants; therefore, antioxidants have a high potential to destroy tumors. In particular, the pathway of the nuclear factor erythroid-2-related factor 2 (Nrf2) controls the genetic expression of important cellular antioxidants and detoxification enzymes. It was found that active compounds isolated from algae appear to suppress the Nrf2 pathway. As a result, inhibition of this pathway in cancer cells is most effective in promoting their death [42].

Konickova *et al.* (2014) observed that *S. platensis* and algae-derived tetrapyrroles were highly potent as

the anti-cell division in pancreatic cancer, and this result was similar to the results of the current study [8]. Interestingly, the bioactive substance tetrapyrroles can potentially improve the glutathione redox status, increasing tumor inhibitory efficacy [43]. In fact, the phycocyanobilin compound inhibits ROS production in mitochondria and is a strong inhibitor of the enzyme NADPH oxidase [8, 44]. The mitochondrial NADPH oxidase has induced superoxide, which represents a carcinogenesis factor affecting the cell cycle and several metabolic pathways [45, 46].

Conclusion

The algae *Spirulina platensis* is rich in nutrients and active components that act as antioxidants. It has therapeutic effects due to its antidiabetic and anticancer activity in alloxan-induced diabetic male rabbits and it could play a significant role in suppressing cancer development in its last stages.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The College of Education for Pure Science, University of Basrah ethics committee reviewed and approved this study (No. 2020/4402).

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