



Further Studies on Taurine and Spirulina Therapeutic Effects on Liver Abnormalities in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Background and objective: Detecting the levels of liver function enzymes in serum is not sufficient to recognize liver injury status or the degree of liver damage. So tracing hepatic enzymes in tissue may be more accurate in diagnosing the case. Taurine and spirulina were selected in this study to test their therapeutic efficacy on hepatocytes mainly suffering from diabetes.

Materials and methods: Rats were divided into five groups. 1-The control group. 2- The diabetic non treated group injected streptozotocin (STZ) 45 mg/kg b.wt. 3-The diabetic group treated with spirulina platensis (15 mg/kg.bd.wt). 4- The diabetic group treated with taurine (500 mg/kg.bd.wt).5-The diabetic group treated with the anti-diabetic drug; amaryl (0.15 mg/ kg.bd.wt).The study tested the influence of STZ (45 mg/kg b.wt) on the ability of the hepatocytes to biosynthesize aminotransferases and lactate dehydrogenase enzymes in addition to the detection of the effect of the chosen dose of STZ on DNA status by using the comet assay in liver cells of diabetic and treated rats. Also the liver total protein content was evaluated in all the groups.

Results and conclusion: Non significant changes in enzyme levels were observed in the liver tissues of the diabetic group treated with spirulina or amaryl drug compared to the diabetic non treated group. On the other hand, taurine treated group showed significant changes in the tissue enzymatic assays. The DNA comet assay of the liver tissue in did not show significant changes in all groups. The study concluded that taurine is suggested as an integrated treatment to compensate the missing targets of the anti-diabetic drugs.

Keywords: Taurine; Spirulina; Hepatocytes; Streptozotocin; DNA

INTRODUCTION

Stabilizing the blood glucose level is mainly one of the fundamental roles of the liver cells. This step can be achieved by storing or breakdown of glycogen in liver cells as a response to insulin signals [1]. Diabetes is characterized by hyperglycemia in blood due insulin insufficiency secreted from the pancreas or insulin insensitivity in several body tissues. Treatments of diabetes mainly are pharmaceutical drugs directed to pancreatic therapy ignoring the liver therapy. So focusing on liver treatment in diabetes is of a vital importance. Nowadays, dietary food and nutrients intake represent a new trend in the prevention of many diseases to avoid the harmful effect of the drugs. Among these important nutrients which acquired much of fame are the blue green algae; Spirulina and then on essential amino acid; taurine. Spirulina is a microalgae which is rich in minerals, pigments, polysaccharides and antioxidants [2].

A great attention was drawn to spirulina species due to its high protein content as it contains mainly about 60-70% of dry weight proteins. These proteins are characterized by the presence of essential amino acids which are digested easily [3]. Spirulina is characterized by the presence of the chromophore; phycocyanobilin (PCB) which is used as a protective and curative agent for diseases when whole spirulina is ingested in murine studies [4]. The anti-hyperglycemic besides the hepatoprotective effect of spirulina species had been proved in many *in vitro* and *in vivo*

studies [5,6]. The clear role of spirulina species as antioxidant preventing from toxicity against lead, mercury and copper was performed in many studies [7-9]. Taurine (2-aminoethylsulphonic acid) is a semi essential sulfur amino acid is synthesized in the human body mainly in the peripheral parts of the liver then it is directed to the circulating blood stream then to some of the body organs (brain, pancreas) also it is found in the bile acids in the liver. Taurine existence is important for the human body as it participates in the metabolism and the growth processes [10]. It was reported that taurine protects the liver from diseases and detoxifies liver cells [11]. Taurine is not abundant in most plant foods (i.e., spirulina algae, green vegetables) [12] but it can be found in meat and sea food [13]. Taurine supplementation is recommended for improving diabetic complications in STZ induced rats [14]. Abnormal mechanisms were detected in the liver cells after streptozotocin (STZ) induced diabetes. So the aim of the study is observing of the liver status after STZ injection at a dose of 45 mg/kg bdwt and its effect on the hepatocytes total protein content, biosynthesis of some enzymes (ALT, AST and LDH) besides exploring the DNA status in the diabetic non treated group and testing the therapeutic efficacy of taurine and spirulina in improving these parameters.

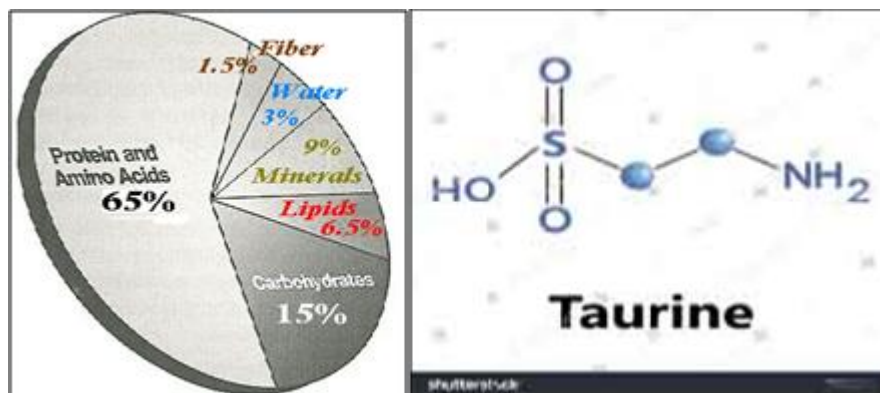


Figure 1: A schematic diagram showing the main constituents of spirulina and taurine

MATERIALS AND METHODS

Materials

Chemicals and kits:

All chemicals used in the experiments were of analytical grade. Kits used for the quantitative determination of different parameters were purchased from Stanbio laboratory, Texas USA and QuinicaClinicaAplicada S.A., Spain. Taurine and streptozotocin were purchased from Sigma Aldrich. Amaryl drug (glimepiride as active ingredient) is a product purchased by Sanofi – Aventis Egypt. *Spirulina platensis* algae were obtained from National Research Center, Cairo, Egypt.

Animals:

Healthy female albino rats (*Rattusrattus*), weighing about 150-200 g were obtained from animal house of the National Research Center, Cairo Egypt. Animals were maintained under standard environmental conditions, i.e., ambient temperature of $(25 \pm 2^\circ\text{C})$, at 45-55% relative humidity, 12 h light/dark cycle and were fed a standard pellet diet and water *ad-libitum*. All the studies were conducted in accordance with the Animal Ethical Committee of the National Research Center under the ethics number (09085).

Methods

Induction of diabetes:

Rats were fasted for 16 hrs and then made diabetic by a single intra peritoneal dose (45 mg/kg/bw) of streptozotocin (STZ) [15]. STZ is dissolved in 0.1 M citrate buffer (pH 4.5) [16]. Three days after STZ injection, rats were screened for fasted blood glucose. Establishment of diabetic state was assured by detecting the blood glucose level in the diabetic non treated group and the treated groups before providing supplementations. Blood samples were taken from lateral tail vein and glucose was determined by the blood glucose monitor Bionime GM100, produced by Taiwan. Rats had blood glucose more than 180 mg/dl were considered diabetic.

Doses preparations:

Spirulinaplantensis algae was suspended in water and administrated orally at a dose of 15 mg/kg/bw [17]. Taurine salt was administrated at a dose of 500 mg/kg/bw. [18]. Taurine was dissolved in distilled water and then administrated orally. Amaryl drug was grinded and dissolved in distilled water then administrated orally at a dose of 0.15 mg/kg/bw.

Experimental design:

Rats were divided into five groups:

Group 1: Rats were not injected with STZ representing the franc control group (negative control) and were sacrificed after a month.

Group 2: Rats were intraperitoneally injected with STZ (45 mg/kg.bw) representing the diabetic non treated group (positive control) and were sacrificed after three days.

Group 3: Rats were injected intraperitoneally with STZ (45 mg/kg.bw) then were supplemented with *Spirulinaplantensis* algae for a month and they were sacrificed.

Group 4: Rats were injected intraperitoneally with STZ (45 mg/kg.bw) then were supplemented with taurine (500 mg/kg.bw) for a month and they were sacrificed.

Group 5: Rats were intraperitoneally injected with STZ (45 mg/kg.bw) and were treated with amaryl drug (0.15 mg/kg.bw) for a month before they were sacrificed.

All the supplements were administrated to rats by gastric intubation besides the standard pellet diet.

Preparation of Liver Homogenates for Enzymatic Assays

1 gram liver from each rat was homogenized in 10 ml of distilled water using an electrical homogenizer with a teflon rod and then centrifuged at 3000 r.p.m for 15 minutes. The supernatant was collected in epindorff tubes and stored at -20°C for enzymatic assays.

Biochemical Assays in Liver Tissue

Alanine and aspartate aminotransferases (ALT & AST) were determined calorimetrically in liver tissue homogenates [19]. Lactate dehydrogenase (LDH) determination was a quantitative, kinetic one [20]. Total protein (Biuret Method) was determined calorimetrically [21].

DNA Comet Assay

DNA comet assay was performed in liver tissues of different groups [22].

Statistical Analysis

All values were expressed as the mean \pm SD. Significant differences between the groups were statistically analyzed using one way ANOVA. *P* value of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

Biochemical Results

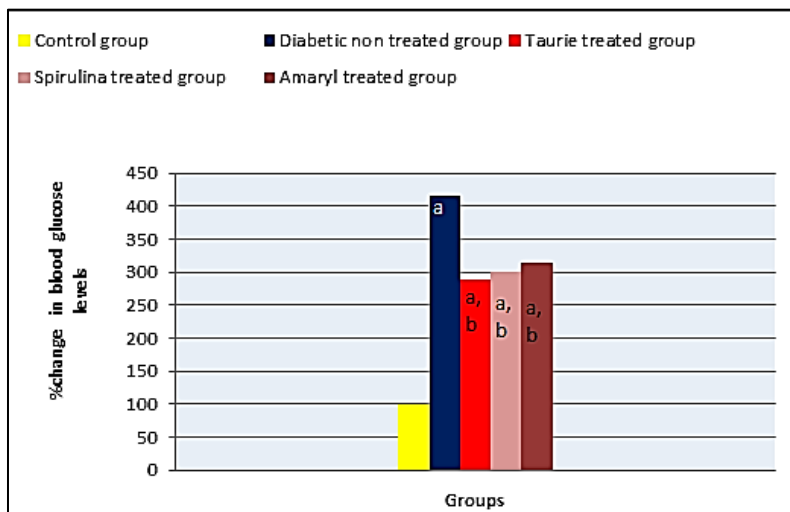


Figure 2: Percent change in blood glucose levels of different groups with significance

^a P < 0.05 compared to control group, ^b P < 0.05 compared to diabetic non treated group.

Figure 2 demonstrated that there was a highly significant increase in blood glucose level in the diabetic non treated group (415%) compared to the control group. Significant decrease in blood glucose levels were observed with similar ranges (-70,-72,-76%) in taurine, spirulina and amaryl treated groups respectively compared to the diabetic non treated group.

Table 1: Effect of STZ and different treatments on alanine aminotransferase (ALT) enzyme activity in rats liver homogenates

Groups\ Parameter	Control	Diabetic non treated group	Spirulina treated group (15mg/kg)	Taurine treated group (500mg/kg)	Amaryl treated group (0.15mg/kg)
ALT (U/mg protein).	0.328 ± 0.11	0.96 ± 0.36 ^a	0.82 ± 0.24 ^a	0.54 ± 0.19 ^b	0.66 ± 0.16 ^a
% change compared to diabetic group.		192.6	-14.6	-43.8	-31.25

Data are represented as Mean ±SD of 5 rats. ^{P^a} Significant at P ≤ 0.05 compared to control group. ^{P^b} Significant at P ≤ 0.05 compared to diabetic group.

Table 2: Effect of STZ and different treatments on aspartate aminotransferase (AST) enzyme activity in rats liver homogenates

Groups\Parameters	Control group	Diabetic non treated group	Spirulina treated group (15 mg/kg)	Taurine treated group (500 mg/kg)	Amaryl treated group (0.15 mg/kg)
AST (U/mg protein)	0.23 ± 0.05	0.86 ± 0.45 ^a	0.54 ± 0.15 ^a	0.32 ± 0.03 ^{abc}	0.59 ± 0.10 ^a
% change compared to diabetic group.		273.91	-37.2	-62.8	-31.4

Data are represented as Mean ±SD of 5 rats. ^{P^a} Significant at P ≤ 0.05 compared to control group. ^{P^b} Significant at P ≤ 0.05 compared to diabetic group. ^{P^c} Significant at P ≤ 0.05 compared to amaryl group.

Elevation of aminotransferases is usually known as a marker for indicating liver abnormal cases. In diabetic case, both the ALT and AST were significantly elevated in hepatocytes with a value reached 192.6% and 273.91% respectively compared to control group as shown in Tables 1 and 2. Treatment with *Spirulina platensis* showed non-significant changes in case of improving the ALT level in hepatocytes with a value reached -14.6% compared to diabetic group as shown in Table 1. Similar non-significant change was also noticed in spirulina treated group in the improvement of AST level where values reached -37.20% compared to diabetic group as shown in Table 2.

Treatment with taurine showed significant improvement in case of ALT level more than that in spirulina treated group. The values of improvement reached -43.8% compared to diabetic group as shown in Table 1. Taurine treated group showed also a significant improvement in AST level with values reached -62.8% compared to diabetic group as shown in Table 2. Surprisingly, the taurine treated group showed a significant improvement in AST level

compared to amaryl groupas shown in Table 2. From the previous data we can conclude the beneficial role of taurine for hepatocytes that were suffering from diabetes

Table 3: Effect of STZ and different treatments on lactate dehydrogenase (LDH) enzyme activity in rats liver homogenates

Groups \ parameter	Control group	Diabetic Non treated group	Spirulina treated group (15mg/kg)	Taurine treated group (500mg/kg)	Amaryl treated group (0.15mg/kg)
LDH (U/mg protein)	4.82 ± 1.75	26.54 ± 9.63 ^a	19.84 ± 8.2 ^a	15.73 ± 6.6 ^{ab}	18.34 ± 6.3 ^a
% change compared to diabetic group.		450.6	-25.24	-40.73	-30.9

Data are represented as Mean ±SD of 5 rats. ^a Significant at $P \leq 0.05$ compared to control group. ^b Significant at $P \leq 0.05$ compared to diabetic group.

Table 3 showed a significant elevation in LDH enzyme level in hepatocytesof diabetic group with values reached 450.6% compared to control group. Non-significant improvement in the level of the enzyme was noticed in the spirulina treated group with values reached -25.24% compared to diabetic group. On the other hand,taurine treated group showed a significant improvement in the LDH enzyme level by values reached -40.73% compared to diabetic group. It seemed that LDH enzyme level in diabetic hepatocytes may be affected by the presence of taurine.

Table 4: Effect of STZ and different treatments on total protein content in rats liver homogenates

Groups/parameter	Control group	Diabetic non treated group	Spirulina treated group (15mg/kg)	Taurine treated group (500mg/kg)	Amaryl treated group (0.15mg/kg)
Protein content (mg protein/g tissue)	343.67 ± 104.6	139.2 ± 53.8 ^a	137 ± 50.8 ^a	175.6 ± 44.6 ^a	176.4 ± 36.5 ^a
% change compared to diabetic group.		-59.5	-1.6	26.14	26.72

Data are represented as Mean ±SD of 5 rats. ^a Significant at $P \leq 0.05$ compared to control group. ^b Significant at $P \leq 0.05$ compared to diabetic group.

Table 4 showed a significant decrease in the total protein content in the hepatocytes of the diabetic group by values equal -59.5% compared to control one. Non-significant improvements in protein content were observed in all treated groups. The taurine and the amaryl treated groups nearly showed similar non-significant improvement with values reached 26.14% and 26.72% respectively compared to the diabetic group. Spirulina treatment seemed to have no effect on improving the protein content in diabetic hepatocytes.

DNA Comet Assay in Liver Tissue of Different Groups

Table 5: Detection of parameters of DNA comet assay in different groups

Parameters\Groups	Tail length	% DNA in tail	Tail moment
Control group (n=4)	4.32 ± 0.27	19.47 ± 3.96	1.06 ± 0.27
Diabetic non treated group (n=4)	4.55 ± 0.52 [#]	21.28 ± 3.64 [#]	1.15 ± 0.13 [#]
Spirulina treated group (n=3)	3.76 ± 0.76 ^{#*}	21 ± 6.6 ^{#*}	0.96 ± 0.52 ^{#*}
Taurine treated group (n=3)	4.2 ± 0.43 ^{#*}	21.6 ± 1.77 ^{#*}	1.13 ± 0.14 ^{#*}
Amaryl treated group (n=3)	4.26 ± 0.45 ^{#*}	21.5 ± 2.41 ^{#*}	1.17 ± 0.20 ^{#*}

Data are represented as Mean ±SD of 3-4 rats. [#] Non Significant at $P \leq 0.05$ compared to control group. ^{*} Non Significant at $P \leq 0.05$ compared to diabetic group.

DNA Comet Assay Images in Liver Tissue of Different Groups

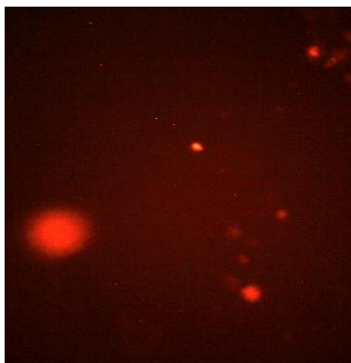


Figure 3: Control group

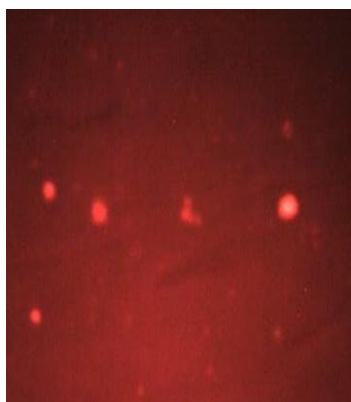


Figure 4: Diabetic on treated group

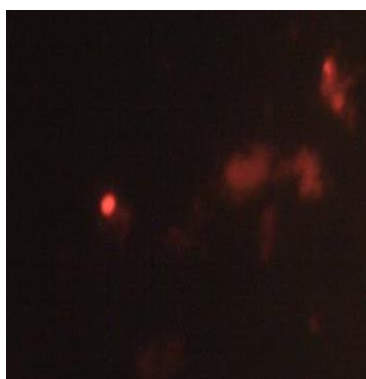


Figure 5: Spirulina treated group

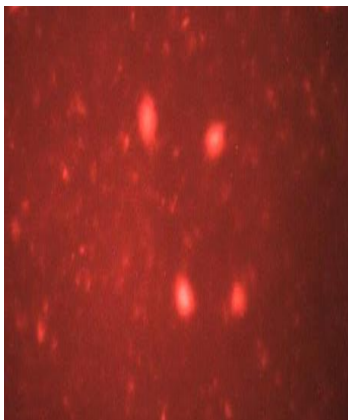


Figure 6: Taurine treated group

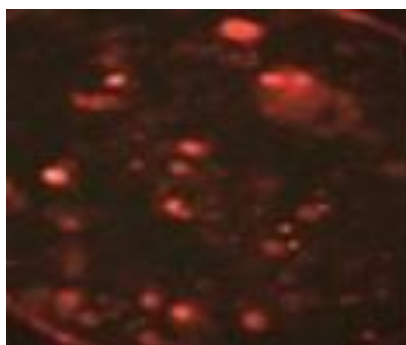


Figure 7: Amaryl treated group

Table 5 clarified the non-significant DNA changes in diabetic group compared to control one as shown in Figures 3 and 4. Different treatment groups did not show any significant change compared to diabetic group or to the control one as shown in Figures 5-7.

DISCUSSION

STZ destroying mechanism depends on the released free radicals that in turn attack the DNA fragments of beta cells in the islets of Langerhans in the pancreas [23]. Applying high doses of STZ is able to cause more destruction in the beta cells besides the expectation to harm other organs as the liver and the kidneys [24].

In this study, it was observed that there was a great elevation in both of the aminotransferases level (ALT & AST) in addition to the elevation of LDH enzyme level in the diabetic non treated group. McAnuff et al. detected the elevation of aminotransferases in hepatocytes during diabetic state which is in accordance with the observed results. According to the comet assay results, the non-significant DNA changes observed in Table 5 and Figures 3 and 4 that lacked a long and clear comet tail in the diabetic non treated group compared to the control one, suggested that this elevation of liver enzymes can be attributed to the diabetes influence (i.e., insulin deficiency and ormonal system dysfunction) and not to the STZ toxic activity. Saandeep et al. approved that DNA damage in diabetic liver tissue is time dependent. So as a result of beginning the treatments in this study after three days from STZ injection up till a month, this lead to a rapid improvement in the diabetic case and approved no changes in DNA status in these groups as shown in Table 5 and Figures 5-7.

Ragavan and Krishnakumari [27] reported that in diabetic cases, usually there is an elevation in liver enzymes due to insulin deficiency and hyperglycemia. Body cells tried to compensate the insulin deficiency and the decreasing levels of the glucose entering them by using amino acids or non-carbohydrate molecules to be a new source to biosynthesize glucose for energy generation (gluconeogenesis which occurs mainly in liver and kidneys). Elevation of ALT and AST played an essential role in this biosynthesis. Oosterveer and Schoonjans discussed that lacking of glucose which entered the cells leads to the deprivation of the cells to energy and oxygen which oblige the cells to change their aerobic mechanism to anaerobic one to obtain energy and this requires the presence of lactate dehydrogenase to do this step. So in diabetics, there is a great elevation of LDH enzyme. These findings are in

accordance with our results. The present study also detected a great decrease in the total protein content in hepatocytes of the diabetic group which is attributed to insulin deficiency [28,29].

Although there was a similar decreasing effect of the three treatments on the blood glucose levels (Figure 2), there was a great difference in their impact on improving liver parameters. The present work detected that spirulina treated group showed non-significant decrease in ALT, AST and LDH enzyme levels compared to diabetic group. It was observed also that spirulina at the dose of 15 mg/kg had no effect on total protein recovery in hepatocytes despite of its high content of proteins. So it was suggested that spirulina may have an indirect role on hepatocytes aminotransferases and lactate dehydrogenase levels. We can attribute the significant decrease in blood level in spirulina treated group to its antioxidant potential in improving the diabetic case [30].

On the other hand, taurine treated group showed significant decrease in AST especially and also in ALT and LDH enzyme levels compared to diabetic non treated group. These data suggested that taurine plays a role in energy metabolism and regulation of mitochondrial function (gluconeogenesis). These results are in accordance with those observed by [31] who suggested the role of taurine incorporation to the mitochondrial proteins for the survival of the mitochondria (the house power of the cell) and in turn the survival of the cell. We can notice the clear role of taurine in improving the tissue liver function enzymes as observed previously besides its antioxidant effect observed in liver tissue [30], we can say that treatment with taurine may be more effective in improving liver diabetic complications more than spirulina treatment in spite of its mild impact on the recovery of total protein content in liver cells.

Amaryl treated group showed non-significant changes in the liver tissue enzymatic assays and in the total protein content. These findings approved that the mechanism of action of the drug on influencing the non-destroyed beta cells in the pancreas to secrete more insulin [32]. Accordingly, it was found that taurine group results compete with the amaryl group in the detected parameters, which encourage us to suggest taurine as an integrated treatment with amaryl drug to compensate the missing targets of the drug and to decrease its undesirable effects. This step may need more studies on taurine action on glycolytic enzymes in liver.

CONCLUSION

The therapeutic effect of taurine treatment appeared clearly in improving parameters related to hepatocytes recovery more than spirulina treatment at these chosen doses. Taurine may play an indirect role in energy production in hepatocytes. Spirulina as a natural nutrient had a mild effect on restoring the hepatocytes enzymes so applying higher doses may affect the liver. Both taurine and spirulina failed in restoring total protein content in diabetic livers. More studies have to be done on the effect of taurine on glycolytic enzymes in liver cells.

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