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Further Studies on Taurine and Spirulinatherapeutic 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 spirulinaplatensis (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 aminotranseferases 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. Spirulinais 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 thechromophore; phycocyanobilin (PCB) which is usedas a protective and curative agentfor diseases when whole spirulina is ingested in murine studies [4]. The anti-hyperglycemicbesides the hepatoprotective effect of spirulina specieshad been proved inmany *in vitro* and *in vivo*

studies [5,6].The clear role of spirulina species as antioxidant preventing from toxicityagainst 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).

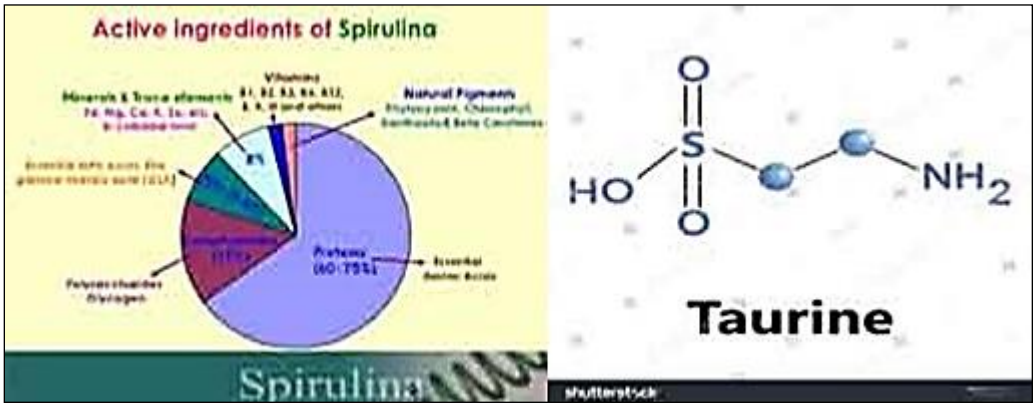

Figure 1: A schematic diagram showing the main constituents of spirulina and taurine

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. *Spirulinaplatensis* 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 ± 2°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 peritonial 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:
Spirulinaplatensis 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 intraperitonially 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 intraperitonially with STZ (45 mg/kg.bw) then were supplemented with*Spirulinaplatensis* algae for a month and they were sacrificed.
Group 4: Rats were injected intraperitonially 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 intraperitonially 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 ± SD. Significant differences between the groups were statistically analyzed using one way ANOVA. *P* value of 0.05 or less was considered statistically significant. *Swietenia mahagoni* seeds were purchased from a local medicinal shop, Dhaka. The seeds were identified at the National Herbarium.

Extract Preparation
Swietenia mahagoni seeds were collected and white inner parts were separated from the brown peel of seed. The seeds were dried, ground and crushed to powder. The dried powder of *Swietenia mahagoni* seeds (3000 g) were extracted with 80% ethanol at room temperature for 3 days and then filtered and evaporate to concentrate by using rotary vacuum evaporator bellow 40°C. The extract was freeze dried. Then 50 g extract was dissolved in a small amount of water and applied to a sephalex LH-20 column (70 × 3.5 cm). The fractions were collected.

Acute Toxicity Study
After brine shrimp lethality assay it was found that Sm-SEF7 fraction was nontoxic (using National Cancer Institute protocol). As the LD50 was found to be more than 4000 mg/kg, so the dose 312.5 mg/(10 ml water)/kg body weight was selected.

Chemicals Used
All chemicals and drugs used were obtained commercially and of analytical grade. Streptozocin and Glibenclamide (Sigma-Aldrich Company, St. Louis, Missouri, USA) &all other chemicals, reagents and kits were purchased from the local market.

Experimental Animals

The experiments were carried out on Long-Evans rats (180-220 gm) of both sexes, bred at BIRDEM animal house and maintained at a constant room temperature of 22 ± 5°C with humidity of 40-70% and the natural 12 hours day-night cycle. The animals are housed in an air-conditioned animal room and fed on pellets and water. Fasted animal were deprived of food for at least twelve hours but allowed free access to plain water.

Induction of Type 2 Diabetes

Type 2 diabetes was induced by a single intraperitoneal injection of STZ at a dose of 90 mg/kg body weight. Experiments were carried out 3 months after STZ injection and rats having blood glucose level 8.5-12 mmol/l at fasting were taken as diabetic rat.

Experimental Design

In the experiment a total number of 18 rats were used. The rats were divided into 3 groups of six each.

Group 1:
Type 2 diabetic control rats received only water.
Group 2:
Type 2 diabetic control rats received standard drug glibenclamide at a dose of 5 mg/kg body weight.
Group 3:
Type 2 diabetic rats were received Fraction Sm-SEF7 at a dose of 312.5 mg/(10 ml water)/kg body weight for 21 days orally.

At the end of the experiment rats were subjected to light ether anaesthesia then blood was collected from the retro-orbital venous plexus following the technique described by Coccheto and Bjornsson [22]. Blood glucose was determined by GOD–POD kit method. The change in body weight was observed once a week [23]. After 15 days, body weight was determined and the animals were sacrificed under theinfluence of anesthetic ether. The blood was collected by heart puncture. The blood sample withdrawn from the sacrificed animals was centrifuged at 3000 rpm for 15 min, [24] and was analyzed for lipid profiles [25-27] (Serum cholesterol, Serum triglyceride, HDL cholesterol).

Statistical Analysis

All the data are expressed as mean ± SD (Standard deviation). The differences between diabetic control and treatment group were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons and the values of P<0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Biochemical Results

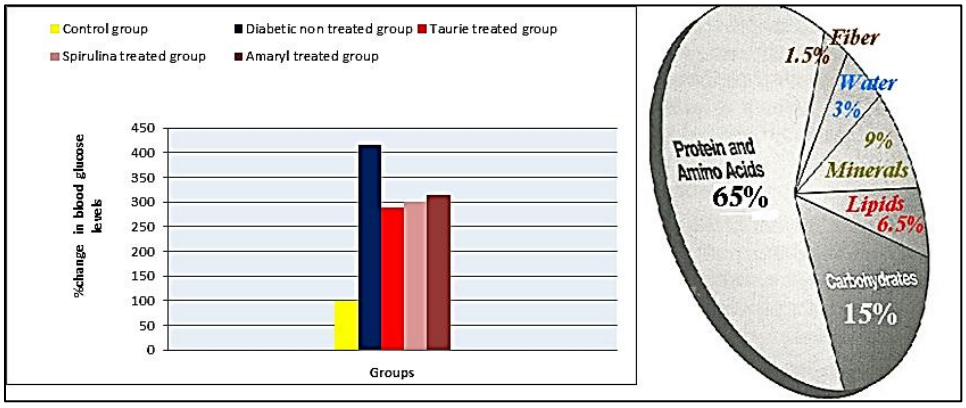

Figure 2: Percent change in blood glucose levels of different groups with significance

[a] P < 0.05 compared to control group, [b] P < 0.05 compared todiabetic 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 observedwith 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 $P \leq 0.05$ compared to control group. P^bSignificant at $P \leq 0.05$ 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 trated 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 $P \leq 0.05$ compared to control group. P^bSignificant at $P \leq 0.05$ compared to diabetic group.P^c Significant at $P \leq 0.05$ 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 *Spirulinaplatensis* 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. P^a Significant at $P \leq 0.05$ compared to control group. P^bSignificant 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. P^a Significant at $P \leq 0.05$ compared to control group. P^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. $P^{\#}$ Non Significant at $P \leq 0.05$ compared to control group. P^{*} Non Significant at $P \leq 0.05$ compared to diabetic group.

DNA Comet Assay Images in Liver Tissue of Different Groups

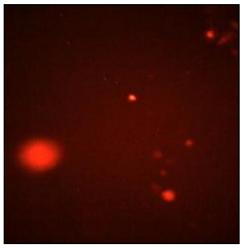

Figure 3: Control group

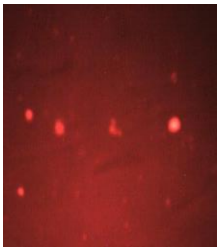

Figure 4: Diabetic on treated group

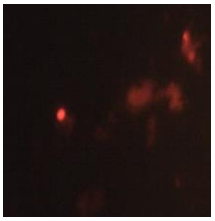

Figure 5: Spirulina treated group

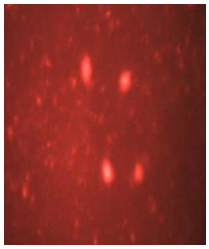

Figure 6: Taurine treated group

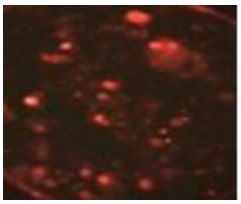

Figure 7: Amaryl treated group

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 aminotranseferases 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-destructed beta cells in the pancreas to secret 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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