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Research Article

# *Spirulina platensis* improves insulin sensitivity and reduces hyperglycemia-mediated oxidative stress in fructose-fed rats

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## Abstract

Oxidative stress, hyperglycemia and insulin resistance are hallmarks of diabetes mellitus. The present study aimed to assess the antidiabetic activity of a local strain of *Spirulina platensis* produced at Pahou (Benin), known as "Spiruline Dou Bogan" (SPD), in fructose-fed rats. Glucose metabolism impairment was induced by feeding 8g/kg, body weight (bw), fructose solution orally to Sprague Dawley rats (n = 8) for 56 days, treated with SPD (18.75; 37.5 and 75 mg/kg, bw), and analyzed for plasma blood glucose, serum biochemistry and the markers of oxidative stress (Ferric Reducing Antioxidant Power Assay (FRAP), Malondialdehyde (MDA), Reduced glutathione (GSH), DPPH radical scavenging assay. SPD concentrations, given orally for 42 days, significantly reversed the elevations in plasma blood glucose, MDA, and the reduction in kidneys glutathione activity. Oral administration of 18.75, 37.5, and 75 mg/kg doses of SPD also lowered serum Aspartate Aminotransferase (ASAT), Alanine Aminotransferase (ALAT), Triglycerides and Creatinine levels. SPD 75 mg/kg treatment in particular has significantly decreased serum Triglycerides level and increased HDL-Cholesterol levels, reversing the atherogenic potential of 56 days fructose administration. The consumption of *S. platensis* produced locally in Benin as a food supplement, easily accessible for low-income populations, may be helpful in the prevention and management of type 2 diabetes.

**Keywords:** Oxidative stress; *Spirulina platensis*; hyperglycemia; insulin resistance, fructose diet

## INTRODUCTION

Cyanobacteria are among the most primitive prokaryotes on Earth. They feature both characteristics of plants (ability to perform photosynthesis) and animals (presence of complex sugars similar to glycogen in their cellular membrane). The principal cyanobacteria for producing natural green-blue pigments are from the genus Arthrospira, also known as Spirulina, a blue-green alga [1]. Specifically, *Spirulina platensis* and *Spirulina maxima* are the species largely used with a wide market such as food additives, health food, cosmetics, pharmaceuticals and medicine [2,3]. Recent reports, focusing on the presence of potential functional ingredients in *S. platensis* such as β-complex, vitamins, minerals, proteins, γ-linoleic acid, nutraceutical pigments like phycocyanin [4], polyphenols, and β-carotene have demonstrated the relevant role of this alga in disease risk reduction [5]. *Spirulina platensis* has a wide range of medicinal properties, including anti-inflammatory, hypolipemiant, neuroprotective, hepatoprotective, immunomodulatory and anticancer activities [6,7]. Heo and Choung [40], and Diniz et al [41] has shown that *Spirulina platensis* consumption prevents obesity and improves the deleterious effects of hypercaloric diets in rats. As a natural product, this alga is non-toxic, cheap and has no side effects, contrary to synthetic drugs [8]. Besides, *S. platensis* can provide significant multi-organ protection against chronic diseases such as diabetes 9. As oral hypoglycemic agents currently used in clinical practice have characteristic profiles of serious side effects [10,11], there is a need to search for new and efficient antidiabetic agents which are devoid of side effects and easily accessible to populations of low income countries such as in Africa. To treat diabetes, traditional healers in Benin often use different plants including *S. platensis*. *S. platensis* is composed of closely related, genetically and physiologically distinct lineages whose differences enable them to adapt to specific ecological niches [12] and have various pharmacological properties. *S. platensis* from IREDESA ("*Institut Régional pour le Développement et la Santé*", Pahou, Benin), a Spirulina of the species *Spirulina platensis* known locally as "Spiruline Dou Bogan" (SPD), is a well-known representative in Benin and south of Togo. A previous work in our laboratory has shown that SPD has significant *in vivo* antihyperglycemic activity and the ability to reduce obesity in rats fed with sub-acute high fructose and fat diet [13]. The present work highlighted the

antioxidant, scavenging activities and insulin sensitizing effects of SPD administration on chronic high fructose diet induced glucose metabolism impairments in rats.

## MATERIALS AND METHODS

### Plant Material

The *S. platensis* strain (SPD) used in this investigation was obtained from IREDESA Institute, Pahou District (Cotonou, Benin) in February 2021, where it is produced in modified Zarrouk's medium at pH 9.5 under a light intensity of 2500 lux at 30 °C for 16 h light and 8 h dark period. On the 20th day, exponential growth phase of the algae is observed, and then the cultures are harvested, filtered, collected, overnight dried below 40 °C and conserved in small aluminium-polyethylene containers for further use. For the tests, SPD powder was weighed and directly diluted in distilled water.

### Chemicals

All the chemicals used in this experiment were of analytical grade and purchased from Sigma- Aldrich (St. Louis, USA).

### Quantification of functional bioactive components

#### *Determination of the total phenols content (TPC)*

Total phenols content in the SPD aqueous solution was determined spectrophotometrically by using Folin-Ciocalteau method, with gallic acid as a standard phenolic compound [14]. 1 mL of SPD solution (0.5 g of the biomass with 10 mL of an 80% aqueous solution of methanol, adjusted to pH 1.5) in a volumetric flask was diluted with distilled water (46 mL). 1 mL Folin-Ciocalteau reagent was added and mixed thoroughly. After 3 minutes, 3 mL of sodium carbonate (2% $Na_2CO_3$) was added and the mixture was allowed to stand with intermittent shaking for 3 h. The absorbance of the mixture was measured at 760 nm (Genesys 20 spectrophotometer, Thermo Scientific). The phenolic content was expressed as mg of gallic acid equivalent (GAE) per 100 mg of the sample.

#### *Determination of total flavonoids compounds*

Flavonoids content was determined by aluminum trichloride method using rutin as reference compound [15]. A volume of 125 µL of SPD aqueous solution (1mg/mL) was added to 75 µL of a sodium acetate solution (50 mg/mL). The mixture was allowed to stand for 6 min, then 150 µL of aluminium trichloride (20 mg/mL) was added and incubated for 5 min, followed by the addition of 750 µL of sodium hydroxide (NaOH 1 M). The final volume of the solution was adjusted to 2500 µL with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm. The total flavonoids content was expressed as mg of rutin equivalent/100 g of the sample.

#### *Determination of total tannins compounds*

Tannin contents were determined by the method of Broadhurst and Jones [16] with slight modification, using gallic acid as a reference compound. A volume of 400µL of SPD aqueous solution was added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm. The condensed tannin was expressed as mg gallic acid/100 g.

#### *Quantitative Analysis of Phycocyanin*

Phycocyanin (PHY) content was determined by a method based on the absorption spectrum of PHY as described previously by Boussiba and Richmond [17]. 0.5g of SPD dry mass was weighed into a 50-mL centrifuge tube; 25 mL of 0.1 mol/L sodium phosphate buffer (pH 6.0) was pipetted into this tube and mixed. The sample was kept at 30 °C for 16 h and then centrifuged at 2000 rpm for 15 min. The supernatant was filtered and distilled water was added to 2 mL of the filtrate to a volume of 50 mL; absorbance was measured at 560, 618, and 650nm. The PHY concentration (mg/100g) was calculated by:

$$PHY= (0.198\times A\_618-0.009\times A\_560-0.133\times A\_650) \times D\times 100/W$$

Where A618 was the absorbance at 618 nm, A560 the absorbance at 560 nm, A650 the absorbance at 650 nm, W is the weight of the sample and D is the dilution ratio.

### Biological analyses

#### *Animals*

Both male and female Sprague Dawley rats (50:50) aged 8-10 weeks (weighing about 150-200g) were used. They were obtained from Nigerian Institute for Medical Research (Lagos, Nigeria) and housed in standard environmental conditions (temperature 24–25 °C, relative humidity (50-70%) and a 12 h/12 h light-dark cycle). They were acclimatized to the laboratory condition for 2 weeks with access to standard diet (3.5 % fat, 16 % protein, 4.5 % fiber, and 5 % ash as source of minerals) and tap water *ad libitum*. All animals were treated according to the ethical guidelines approved by Institutional Animal Ethics Committee, University of Lomé, Togo (approval no 006/2021/CB-FDS-UL).

#### *Induction of glucose metabolism impairment and treatments*

The experimental animals were fed orally with fructose solution (8 g/kg, body weight) for 56 days and blood glucose was monitored at an interval of 14 days until the 56th day while various antioxidant and serum biochemistry parameters were analyzed at the end of the 56-day period.

Forty Sprague Dawley rats were divided into the following five groups (n = 8): normal control group (NC), fructose-fed control group (FC), fructose-fed rats treated with 18.75 mg/kg of SPD group (FSPD1), fructose-fed rats treated with 37.5 mg/kg of SPD group (FSPD2) and fructose-fed rats treated with 75 mg/kg of SPD group (FSPD3). These concentrations were chosen basing on our previous studies [13] in which 75 and 150 mg/kg of SPD prevented metabolic syndrome induced by high fructose and fat diet. The groups were treated as below (Fig. 1): Normal control (NC): rats received only tap water and standard feed; FC: received 8 g/kg body weight fructose for 56 days and was treated with distilled water over a period of 42 days starting from day 14; FSPD1: rats were fed with fructose (8 g/kg) for 56 days and treated orally with 18.75 mg/kg SPD once daily over a period of 42 days starting from day 14; FSPD2: these rats were fed with fructose (8 g/kg) for 56 days and treated orally with 37.5 mg/kg SPD once daily over a period of 42 days starting from day 14; FSPD3: rats of this group were fed with fructose (8 g/kg) for 56 days and treated orally with 75 mg/kg SPD once daily over a period of 42 days starting from day 14.

#### *Body weight and blood glucose estimation*

Rats were weighed at 7-day intervals till the end of the study. Blood was collected from the tail vein at 0, 14, 28, 42, 56th day, and blood glucose levels were measured with a glucometer (One Touch Ultra Easy, LifeScan, UK/Ireland).

#### *Insulin resistance test (IRT)*

On the 49th day (Fig.1), IRT was performed on all the animals. Rats were fasted for 6 hours and subcutaneously injected recombinant human insulin (4 IU/kg, bw) freshly prepared in Tris buffer (pH 7). Blood glucose level was then determined at 0 (prior to insulin injection), 15-, 30-, 45- and 60-min

following insulin administration using a glucometer (One Touch Ultra Easy).

***Oral glucose tolerance test (OGTT)***

Glucose tolerance test (OGTT) evaluates the ability to respond appropriately to a glucose challenge. OGTT was conducted in the five groups at 56th day (Fig. 1). Animals were fasted overnight to obtain baseline blood glucose levels. Subsequently, rats of all the groups received orally glucose solution (4 g/kg, bw) 60 min after the last daily treatments. Blood glucose level was determined at 0 (before glucose administration), 30, 60, and 120 min after glucose administration using glucometer (One Touch Ultra Easy).

***Serum biochemistry***

On the last day of the experiment, following OGTT, rats were anaesthetized with ethyl ether and blood was collected from retro orbital plexus into non heparinized vial. Samples were centrifuged at 3000 rpm (Shimadzu, Japan) for 10 min. The sera were stored at -20 °C until they were analyzed. Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatinine, triglycerides, total cholesterol (T-Ch) and HDL-cholesterol (HDL-Ch) in blood serum were evaluated by an autoanalyzer (Prietest Touch Plus, Robonik India PVT LTD), using specific spectrophotometric diagnostic kits (PharmaLab, India). The triglyceride/high-density lipoprotein cholesterol (Trigly/HDL-Ch) ratio was calculated, and atherogenic index (AI) was determined according to the Friedewald equation [18]:

$$AI = (T\text{-}Ch - HDL\text{-}Ch) / HDL\text{-}Ch.$$

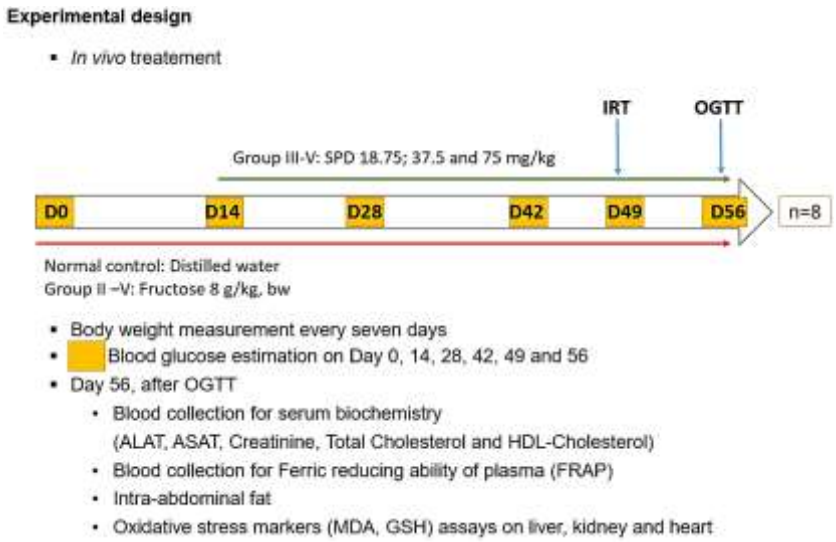


Figure 1: Experimental design of the study showing treatments and assays

***Ferric reducing ability of plasma (FRAP)***

On the last day of experiment, blood was also collected from retro orbital plexus through heparinized capillary into heparinized vials for ferric reducing antioxidant power (FRAP) test on plasma. Blood samples were centrifuged at 3000 rpm (Shimadzu, Japan) for 10 min within 1h after collection. The plasmas were stored at -20 °C until they were analyzed. The reducing power of plasma was determined according to the method of Benzie and Strain [19]. In brief, freshly prepared FRAP reagent [(300 µL, 10 mM tripyridyltriazine, 20 mM ferric chloride, and 500 mM acetate buffer, pH 3,6), ratio 01:01:10] was mixed with plasma (10 µL), and the absorbance was measured at 593 nm (Genesys 20 spectrophotometer, Thermo Scientific).

***Abdominal fat and oxidative stress markers***

On the 56th day of experiment, after blood collection, the animals were weighed and sacrificed by cervical dislocation. Livers, kidneys and hearts were quickly excised, weighed, frozen and stored at -20°C until glutathione (GSH) content and lipid peroxidation (MDA) analyses. Intra-abdominal fat was also dissected and weighed. Livers, kidneys and hearts were then washed three times in extraction buffer (10 mM Tris-HCl, pH 7.5), ground, and homogenates obtained were used for the assays. GSH levels were estimated calorimetrically using Ellman's reagent as described previously by Sedlak and Lindsay [20]. Malondialdehyde (MDA) concentration was measured in 10% homogenates of liver, heart and kidney using LPO assay kit from Calbiochem (San Diego, CA) according to the method described by Patlolla et al [21]. Protein concentrations were determined using the Bradford method with bovine serum albumin as the standard [22].

**In vitro antioxidant activity**

The free radical scavenging activity of SPD aqueous solution was measured by DPPH, using the method previously described by Shimada et al [23]. A 0.1 mM solution of DPPH in ethanol was prepared and 1 mL of this solution was added to aqueous SPD solution (3 mL) at different concentrations (50-200 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm. Quercetin was used as standard antioxidant compound. The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A0 - At / A0) \times 100]$$

Where, A0 was the absorbance of the control reaction and At was the absorbance in the presence of the standard or SPD.

**Statistical analysis**

Results were expressed as mean ± SEM (n = 8). Statistical analyses were performed with one-way and two-way analysis of variance (ANOVA) followed by post hoc "Dunnett's Multiple Comparison Test" using GraphPad prism software package. $P<0.05$ were considered statistically significant.

## RESULTS

**SPD functional bioactive components**

Total phenolic, flavonoid, and tannin and phycocyanin content were measured and shown in Table 1. Mean concentration of

total phenols and flavonoids were 135 ± 4.2 mg GAE/100 g and 103.57 ± 12.92 mg rutin equivalent/100 g. The content of the phycocyanin in the sample was 311.9 ± 7.70 mg/100 g.

Table 1: Functional bioactive components of SPD

| Bioactive compound | Concentration (mg/100 g of SPD) | Standard Drug |
|---|---|---|
| Total phenols | 135 ± 4.2 | Gallic acid |
| Tannins | 102.50 ± 4.25 | Gallic acid |
| Flavonoids | 103.57 ± 12.92 | Rutin |
| Phycocyanin | 311.9 ± 7.70 | - |

Abbreviations: SPD – "Spiruline Dou Bogan". Units: mg/100 g. The values are expressed as Mean ± SEM equivalents of Standard Drugs when used, n=3.

**Biological tests**

***Effect of SPD on body weight variation***

At day 0 (baseline) body weights were not statistically different in the five groups. At the end of the study, body weights were significantly higher in FC group (162.50 ± 6.49 g, P < 0.001) compared to NC group (128.00 ± 3.82 g). After six-week treatment with SPD, lower body weight was observed in FSPD3 group as compared to FC group. The highest dose (75 mg/kg) demonstrated a statistically significant body weight attenuation effect throughout the study (Fig. 2).

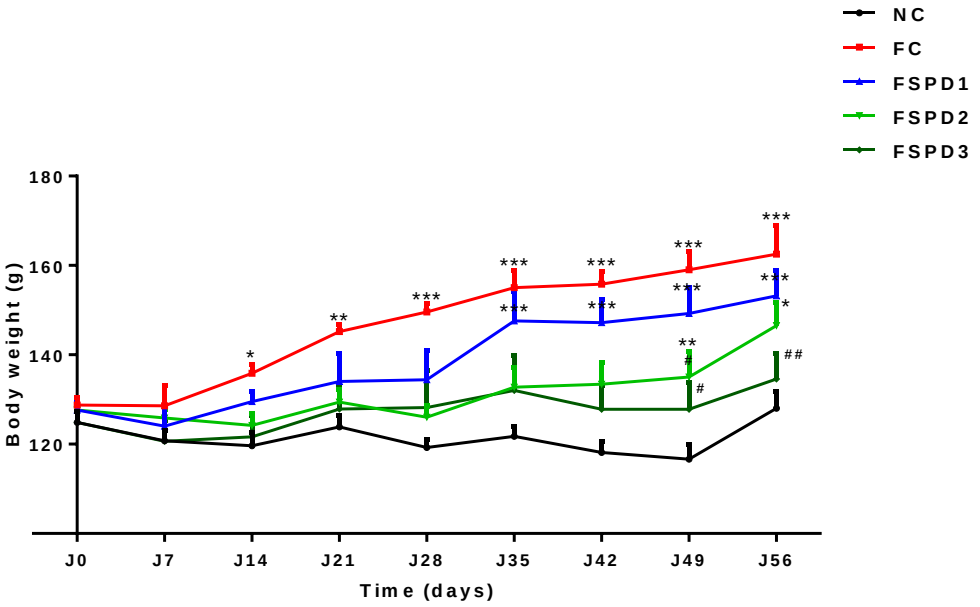


Figure 2: Effect of SPD on the variation of body weight after 56-days treatment

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: g. The values are expressed as Mean ± SEM, n = 8. *p<0.05, **p<0.01, ***p< 0.001 significant when compared with normal control group. #p< 0.05, ## p < 0.01 significant when compared with fructose control.

***Effect of SPD on intra-abdominal fat***

After 8 weeks of the experiment, the FC rats showed a significant increase of intra-abdominal fat (Fig. 3), as compared to NC group (P<0.01). SPD (37.5 and 75 mg/kg) significantly reversed the intra-abdominal fat increase compared to FC animals (P<0.05).

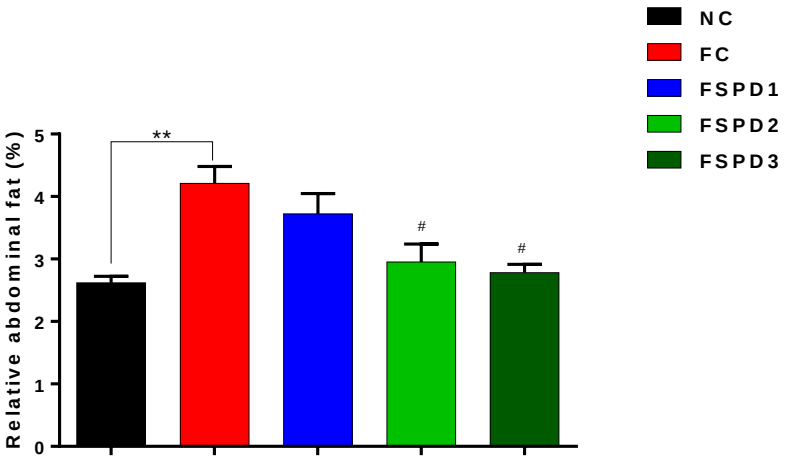


Figure 3: Effect of SPD on the ratio of intra-abdominal fat/body weight

The relative weight of fat is the ratio of the weight of the fat collected on the final weight of the animal x 100. NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: %. The values are expressed as Mean ± SEM, n = 8. **p< 0.01 significant when compared with normal control group. #p< 0.05 significant when compared to fructose control.

***Effect of SPD on blood glucose level in fructose-induced hyperglycemia in rats***

Fructose feeding was accompanied with a gradual fasting blood glucose level which ended on day 56 with a significantly high value (5.72 ± 0.16 mmol/L, p < 0.001) in FC compared to NC rats (4.14 ± 0.29 mmol/L). SPD administration attenuated the increase of fasting blood glucose level with the highest glucose lowering effect in FSPD3 group (Fig. 4). This was evidenced by overall lower glucose AUC in FSPD groups compared FC group.

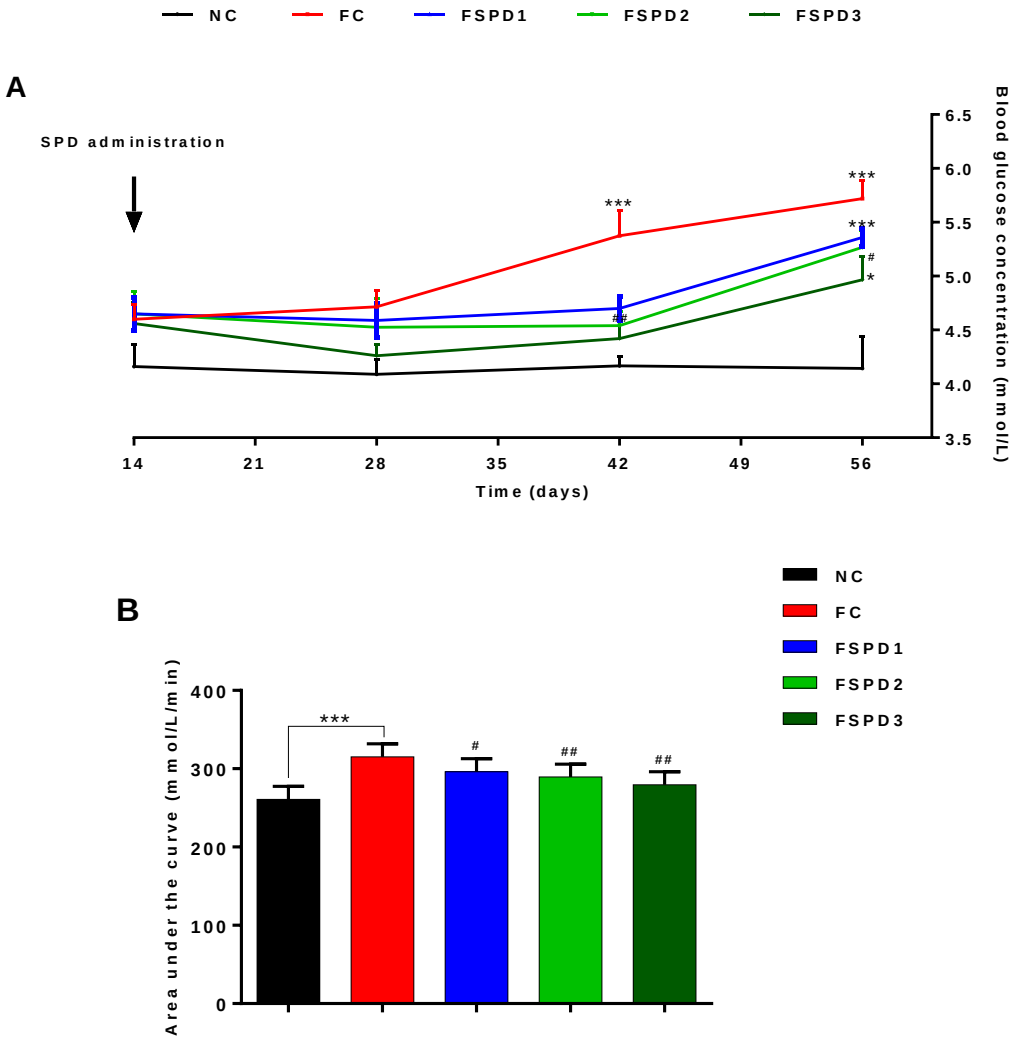


Figure 4: Effect of SPD administration on blood glucose level (A: blood glucose concentration; B: area under the curve (AUC))

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: mmol/L. The values are expressed as Mean ± SEM, n = 8. *p< 0.05, **p < 0.01, ***p< 0.001 significant when compared with normal control group. #p< 0.05, ##p< 0.01 significant when compared with fructose control.

***Effect of SPD on insulin resistance***

After administration of insulin, blood glucose levels in FC rats remained higher within the first 45 min (+44% at t = 15 min and +69% at t = 30; P< 0.05 compared to NC group), suggesting insulin resistance in FC rats (Fig. 5A). Interestingly, blood glucose levels and overall glucose area under the curve (Figure 5 B) in FSPD3 group remained lower than the FC group and no significant difference was found when compared to NC group. The lowest glucose level was observed at dose of 75 mg/kg (-40% at t = 15min, and -53% at t = 30; P< 0.05 compared to FC group).

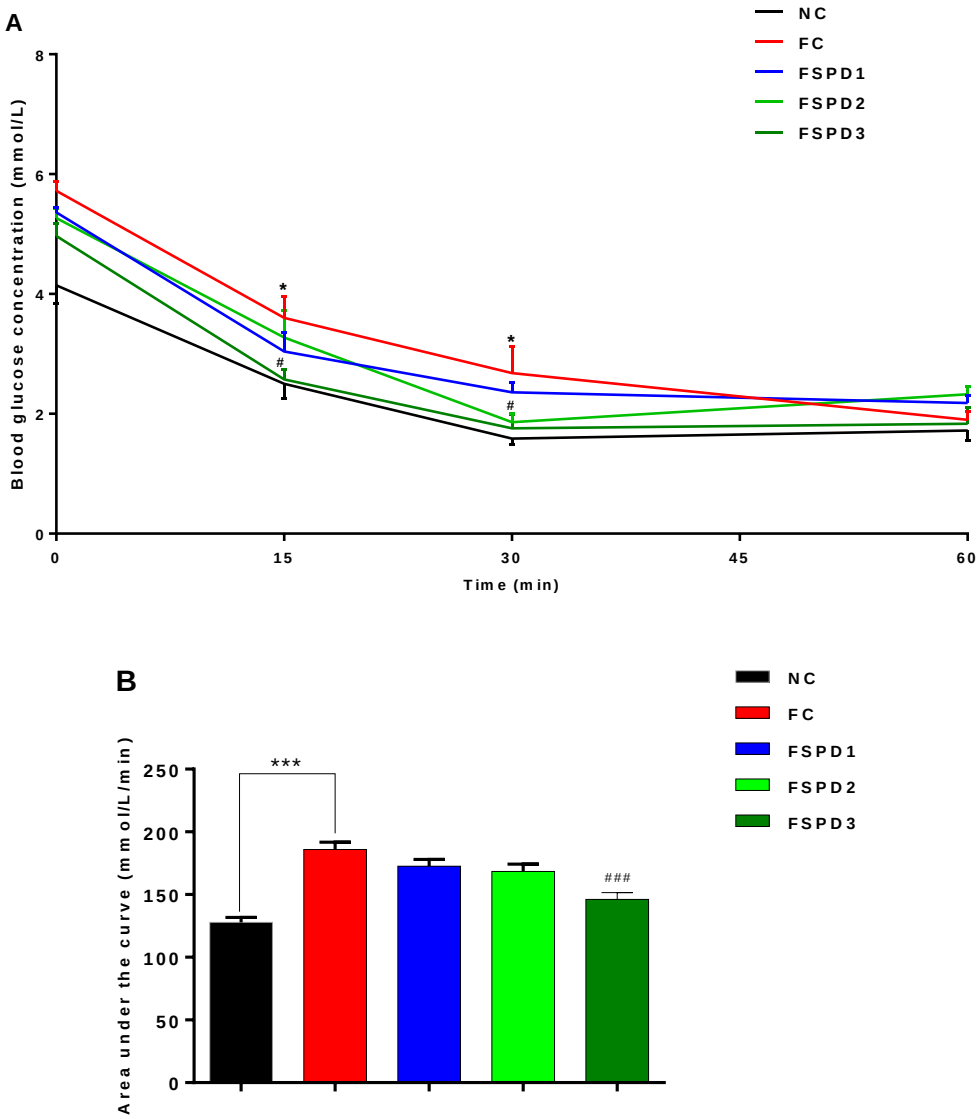


Figure 5: Effect of SPD on insulin resistance (A: blood glucose level; B: area under the curve (AUC))

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: mmol/L (A), mmol/L/min (B). The values are expressed as Mean ± SEM, n = 8. *p< 0.05, ***p< 0.001 significant when compared with normal control group. #p< 0.05, ###p< 0.001 significant when compared with fructose control.

***Effect of SPD on Oral glucose tolerance test (OGTT)***

The effect of SPD on oral glucose tolerance test (OGTT) is presented in Figure 6. Thirty (30) min after the glucose load, blood glucose level increased in all animals. Blood glucose level in FC group peaked 1 h after glucose administration (+24%; P< 0.05 compared to NC group). Although glucose level in FC group declined thereafter, it remained statistically higher than in NC group (+27%; P< 0.01). Conversely, in FSPD groups, glucose level remained low and the maximum reduction was observed at dose of 75 mg/kg (-28%; P< 0.01 compared to FC group). Blood glucose lowering effect in glucose loaded animals was evidenced by significantly lower values of glucose AUC in FSPD groups compared to FC rats (Fig. 6B).

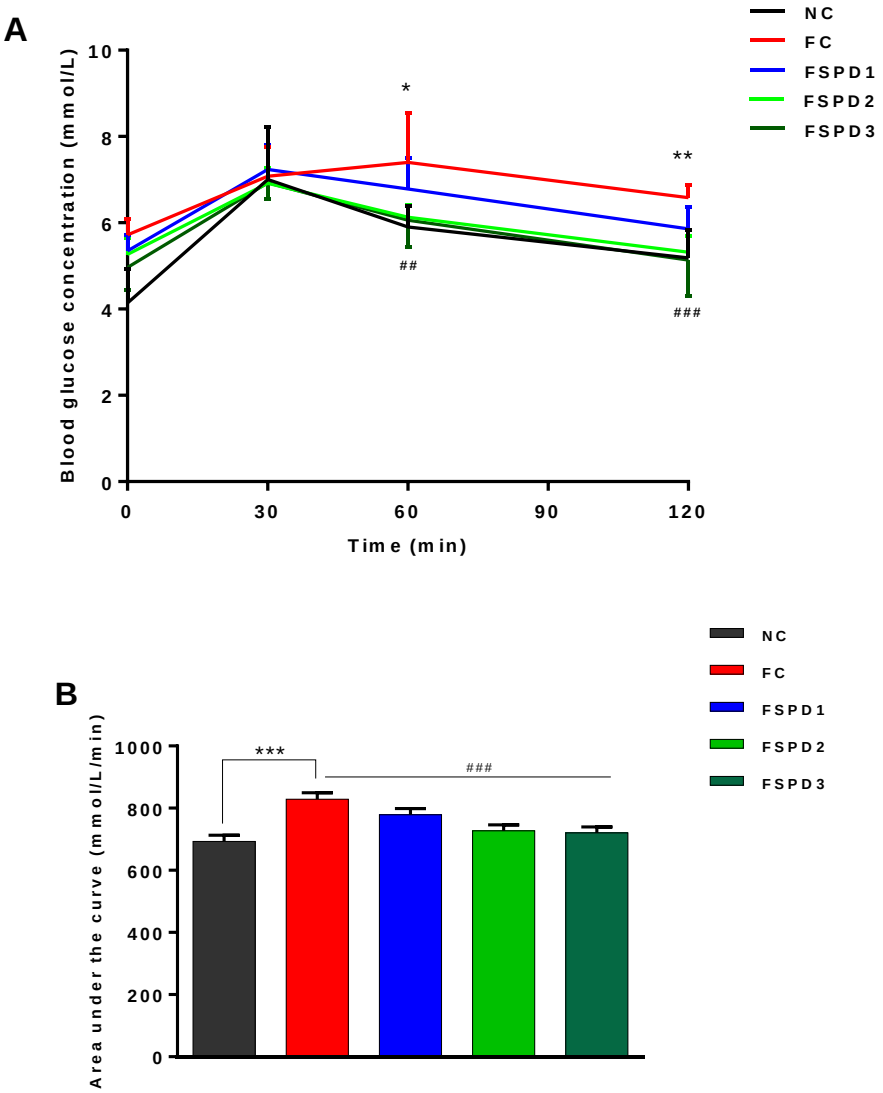


Figure 6: Effect of SPD on glucose tolerance test (A: Blood glucose level; B: area under the curve (AUC))

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: mmol/L (A), mmol/L /min (B). The values are expressed as Mean ± SEM, n = 8. *p< 0.05, **p< 0.01, ***p< 0.001 significant when compared with normal control group. ##p< 0.01, ### p < 0.001 significant when compared with FC rats.

***Effect of SPD on blood serum ALAT, ASAT, Creatinine, Triglycerides, Total cholesterol (T-Ch) and HDL-Cholesterol (HDL-Ch) levels***

Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) serum activities were measured to assess hepatotoxicity. Administration of fructose (8g/kg/day) induced significant elevation of serum ALAT and ASAT activities compared to NC group (Fig. 7A and 7B). Treatment with SPD (75 mg/kg/day, orally, for 42 days) reversed significantly serum ALAT and ASAT activities compared to FC group. To evaluate the effect of fructose-induced abnormalities in renal function, we monitored creatinine levels. Consistent with the observed attenuation of fructose-induced increases in ALAT and ASAT serum levels by SPD, the highest dose (75 mg/kg/day), also significantly reduced creatinine levels when compared to FC group (Figure 7C).

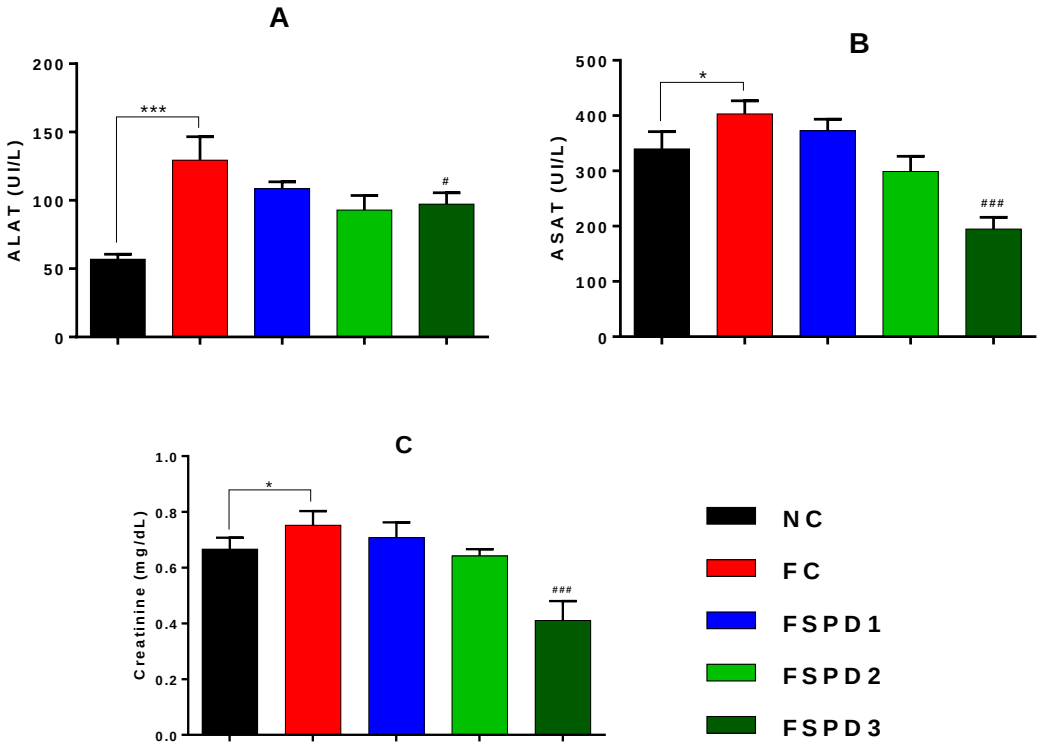


Figure 7: Effect of SPD on blood serum ALAT (A), ASAT (B) and Creatinine levels (C)

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: UI/L (A-B), mg/dL (C). The values are expressed as Mean ± SEM, n = 8. *p< 0.05, ***p< 0,001 significant when compared with normal control group. #p< 0.05, ###p< 0,001 significant when compared with fructose control.

The deleterious effect of fructose administration on lipid profile are reported in Table 2. Serum triglycerides (TG) and total cholesterol (T-Ch) were increased in FC group when compared to NC group. Conversely, HDL-cholesterol (HDL-Ch) level was significantly reduced (-31%; P< 0.05) in comparison to the NC rats after 56 days of fructose feeding. However, FSPD rats exhibited a marked reversal of the serum lipid profile compared to the FC group. This trend was more marked in FSPD3 rats, resulting in the lowest mean values of serum TG, T-Ch and the highest mean value of serum HDL-Ch after 42 days of treatment. In addition, a corresponding decrease in (TG/HDL-Ch) ratio and atherogenic index (AI) were observed in FSPD rats suggesting SPD beneficial effect on fructose induced insulin resistance and atherosclerosis.

Table 2: Effect of SPD on serum lipids (Triglycerides, T-Ch and HDL-Ch) levels

| | | | FSPD | | |
|---|---|---|---|---|---|
| | **NC** | **FC** | **FSPD1** | **FSPD2** | **FSPD3** |
| **TG (mg/dL)** | 62.200 ± 5.297 | 71.580 ± 3.811 | 68.675 ± 4.432 | 67.580 ± 5.741 | 59.500 ± 4.001 |
| **T-Ch (mg/dL)** | 63.760 ± 5.034 | 75.020 ± 6.477 | 63.325 ± 6.045 | 55.840 ± 2.031 | 52.780 ± 2.633 |
| **HDL-Ch (mg/dL)** | 29.264 ± 1.865 | 22.326 ± 1.336* | 25.878 ± 1.531 | 28.455 ± 0.582 | 29.210 ± 0.680# |
| **TG/HDL-Ch ratio** | 2.125 ± 0.181 | 3.206 ± 0.170** | 2.653 ± 0.171 | 2.374 ± 0.201# | 2.036 ± 0.136## |
| **Atherogenic index (AI)** | 1.179 ± 0.064 | 2.360 ± 0.060*** | 1.447 ± 0.059### | 0.962 ± 0.020### | 0.806 ± 0.023### |

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis* 18.45 mg/kg (FSPD1), 37.5 mg/kg (FSPD2), 75 mg/kg (FSPD3). Units: mg/dL. The values are expressed as Mean ± SEM, n = 8. *p< 0.05, **p< 0.01, ***p< 0.001 significant when compared with normal control group. #p< 0.05, ##p< 0.01, ###p< 0.001 significant when compared with fructose control. Atherogenic index (AI) was calculated using Friedewald equation: AI = (T-Ch - HDL-Ch)/HDL-Ch.

***Effect on ferric reducing ability of plasma (FRAP)***

Ferric reducing ability of the plasma (FRAP) was significantly low following administration of fructose (Fig. 8). Treatment with SPD at all the doses (18.75 mg/kg, 37,5 mg/kg and 75 mg/kg) reversed this effect.

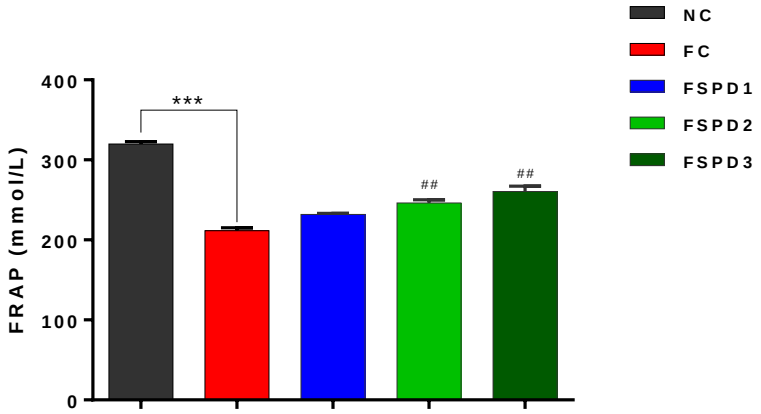


Figure 8: Effect of SPD on the ferric reducing ability of the plasma (FRAP) following chronic fructose administration

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: mmol/L. The values are expressed as Mean ± SEM, n = 8. ***p< 0.001 significant when compared with normal control group. ##p< 0.01significant when compared with fructose control.

***Effects of SPD on oxidative stress markers***

Fructose administration resulted in oxidative stress state manifested in FC rats by a significant increase in lipid peroxidation products measured by MDA in liver (0.105 ± 0.028 nM/mg protein; P< 0.05) and kidney (0.234 ± 0.010 nM/mg protein; P< 0.05) compared to NC rats with respective values of 0.045 ± 0.001 nM/mg protein and 0.197 ± 0.011 nM/mg protein. SPD (75 mg/kg) decreased significantly (-53%; P< 0.05) MDA levels in kidney homogenates when compared to FC rats (Fig. 9). Consistently, kidney GSH level was significantly depleted in FC rats (0.635 ± 0.052 nM/mg protein; P<0.001) as compared to NC rats (1.232 ± 0.071 nM/mg protein). Treatment with SPD (18.75-75 mg/kg) significantly protected rat's kidneys against the GSH depletion induced by fructose (Fig. 10).

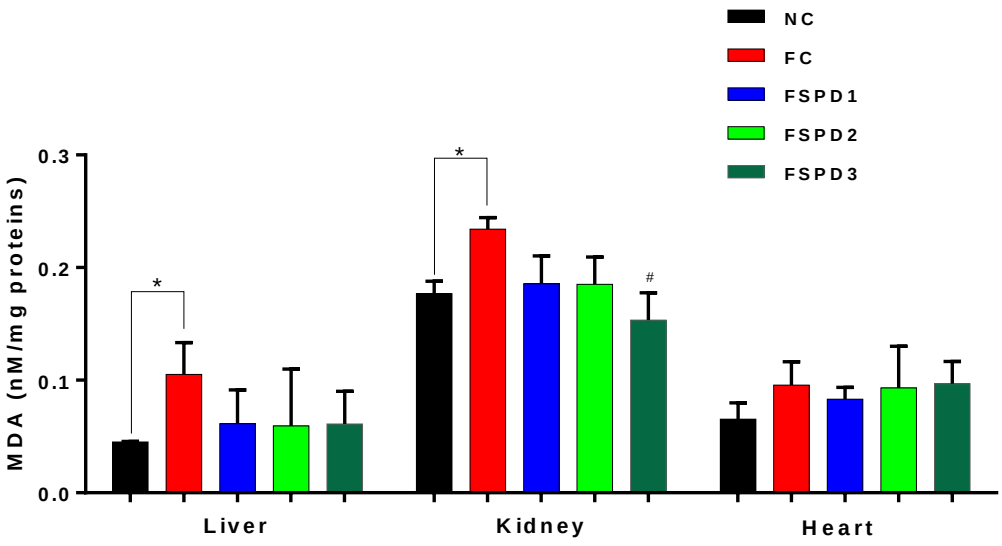


Figure 9: Effects of SPD on lipid peroxidation levels

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: nM/mg proteins. The values are expressed as Mean ± SEM, n = 8. *p< 0.05 significant when compared with normal control group. #p< 0.05 significant when compared with fructose control.

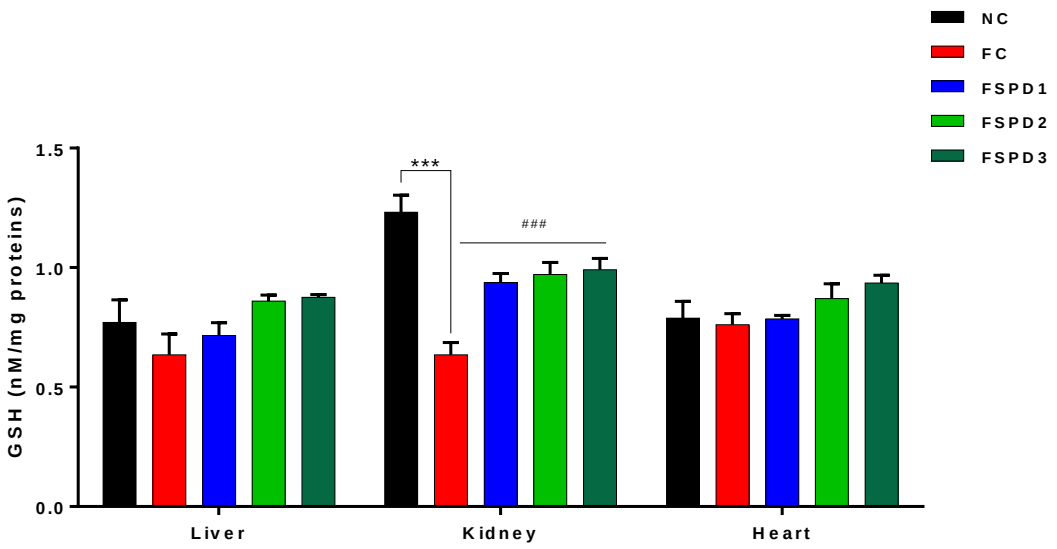


Figure 10: Effects of SPD on GSH levels

Abbreviations: NC-Normal control, FC-Fructose control, FSPD – Fructose + *Spirulina platensis*. Units: nM/mg proteins. The values are expressed as Mean ± SEM, n = 8. ***p< 0.001 significant when compared with normal control group. ###p< 0.001 significant when compared with fructose control.

***DPPH radical scavenging activity***

The scavenging ability of SPD on the DPPH radical was measured using quercetin as reference drug. At the concentration of 200 µg/mL, the scavenging abilities on DPPH radicals of SPD and quercetin reached respectively 59.93 ± 2.14% and 61.22 ± 1.52% (Fig. 11).

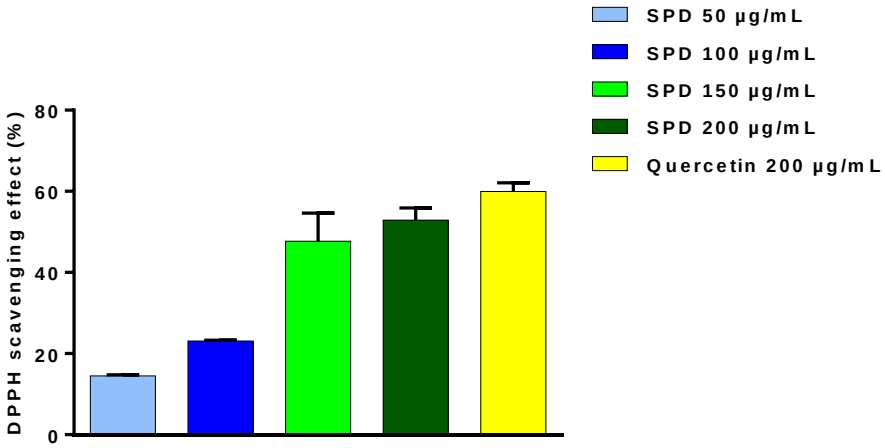


Figure 11: DPPH radical scavenging activity of SPD

Abbreviations: SPD - *Spirulina platensis*. Units: %. The values are expressed as percentage of DPPH radical inhibited, n = 3.

## DISCUSSION

Insulin resistance is one of the most salient features of T2D, which results in the need of greater amounts of insulin to maintain carbohydrate homeostasis [24]. It therefore seemed relevant to verify in the present work the SPD's ability to improve the use of insulin by the body. A high fructose diet over a long period (56 days) permitted to establish in Sprague Dawley (SD) rats a state of insulin resistance similar to that observed in humans. It is now clearly established that chronic administration of large amounts of fructose can mimic a state of insulin resistance in rodent models [25,26, 4] associated with arterial hypertension, as confirmed by observations in humans [27,28]. Johnson et al [42], in their review on the potential role of fructose in the epidemic of hypertension, obesity and diabetes, have clearly exposed the role and the mechanisms for fructose-induced metabolic syndrome. Fructose metabolism is known to be rapid and continuous, thus leading to excessive fatty acid synthesis, precursors of triglycerides [43]. It is therefore on a validated and very well-known model of insulin resistance that the effects of SPD on insulin sensitization have been carried out.

After 56 days, blood glucose monitoring clearly indicated a significant downward trend in blood glucose in all animals that received spirulina and fructose concomitantly compared to those that received fructose alone. The best antihyperglycemic activity was observed at a dose of 75 mg/kg. This result confirms on one hand our previous work on SPD [13] in which hyperglycemia and dyslipidemia were induced in SD rats by administration over 28 days of a mixture of

fructose and cholesterol. On the other hand, this result is similar to those of Jarouliya et al [29] using *Spirulina maxima* and Hozayen et al [30] using *Spirulina versicolor* on fructose-induced diabetic rats. In addition, numerous clinical and experimental studies have shown that chronic hyperglycemia progressively impairs insulin secretion and decreases the peripheral action of insulin, leading to auto-aggravation of non-insulin-dependent diabetes or T2D. Thirty (30) min after an injection of insulin, the administration of SPD to the rats perfectly allowed resensitization of rat's tissues to the hormone and brought blood sugar levels back to values close to control levels, while blood sugar levels remained high in FC group. SPD at a dose of 75 mg/kg therefore reduced insulin resistance, probably by increasing insulin sensitivity. The significant decrease in glycaemia in FSPD groups would therefore reflect a better utilization of glucose in comparison with those of FC group.

The oral glucose tolerance test presented in Figure 6 confirms these observations and highlighted the effect of SPD on glucose overload caused by oral administration of high amount of glucose (4g/kg, b.w). Thirty minutes (T30) after the administration of glucose, the glycaemia of all groups experienced a significant increase. But then, a gradual decrease in blood sugar in the spirulina-treated groups was observed, while the upward trend was maintained in the insulin-resistant animals treated with fructose alone. Even in the presence of a significant amount of glucose in the blood, SPD allowed the rats of FSPD groups to better metabolize this sugar than their congeners of FC group. SPD would therefore act like a “potentiator of the effects of insulin”, a mode of action similar to that of biguanides.

Furthermore, an increase in the total cholesterol level and a decrease in HDL cholesterol in the serum of FC rats were observed. The administration of spirulina to animals on one hand reduced total cholesterolemia, and on the other hand significantly increased the level of HDL cholesterol at the dose of 75 mg/kg. The atherogenic index (AI) calculated using the Friedewald equation confirmed this trend towards an overall drop in cholesterolemia. Thus, as abundantly reported in the scientific literature, fructose-enriched diet caused in rats an increase in plasma concentrations of total cholesterol (T-Ch) and a decrease in HDL cholesterol, characteristic of states of dyslipidemia [9, 31] and leading to an increase in the atherogenic index. The increase in T-Ch and the atherogenic index observed in our study may be related to the mobilization of fructose by the liver and its rapid metabolism into precursors used for *de novo* lipid synthesis. This generally leads to a pathological accumulation of triglycerides in this organ (hepatic steatosis) and in the plasma (peripheral hyperlipidemia), consequently contributing to aggravation of insulin resistance and favoring the onset of cardiovascular diseases [32]. Taghibiglou et al [33] provided evidence of an increase in the synthesis of low molecular weight lipoproteins (LDL and VLDL) in hamsters fed fructose, confirming since then, the complexity existing between the metabolism of excess fructose, the resistance to insulin and dyslipidemia. Interestingly, SPD supplementation in this study did not only lowered T-Ch, but also increased HDL-cholesterol, an actor known to play an important role in transporting cholesterol from peripheral cells to the liver through a pathway called "reverse cholesterol transport." The current results are consistent with those of Parikh et al and Torres-Duran et al [9, 34] obtained during clinical studies in type 2 diabetic patients. Nagaoka et al [35] also reported hypocholesterolemic action of *Spirulina platensis* in rats. Basing on this present data, SPD can be qualified as an antiatherogenic agent

The beneficial effects of SPD observed may also be related to antioxidant activities *in vivo* as shown in Figures 8,9,10. Spirulina has been shown to be very active in vivo also against lipoperoxidation by reducing the level of MDA, particularly in the kidneys. Glutathione is an endogenous antioxidant of the cell's antiradical detoxification system. Its increase in the organs of animals of FSPD groups testify to an inducing effect of spirulina on the expression of genes and/or the activity of glutathione, as also demonstrated by the studies of Aissaoui et al [36] and Nasirian et al [37] on other strains of *Spirulina platensis*, or even Jarouliya et al [38] on a related species, *Spirulina maxima*.

Additionally, this improvement in antioxidant status induced by SPD partly explains the attenuation of the negative effects of the fructose-enriched diet on hepatic and renal activities. In fact, a relative protective activity on the liver and kidneys was observed in the presence of SPD 75 mg/kg as shown in Figure 7. Compared to normal controls (NC group), ALAT and ASAT levels increased significantly in animals of FC group. SPD significantly corrected these effects on the liver, especially at a dose of 18.75 mg/kg for ALAT levels and at a dose of 75 mg/kg for ASAT. Transaminases (ASAT and ALAT) in particular are excellent predictive markers of impaired liver function. Their increase in animals of FC group confirms the efficacy of this diet in establishing a state of insulin resistance with impaired liver function. The decrease observed later in the presence of SPD corroborates the restoration of hepatic sensitivity to insulin and glucose utilization, as observed earlier in the oral glucose tolerance test (OGTT).

Creatinine levels were measured to explore kidney function and to determine whether the hyperglycemia and high blood pressure commonly associated with a diet enriched in fructose [26, 39] have impacted the kidneys of rats. As with liver markers, kidney marker (creatinine levels) increased in animals of FC group. Treatment with SPD 75 mg/kg reversed significantly this effect on creatinine levels.

The consumption of SPD as supplement could prevent diabetic pathological conditions induced by hyperlipidemia through lowering TG and total cholesterol. This is in line with our previous observations [13] on hypocholesterolemic action of S. platensis in rats. Thus, in accord with these present results, SPD may be qualified as an anti-atherogenic agent. The antioxidant and protective effects of SPD should be attributed to its functional bioactive components found, namely phycocyanin, flavonoids, tannins and various phenolic compounds.

## CONCLUSION

“Spiruline Dou Bogan” (SPD) effectively alleviated the abnormal biochemical parameters, especially glycaemia and insulin resistance. It also prevents body-weight gain, intra-abdominal grease accumulation and hyperlipidemia by lowering TG and total cholesterol. SPD contains functional phytochemicals, which support its activities. Thus, SPD may be used as a potent low-cost phytomedicine for type 2 diabetes. Other investigations, *in vitro* study of the fructose induced ROS condition and deep analysis of the molecular mechanisms in particular, are needed to elucidate the exact mechanism of action of the SPD and its possible effects on other complications of diabetes mellitus.

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## INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Institutional Review Board of UNIVERSITY OF LOME-TOGO (Approval no 006/2021/CB-FDS-UL; January 22, 2021).

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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