IJMPR 2022, 6(8), 01-07

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

EFFECT OF *SPIRULINA PLATENSIS* AND ATORVASTATIN ON SERUM ENDOTHELIAL NITRIC OXIDE SYNTHASE ACTIVITY IN NON-ALCOHOLIC FATTY LIVER DISEASE IN WISTAR RATS

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Received on: 27/06/2022
Revised on: 17/07/2022
Accepted on: 07/08/2022

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ABSTRACT

The *Spirulina platensis* has been extensively used in the therapeutic management of chronic metabolic and non-metabolic disorders. However, Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease known as hepatic module of the metabolic syndrome. In this study, the efficacy of atorvastatin treatment and graded doses of *S. platensis* on the NAFLD rats were assessed. Forty-six (46) Wistar rats were randomly grouped into 2 groups comprising of 8 rats as a controls and 38 rats as test group. Control group received only basal diet and water while the test group was fed with high fat diet (HFD) and carbon tetrachloride (CCL_4) intragastric twice a week for 8 weeks. Thereafter, 2 rats were sacrificed from each group and hepatic histological examination carried out to establish the occurrence of non-alcoholic liver disease. The negative control and test group were regrouped into six groups (n=6). Positive control (NAFLD), received high fatty diet and CCL_4 intragastric, group 3 received 10 mg/kg b.w. of atorvastatin and groups 4, 5, 6 and 7 received (200 mg, 400 mg, 600 mg and 800 mg/kg b.w respectively) of *S. platensis* extract intragastric for 4 weeks. Serum nitric oxide (NO), endothelial nitric oxide synthase activity (eNOS), liver function test, lipid profile and histologic analysis were performed using standard techniques. Administration of atorvastatin and high doses of *S. platensis* exerted more potent effects against necrosis of the hepatocytes, steatohepatitis, hyperlipidemia, and obesity in NAFLD rats, and this may be a future aid tool for developing a novel challenge against NAFLD impacts. The results indicated that high doses of *spirulina* exhibited better effects comparing to the atorvastatin.

KEYWORDS: Non-alcoholic fatty liver disease, *Spirulina Platensis*, Atorvastatin, eNOS.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a band of clinical and pathological conditions characterized by excessive lipid deposition in the liver parenchyma in the absence of significant alcohol consumption.[1] Several literatures have pointed out the NAFLD a hepatic module of the metabolic syndrome, a syndrome that also includes central obesity, dyslipidaemia, hypertension and insulin resistance/ impaired glucose tolerance.[2]

Epidemiological data shows the global prevalence of NAFLD in diverse population as follows: In the United States, the prevalence of NAFLD in adults is 24.13%, and it is projected to be 33.5% in 2030 and cases will reach 100.9 million in the general population. In Asian, the prevalence of NAFLD is 27.37%, with 20.09% in China. In South America 30%, Europe 24% with wide variations in the prevalence identified among different ethnic groups of these populations.[3] In some developing countries, such as Sudan and Nigeria is 8.7% and 20% respectively.[4]

It has been hypothesized that NAFLD may present an endothelial dysfunction that could be one of the earliest factors associated to fat accumulation and liver damage.[5] Since its discovery, NAFLD has been broadly associated to cardiometabolic syndrome and its components: hepatic and systemic insulin resistance, dyslipidaemia, visceral obesity, hypertension and impaired fasting glucose.[6]

Spirulina platensis is a microscopic blue green algae (microalgae) that is reported to have hepatoprotective properties by decreasing liver lipid profile, lipoperoxidation products and it also has a hypolipaemic effect, especially on the concentrations of triglyceride and the cholesterol associated to low density lipoprotein and indirectly on total cholesterol and cholesterol associated to high density lipoprotein.[7]

Atorvastatin has cholesterol-lowering properties, statins exert cholesterol-independent pleiotropic effects that are not mediated by HMG-CoA reductase inhibition, which include up-regulation of hepatic low density lipoprotein receptors which reduce proatherogenic circulating LDL cholesterol.[7]

Carbon tetrachloride (CCl4) is reported to induce chemical hepatitis and liver injuries in experimental animals. An exposure to CCl4 as being a strong hepatotoxic xenobiotic will directly leads to severe liver necrosis and steatosis through its strongly reactive free radical metabolites, trichloromethyl and trichloromethyl peroxy.[8] And treatment with spirulina platensis and atorvastatin was reported to alleviate oxidative stress. Therefore the present study was designed to investigate the effect of *spirulina platensis* and atorvastatin on serum endothelial nitric oxide synthase activity in NAFLD in wistar rats.

MATERIALS AND METHODS

All chemicals and reagents used in this study were of analytical grade and used before their expiration date. Endothelial Nitric oxide synthase and Nitric oxide ELISA Kits were products of Glory Science Co.Ltd China, while Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline phosphatase, Total protein, Albumin, Bilirubin, and lipid profile (Total cholesterol, HDL-c and Triglyceride) assay kits were products of Agappe Diagnostics Switzerland. The study was conducted at the Department of Chemical Pathology, School of Medical Laboratory Science and Animal House of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto-Nigeria. Based on sample size calculation, forty-six.[46] wistar rats (aged 8-12 weeks old), weighing between 120-140 g were purchased from the Animal House of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were allowed to acclimatize for a period of 2 weeks and allowed access to drinking water *ad libitum*.

Plant Collection: A 100% pure *Spirulina Platensis* in powder form was purchased from Neneeys Optimal Skincare, 32 Road, Kenjul Plaza, Opposite 321 Junction, Festac Town, Lagos and was aqueously extracted and administered in graded doses of 200mg/kg body weight (b.w), 400 mg/kg b.w., 600 mg/kg b.w., and 800 mg/kg b.w. to the experimental rats.

Preparation of Extract: The *spirulina* powder (100 g) was soaked in 1 litre of ultrapure water and shaken occasionally for 24 hours at room temperature. The mixture was then centrifuged at 5,000 rpm for 10 minutes and the supernatant was filtered using Whatman No. 1 to remove the cell debris. The sample was freeze-dried and the dried extract was stored at 4^0C until use for the experiment.

Formulation of Non-alcoholic Fatty Liver Disease in Rats: Non-alcoholic fatty liver disease (NAFLD) was formulated in accordance with Neoman *et al*., 2011 with some modifications. The formulation consist of 54.7% fat, 20% carbohydrates, 20.3% protein and 5% fibre. And carbontetrachloride (CCl4) intragastric twice a week for 8 weeks. Formulation induce high fatty liver and liver damage was induced by intragastric administration of 30% CCl4 (dissolved in olive oil, v/v) at 1.0 ml/kg b.w. twice a week for 8 weeks as described by 9; 10).

Experimental Design: The forty-six (46) wistar albino rats were randomly grouped into two groups comprising of 8 rats as control and 38 rats as a test groups. The control group received only basal diet and water. The test groups were fed with high fat diet (HFD) and CCl4 intragastric twice a week for 8 weeks. After 8 weeks, two rats were sacrifificed from each group and hepatic histological examination was carried out to establish the occurrence of non-alcoholic fatty liver disease. The animals were regrouped and treated as follows:

Group 1(n=6): Control rats,received clean distilled water and rodent chow *ad-libitum*
Group 2 (n=6): NAFLD Control rats, received high fatty diet and I ml/kg CCl4 intragastric
Group 3 (n=6): Test rats, received 10 mg/kg b.w. of atorvastatin
Group 4 (n=6): Test rats, received 200 mg/kg b.w. of *S. Platensis* extract
Group 5 (n=6): Test rats, received 400 mg/kg b.w. of *S. Platensis* extract
Group 6 (n=6): Test rats, received 600 mg/kg b.w. of *S. Platensis* extract
Group 7 (n=6): Test rats, received 800 mg/kg b.w. of *S. Platensis* extract

The appropriate doses of the treatment regiments were administered orally/intragastric to the animals according to their body weight once daily by intubation using intravenous cannula tube for 4 weeks. The rats were fasted for 12 hours, and anaesthetized in a glass jar containing wool soaked with chloroform. Blood samples were collected from the animals through cardiac puncture into clean plain bottles. The rats were later sacrificed through lumbar dislocation and their liver was removed. The sample collected in the plain tubes were allowed to clot at room temperature and later centrifuged at 4000 rpm for 10 minutes. The sera were transferred into labelled clean serum bottles with tighten cap and stored at -20^0C until required.

The Ethical approval was sorted from the ethics and research committee, Department of Pharmacy and Toxicology, Usmanu Danfodiyo University, Sokoto, with the approved number PTAC/SP/0T/30-21. The following methods were adopted in the measurement of biochemical parameters: Serum Endothelial Nitric oxide synthase described by Engvall and Peter (1971),[11] Nitric oxide was determined by method of Mesaros and Grunfeld (1997),[12] Aspartate Aminotransferase and Alanine Aminotransferase activities were determined by method of Reitman and Frankel (1957),[13] Alkaline

phosphatase activity was determined by method of Belfield and Goldberg (1971),[14] Total protein was determined by method of Lowry *et al*. (1951),[15] Albumin concentration determined by method of Spencer and Price (1977),[16] Bilirubin was determined by method of Basil *et al*. (1958),[17] LDL-cholesterol was calculated using Friedwald *et al*. (1972),[18] formular: Serum LDL-c = Total cholesterol- (HDL-c +VLDL), Atherogenic index (AIX) was calculated using Kazemi *et al.* (2018),[19] formular: Serum AIX = (LDL-c/HDL-c).

The results of the data were presented as mean ± Standard Error of Mean (SEM). Group comparisons were made using one-way analysis of variance (ANOVA) and Pearson's correlation was used to correlate the parameters. Significant difference was taken at 5% (P <0.05).

RESULTS

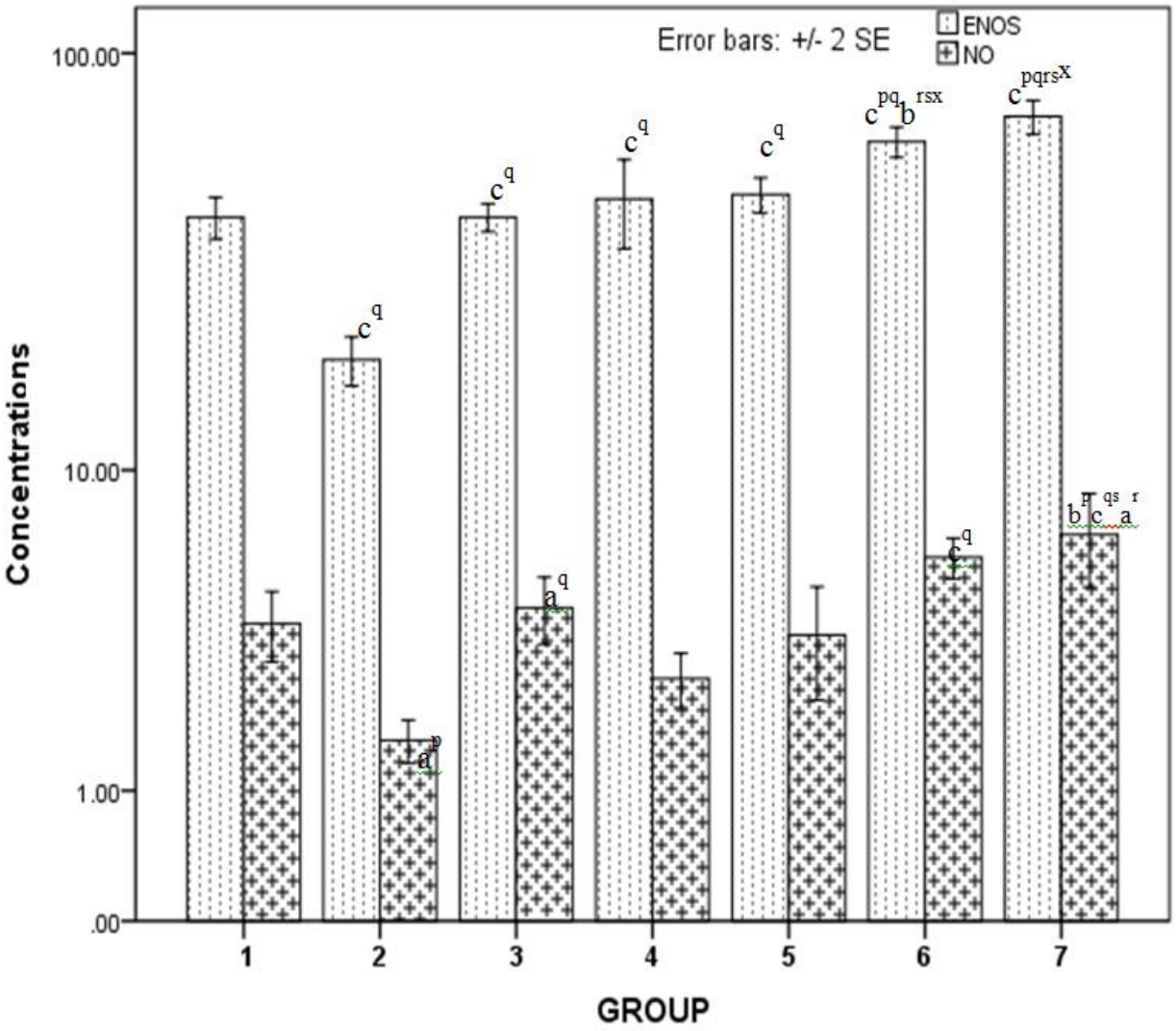

Figure 1: Effects of *S. platensis* and atorvastatin treatment on serum endothelial nitric oxide synthase (eNOS) (pg/mL) activity and nitric oxide (NO) (µmol/L) level in non-alcoholic fatty liver disease Wistar rats.

Grp1: Negative control; **Grp2:** Positive control; **Grp3:** Test received 10mg/kg Atorvastatin **Grp4:** Test received 200mg/kg *Spirulina platensis;* **Grp5:** Test received 400mg/kg *Spirulina platensis*; **Grp6:** Test received 600mg/kg *Spirulina platensis*; **Grp7:** Test received 800mg/kg *Spirulina platensis*. Values are expressed as mean ± standard error; Data analysis was carried out using One Way ANOVA followed by Tukey's test. Values with superscript differ significantly at $^{a}p < 0.05$ and $^{b}p < 0.01$ and $^{c}p < 0.001$ when compared within groups (p, q, r, s, x, y and z represented group 1, 2, 3, 4, 5, 6 and 7).

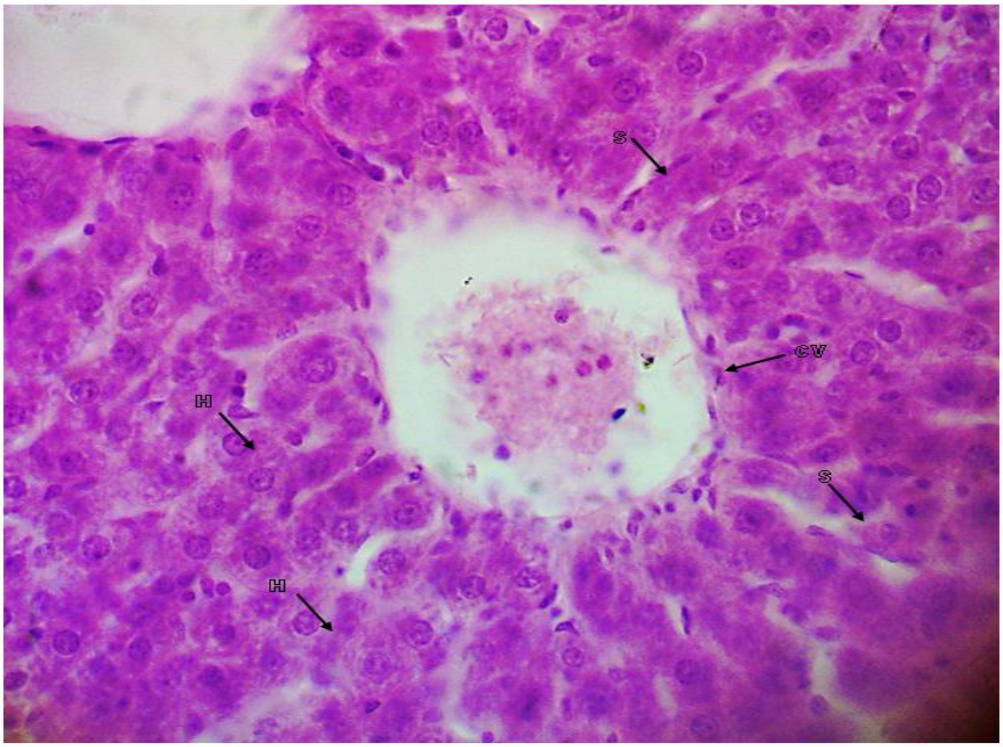

Plate 1: Negative Control showed radiating chords of hepatocytes (H0 and sinusoid (S) converging into the central vein (CV) within the portal area. (H&E. x 400).

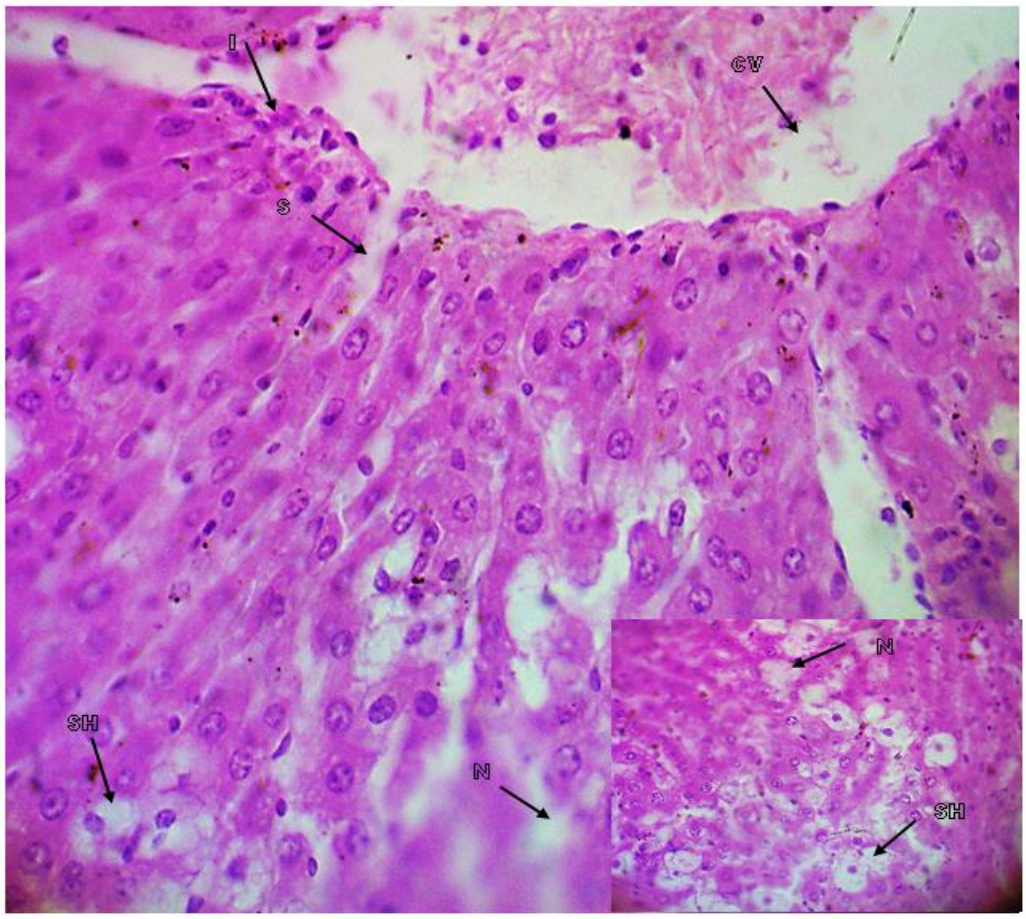

Plate 2: Positive Control showed HFD and CCl_4 induced necrosis of the hepatocytes (N), infiltrating leukocytes, disorganized sinusoids (S) surrounding the central vein, and steatohepatitis (SH). (H&E. x 400).

Table 1: Effects of *S. platensis* and atorvastatin treatment on liver function test in non-alcoholic fatty liver disease Wistar rats.

Group	N	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)	TBIL (µmol/l)	DBIL (µmol/l)
1	6	45.69 ± 3.10	30.66 ± 1.24	170.91 ± 8.52	62.04 ± 2.40	38.23 ± 1.44	8.41 ± 1.61	2.32 ± 0.69
2	6	79.63 ± 2.91^{c}	109.63 ± 3.45^{c}	402.93 ± 38.81^{c}	49.22 ± 2.20^{a}	31.00 ± 0.78	16.68 ± 1.77^{b}	1.83 ± 0.55
3	6	63.66 ± 4.16^{b,a}	99.83 ± 3.30^{c}	276.04 ± 22.65^{a,b}	64.13 ± 1.77^{a}	40.65 ± 2.82^{a}	5.13 ± 0.70^{c}	2.87 ± 0.86
4	6	61.39 ± 1.91^{a,b}	91.04 ± 3.39^{c,a}	216.75 ± 12.59^{c}	63.43 ± 2.25^{a}	38.79 ± 2.14	11.76 ± 0.62	2.87 ± 0.86
5	6	64.01 ± 2.24^{b,c}	81.04 ± 0.70^{c,a}	195.35 ± 6.94^{c}	69.62 ± 3.11^{c}	37.77 ± 2.33	11.43 ± 1.21	3.29 ± 1.40
6	6	50.49 ± 3.39^{c}	67.87 ± 7.56^{c,b}	161.24 ± 3.44^{c,a}	77.03 ± 4.81^{b,c}	50.90 ± 4.22^{c,b,a}	11.20 ± 1.03	1.99 ± 0.28
7	6	45.40 ± 3.37^{a,b}	55.68 ± 5.04^{c,b}	138.58 ± 5.62^{c,b}	81.71 ± 2.46^{c,b}	53.93 ± 1.69^{c,b}	9.09 ± 1.91^{a}	2.66 ± 0.72
P-values		0.000	0.000	0.000	0.000	0.000	0.001	0.702
F values		17.493	63.410	21.714	16.248	14.725	5.629	0.634

Values are expressed as mean ± standard errors; **N:** Number of rats; **ALT:** Alanine transaminase; **AST:** Aspartate transaminase; **ALP:** Alkaline phosphatase; **TP:** Total protein; **ALB:** Albumin; **TBIL:** Total bilirubin; **DBIL:** Direct bilirubin; **Grp 1:** Negative control; **Grp 2:** Positive control; **Grp3:** Test received 10mg/kg Atorvastatin; **Grp4:** Test received 200mg/kg *Spirulina platensis*; **Grp5:** Test received 400mg/kg *Spirulina platensis*; **Grp6:** Test received 600mg/kg *Spirulina platensis*; **Grp7:** Test received 800mg/kg *Spirulina platensis*. Data

Table 2: Serum Lipid profile in non-alcoholic fatty liver disease wistar rats treated with *S. platensis* and atorvastatin.

Group	N	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	AIX
1	6	1.09 ± 0.08	0.72 ± 0.07	0.53 ± 0.05	0.42 ± 0.06	0.14 ± 0.02	0.83 ± 0.14
2	6	2.57 ± 0.25^{c}	1.67 ± 0.21^{c}	0.24 ± 0.04^{b}	1.95 ± 0.24^{c}	0.33 ± 0.04^{c}	8.80 ± 1.27^{c}
3	6	1.59 ± 0.10^{b}	1.03 ± 0.08^{a}	0.50 ± 0.09^{a}	0.88 ± 0.39^{c}	0.21 ± 0.02^{a}	2.11 ± 0.67^{c}
4	6	1.44 ± 0.09^{c}	1.14 ± 0.19	0.59 ± 0.05^{b}	0.62 ± 0.05^{c}	0.23 ± 0.04	1.05 ± 0.06^{c}
5	6	1.11 ± 0.12^{c}	0.69 ± 0.11^{c}	0.52 ± 0.06^{a}	0.45 ± 0.11^{c}	0.14 ± 0.02^{c}	0.93 ± 0.23^{c}
6	6	1.17 ± 0.14^{c}	0.64 ± 0.11^{c}	0.53 ± 0.06^{a}	0.51 ± 0.10^{c}	0.13 ± 0.02^{c}	0.96 ± 0.11^{c}
7	6	0.83 ± 0.07^{c,a}	0.55 ± 0.07^{c}	0.58 ± 0.03^{b}	0.15 ± 0.05^{c,a}	0.11 ± 0.02^{c}	0.28 ± 0.10^{c}
P value		0.000	0.000	0.000	0.000	0.000	0.000
F value		17.418	9.496	6.059	21.951	9.608	25.104

Values are expressed as mean ± standard error; **N:** number of rats; **TC:** Total cholesterol; **TG:** Triglyceride; **HDL:** High density lipoprotein; **LDL:** Low density lipoprotein; **VLDL:** Very low density lipoproteins; AIX: Artherogenic index; **Grp1:** Negative control; **Grp2:** Positive control; **Grp3:** Test received 10mg/kg Atorvastatin; **Grp4:** Test received 200mg/kg *Spirulina platensis*; **Grp5:** Test received 400mg/kg *Spirulina platensis*; **Grp6:** Test received 600mg/kg *Spirulina platensis*; **Grp7:** Test received 800mg/kg *Spirulina platensis*. Data analysis was carried out using One Way ANOVA followed by Tukey's test. Value differs significantly when $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0$.

DISCUSSION

Plate 2 results evidence the confirmation of NAFLD in wister rats, which is consistent with the work of,[9] characterized by necrosis of the hepatocytes, infiltrating leukocytes, disorganized sinusoids surrounding the central vein, steatohepatitis and infiltration of inflammatory cells. The hepatic damage causes elevated liver enzymes and reduced TP and ALB level, through endothelial cell capillarization, activation of HSCs, accompanied by extracellular matrix (ECM) deposition,[22] and associated endothelial dysfunction with impaired NO production. Treatment with atorvastatin and different doses of *Spirulina platensis* extracts significantly improved the liver damage, indicated a proliferation of undamaged hepatocytes, revascularization and sinusoid remodel in necrotic area crucial for repair processes via increase protein and albumin and consequently improving eNOS and NO (plate 1).

Following post *S. Platensis* intervention we reported the following important findings: there was significantly decreased MDA, eNOS, NO, AST, ALT, ALP, total bilirubin, total cholesterol, triglyceride, LDL-C, and AIX and increased SOD, GPx, CAT, protein, albumin, and HDL-C levels.

Following treatment of NAFLD rats with atorvastatin and *Spirulina* extracts for 4 weeks, there was decreased in oxidative damage produced by HFD + CCL_4 via enhancement of eNOS activities and NO levels. This is in line with,[22] that reported the increased effect of statins on NO level and eNOS expression which has been observed in several species, including human cell culture, in mice, rats, rabbits and dogs.

There were also significantly decreased serum concentrations of ALT, ALP and total bilirubin and increased total proteins and albumin concentrations.

These findings are consistent with those of.[25] This functional activity may be related to its hypolipidaemic activity.[26]

Also, graded doses of *S. platensis* considerably decreased the concentrations of liver enzymes, increased total proteins, albumin and total bilirubin concentrations compared to positive control. This is in line with the study of.[27] Other studies have reported that, *Spirulina platensis* prevents the development of fatty liver, and its antioxidant properties seem to mediate such a protective effect, indicated by the reduction of malondialdehyde (MDA) as well as the elevation of reduced glutathione (GSH) and superoxide dismutase levels in liver tissue.[28]

Furthermore, treatment with atorvastatin and *S. platensis* mitigated the hepatic damages, which was associated with the improvement of liver function, and the higher concentration of *spirulina platensis* shows a remarkable significance against atorvastatin group leading to reduction in the severity in NAFLD rats. Therefore, the result in this study, present that *S. platensis* is critical for the control of liver damage associated with NAFLD in a rat model study.

This study also presented the hypolipidaemic activity of atorvastatin and graded doses of *S. platensis* which is supported with the decreased in serum TC TG, LDL-C, VLDL-C, AIX and increased level of HDL-C. The result of this study is in line with the previous studies of.[25,29,30]

CONCLUSION

The study reported that, atorvastatin and *spirulina* treatment exerts a protective effect on liver through enhancement of liver function test parameters and reduced hyperlipidaemia in NAFLD rats. Conversely, administration of atorvastatin and high doses of *S. platensis* exerted more potent effects against necrosis of the hepatocytes and steatohepatitis in NAFLD rats indicating their antioxidant and anti-inflammatory activity. The results indicated that high doses of *spirulina* exhibited better effects comparing to the atorvastatin.

However, the multifunctional role of *Spirulina* species makes it an ideal natural drug with enormous prophylactic and therapeutic properties.

Recommendation

We recommend use of *S.Platensis* instead of conventional drugs in the treatment of NAFLD since is cheaper, affordable and more effective compare to atorvastatin.

ACKNOWLEDGMENT

This study was supported by a grant from the Tertiary Education Trust Fund, Nigeria with grant number TET/DR&D/CE/UNI/SOKOTO/IBR/2020/VOL1.

Conflict of interest

There is no conflict of interest among authors.

REFERENCES

1. Olusanya, T. O., Lesi, O. A. Adeyomoye, A. A. and Fasanmade, O. A. (2016). Non-alcoholic fatty liver disease in Nigerian population with type II diabetes mellitus. *The Pan African Medical Journal*, 24: 20.
2. Younossi, Z, M. Non-alcoholic fatty liver disease – A global public health perspective. *Journal of Hepatology*, 2019; (70): 531–544.
3. Carr, R. M., Oranu, A. and Khungar, V. Nonalcoholic fatty liver disease: pathophysiology and management. Gastroenterology Clinics North America, 2016; 45: 639–652.
4. Jiang-Hua, Z., Cai, J-J., She, Z-G. and Li, H-L. Noninvasive evaluation of nonalcoholic fatty liver disease: Current evidence and practice. *World Journal of Gastroenterology*, 2019; 25(11): 1307-1326.
5. Mura, L. V., Pasarín, M., Rodriguez-Vilarrupla, A., García-Pagán, J. C., Bosch, J. and Abraldes, J G. Liver sinusoidal endothelial dysfunction after LPS administration: a role for inducible-nitric oxide synthase. *Journal of Hepatology*, 2014; 61(6): 1321–1327.
6. Perlemuter, G., Bigorgne, A., Cassard-Doulcier, A. M. and Naveau, S. Nonalcoholic fatty liver disease: from pathogenesis to patient care. *Nature Clinical Practice Endocrinology* and *Metabolism*, 2007; 3(6): 458–69.
7. Elisa, P., Trebicka, J., Mookerjee, R. P., Angeli, P. and Ginès, P. Statins: Old drugs as new therapy for liver diseases? *Journal of Hepatology*, 2019; 70: 194–202.
8. Mazin, A., Zamzami, O., Baothman, A. S., Samy, F. and Abo-Golayel, M. K. Amelioration of CCl_4-Induced Hepatotoxicity in Rabbits by *Lepidium sativum* Seeds. *Evidence-Based Complementary* and *Alternative Medicine*, 2019; 17.
9. Liu, Q., Wang, C. Y., Liu, Z., Ma, X. S., He, Y. H., Chen, S. S. and Bai, X. Y. Hydroxysafflor yellow A suppresses liver fibrosis induced by carbon tetrachloride with high-fat diet by regulating PPAR-γ/p38 MAPK signaling. *Pharmaceutical Biology*, 2014; 52(9): 1085-1093.
10. Dong, S., Chen, Q.-L., Song, Y.-N., Sun, Y., Wei, B., Li, X.-Y., Hu, Y-Y., Liu, P. and Su, S.-B. Mechanisms of CCl_4-induced liver fibrosis with combined transcriptomic and proteomic analysis. *The Journal of Toxicological Sciences*, 2016; 41(4): 561–572.
11. Engvall, E. and Peter, P. "Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G". Immunochemistry, 1971; 8(9): 871–874.
12. Mesároš, S. and Grunfeld, S. Determination of nitric oxide in biological samples using *N, N, N′, N′*-Tetramethyl-p-phenylenediamine by UV VIS Spectrophotometry, *Chemical Papers*, 1997; 52(6): 741-746.
13. Reitman and Frankel, 1957.

14. Belfield, A. and Goldberg, D. M. "Colorimetric Determination of Alkaline Phosphatase Activity". Enzyme, 1971; 12(5): 561-568.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. I. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 1951; 193: 265- 275.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. I. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 1951; 193: 265- 275.
17. Spencer K. and Price C. P. Influence of reagent quality and reaction conditions on the determination of serum albumin by the bromcresol green dye-binding method. *Annals of Clinical Biochemistry*, 1977; 14: 105–115.
18. Basil, T., Doumas, P., Kwok-Cheung, P., Billy, W., Schaffer, R. and Lawrence, L., K. Candidate Reference Method for Determination of Total Bilirubinin Serum: Development and Validation. *Clinical Chemistry*, 1958; 31(11): 1779-1789.
19. Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, 1972; 18(6): 499–502.
20. Kazemi, T., Maryam, M., Mina, H., and Masood, Z. Cardiovascular risk factors and atherogenic indices in an Iranian population: Birjand East of Iran. *Clinical Medicine Insights Cardiol*ogy, 2018; 12: 1-6.
21. Liu, Q., Wang, C. Y., Liu, Z., Ma, X. S., He, Y. H., Chen, S. S. and Bai, X. Y. Hydroxysafflor yellow A suppresses liver fibrosis induced by carbon tetrachloride with high-fat diet by regulating PPAR-γ/p38 MAPK signaling. *Pharmaceutical Biology*, 2014; 52(9): 1085-1093.
22. Iwakiri, Y and Kim, M. Y. Nitric oxide in liver diseases. *Biology* and *Chemistry*, 2015; 5(1): 62–71.
23. Ulrich, L. Beyond lipid-lowering: effects of statins on endothelial nitric oxide. *The European Journal of Clinical Pharmacology*, 2003; 58: 719–731.
24. Mohamed, A. S, Ibrahim, W. M., Zaki, N. I., Ali, S. B. and Soliman, M. Effectiveness of *Coelatura aegyptiaca* Extract Combination with Atorvastatin on Experimentally Induced Hyperlipidemia in Rats. *Evidence-Based Complementary* and *Alternative Medicine*, 2019; 1-9.
25. Thompson, P. D., Panza, G., Zaleski, A. and Taylor, B. "Statin associated side effects," *Journal of the American College of Cardiology*, 2016; 67(20): 2395–2410.
26. El-Sheekh, M. M., Hamad. S. M. and Gomaa, M. Protective Effects of *Spirulina* on the Liver Function and Hyperlipidemia of Rats and Human. *Brazilian Archives of Biology* and *Technology*, 2014; 57(1): 77-86.
27. Karadeniz, A., Cemek, M. and Simsek, N. The effects of Panax ginseng and *Spirulina* platensis on hepatoxicity induced by cadmium in rats. *Ecotoxicology and Environmental Safety*, 2009; 72: 231-235.
28. Li, T.-T., Liu, Y.-Y., Wan, X.-Z., Huang, Z.-R., Liu, B. and Zhao, C. Regulatory efficacy of the polyunsaturated fatty acids from microalgae *spirulina* platensis on lipid metabolism and gut microbiota in high-fat diet rats. *International Journal of Molecular Sciences*, 2018; 19(10): 3075. doi:10.3390/ijms19103075
29. Mohamed, A. S, Ibrahim, W. M., Zaki, N. I., Ali, S. B. and Soliman, M. Effectiveness of *Coelatura aegyptiaca* Extract Combination with Atorvastatin on Experimentally Induced Hyperlipidemia in Rats. *Evidence-Based Complementary* and *Alternative Medicine*, 2019; 1-9.
30. DiNicolantonio, J. J., Bhat, A. G and OKeefe, J. Effects of *Spirulina* on weight loss and blood lipids: a review. *Open Heart*, 2020; 7: e001003.