African Scientist Vol. 18, No. 3, September 30, 2017
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2017032/18302

Histological and Biochemical effects of Aqueous Extracts of *Tectona grandis* leaf on high-fat induced non-alcoholic fatty liver in albino rats

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(Received April 23, 2017; Accepted in revised form June 21, 2017)

Abstract: This study was done to determine the histological and biochemical effects of aqueous leaf extract of *Tectona grandis* on egg yolk induced non- alcoholic liver damage. The rats were randomly divided into four groups A, B, C, and D comprising of 8 rats each. Group A served as the control. Group B received egg yolk daily for 30 days, Group C was treated with 300 mg/kg body weight of aqueous extract of *T. grandis* alone while Group D was fed egg yolk and was treated with aqueous leaf extract of *T. grandis* for 30days. Oxidative stress parameters (superoxide dismutase and catalase activities), cholesterol, alanine transaminase levels were determined in serum. Significant ($P<0.05$) increase in superoxide dismutase (SOD), catalase (CAT), cholesterol and alanine transaminase activities were observed in group B. SOD, CAT, Cholesterol and alanine transaminase levels in serum of rats treated groups(groups C and D)were generally lower or statistically significant to control. Histological examination of the liver tissue confirmed the therapeutic effect of *T. grandis* leaf extract as evidenced by the complete reversal of the significant macrovascular steatosis seen in group B compared to the normal liver architecture seen in the control and treated group. In conclusion, our study showed that *T. grandis* leaf can act as a protective agent against oxidative stress and liver injury caused by high fat diet.

Keywords: *Tectona grandis*, macrovascular steatosis, liver damage, oxidative stress.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is seen as an important manifestation of the metabolic syndrome and obesity, is the most common form of chronic liver disease in the Western world(Vernon *et al*., 2011). In African populations, there is a dearth of evidence on the prevalence and characteristics of NAFLD (Kruger *et al*., 2010). Onyekwere *et al*., (2011) reported a prevalence of NAFLD in 9.5% of diabetics and 4.5% of non-diabetic controls in West Africa. Nonalcoholic fatty liver disease (NAFLD) is defined as the accumulation of triglycerides in liver hepatocytes of patients who do not consume large quantities of alcohol (Paschos *et al*., 2009). It includes nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). Steatosis is determined by estimating the proportion of hepatocytes containing fat droplets. With an increase in high-fat food intake, diagnosis of NAFLD has increased substantially over the past decade and, due to alterations in human's dietary regimen and lifestyle. NAFLD is a common cause of chronic liver disease and its prevalence has approximately doubled in the past decade with the growing epidemic of obesity (Bugianesi *et al*., 2005). Fatty liver is classified as either macrovesicular or microvesicular steatosis depending on the size of the lipid vacuoles (Amacher, 2011). The undesirable side effects, disease recurrence and drug interaction have led to the search of alternative source of treatment. Several species of medicinal plants used in traditional treatment and prevention of hepatic lipid accumulation has been investigated (Farzaei *et al*., 2015)

Tectona grandis (Verbenaceae) is a large deciduous tree. The tree is found growing in higher situations, native to central India, Konkan, Western Deccan peninsula, South India and Burma. *T. grandis* is known as *saka* in Sanskrit, *sagun* in Hindi, *sagwan* in Marathi and teak tree in English (Sharma *et al*., 1986). It is also called obe-

agbon among Owan people in Edo state. Teak belongs to the family Lamiaceae (in older classifications in Verbenaceae) Heywood *et al*., 2007). *T. grandis* has been investigated for nitric oxide scavenging activity (Jadetia *et al*., 2004), cardioprotective activity (Ehimigbai *et al*., 2016) and wound healing activity in rats (Majumdar *et al*., 2007). Tannin found in this plant has anti-inflammatory effects and is used topically for treatment of burn wounds (Jaybhaye *et al*., 2010. This study was therefore done to determine the histological and biochemical effects of aqueous leaves extracts of *Tectona grandis* on high fat induced non -alcoholic liver disease.

Materials and methods

Collection of plant materials: Fresh leaves of *Tectona grandis* were collected from the botanical garden University of Benin. The fresh leaves were identified and authenticated in the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria. Herbarium specimen was deposited at the University of Benin Herbarium.
Preparation of plant extract: The shade dried leaves were pulverized and then soaked in distilled water for 72 hours. The contents was stirred several times a day and at the end of the third day, was filtered through two layers of cheese cloth and the filtrate evaporated to dryness by a rotary evaporator and then freeze dried using a freeze dryer (Onoagbe *et al.* (1999b). The concentrated extract was weighed and stored in an air-tight container and kept in the freezer until use. The volume of the extract given to each animal was determined by the concentration of the extract and body weight of the animal.
Experimental animals: A total of 32 Male albino rats weighing 180-250g were used for this study. The rats were obtained from the animal house, Department of Anatomy, University of Benin. The animals were housed in galvanized rat cages and acclimatized for two weeks on guinea growers mash (Bendel Feed and Flour Mill, Ltd, Ewu, Nigeria) and allowed to drink water freely. Treatment of the animals was in accordance with the principle of Laboratory Animal Care (NIH Publication 85-93, revised 1985).
Experimental design: 32 albino rats after two weeks of acclimatization were randomly divided into four groups of eight (8) animals each, Groups A-D.
Group **A** served as the control and was fed with the normal diet and distilled water *ad libitum*.
Group **B** with four rats which served as the negative control was fed with 300 mg/kg body weight of egg yolk for thirty days.
Group **C** was given 300 mg/kg body weight of aqueous leaf extract of *Tectona grandis* only
Group **D** was given 300 mg/kg body weight of egg yolk concurrently with 300 mg/kg body weight of the extract. Administration of the aqueous leaf extract of *Tectona grandis* was done using an oral gavage for 30 days. After 30 days, the thirty surviving animals were fasted overnight and sacrificed thereafter. The liver was excised and fixed in 10 % formal saline for histopathological examinations.
Determination of biochemical parameters: Blood samples were collected into plain tubes and were centrifuged at 1000g for 15 min for evaluating oxidative stress biomarkers (superoxide dismutase and catalase). Superoxide dismutase activity was determined using the methods of Misra and Fridovich (1972) while catalase activity was determined using the method as described by Sinha 1972. Cholesterol (kit no: BT29 4QY) and alanine transferase were determined using the methods of Richmond, 1973, Reitman & Frankel 1975 respectively as described in the manual of Randox kits. Other chemical used was also of analytical grade.
Statistical analysis: Results were expressed as mean ± standard error of mean. The differences among groups were analyzed by the one-way analysis of variance (ANOVA). The Duncan's Post Hoc test was used to assess the significant difference between means. $P<0.05$ was accepted as significant.

Results

Results of the effect of aqueous leaf extracts of *Tectona grandis* on high-fat induced non-alcoholic fatty liver on serum superoxide dismutase, catalase, alanine amino transferase activities and cholesterol content in albino rats are presented in Table 1. There was significant increase in enzyme activity ($P<0.05$) in serum superoxide dismutase, SOD (148.50±3.82 ng/ml), catalase, CAT (266.32±5.74 µm/L), alanine transaminase, ALT (100.49±6.24 U/l) and cholesterol content (294.21±4.92 mg/dl) in the group in which four rats were fed with 300 mg/kg body weight of egg yolk for thirty days which served as the negative control (Group B). However, compared to the control, the administration of 300 mg/kg body weight of aqueous leaf extract of *Tectona grandis* only (Group C) showed significant ($p<0.05$) reduction in the activities of SOD (65.43±1.63 ng/ml), CAT (152.74±3.98 µm/L), ALT (28.87±2.92 U/l) and cholesterol content (156.54±4.13 mg/dl). Similarly, there was significant decrease ($p<0.05$) in the activities of SOD (78.38±2.76 ng/ml), CAT (156.50±4.32 µm/L), ALT

(30.73±4.12 U/l) and cholesterol content (173.50±1.69 mg/dl) when the rats were fed with 300 mg/kg body weight of egg yolk concurrently with 300 mg/kg body weight of the extract (Group D).

Table 1: Effects of aqueous extracts of *Tectona grandis* leaf on high-fat induced non-alcoholic fatty liver on the activities of serum superoxide dismutase, catalase, alanine amino transferase and cholesterol content

Groups	SOD (ng/ml)	CAT(µm/L)	ALT(U/l)	CHOL (mg/dl)
A	60.20 ± 1.28	140.30 ± 4.28	25.16 ±5.21	152.00 ±6.41
B	148.50 ± 3.82[a]	266.32 ± 5.74[a]	100.49 ± 6.24[a]	294.21 ± 4.92[a]
C	65.43 ± 1.63[b]	152.74 ± 3.98[b]	28.87 ±2.92[b]	156.54 ± 4.13[b]
D	78.38 ± 2.76[b]	156.50 ± 4.32[b]	30.73 ±4.12[b]	173.50 ± 1.69[b]

SOD: Superoxide dismutase, CAT: Catalase: CHOL: Cholesterol, ALT: Alanine transaminase.
Legend: Group A: Normal control; Group B: fed with 300 mg/kg body weight of egg yolk Group C: treated with 300 mg/kg body weight of aqueous leaf extract of *Tectona grandis* only; Group D was given 300 mg/kg body weight of egg yolk concurrently with 300 mg/kg body weight of the extract
[a] Significantly different from the normal group (Group A) at $p < 0.05$. [b] Significantly different from the HFD-fed group (Group B) at $p < 0.05$.

Histological examinations of the liver of rats fed with aqueous leaf extracts of *Tectona grandis* on high-fat induced non-alcoholic fatty are presented in Plates 1-4. Liver section of rats in Group A shows normal histology of hepatocyte, sinusoid and central vein, while the section in Group B shows hepatic steatosis and excess inflammatory cells. Section of the liver in Group C reveal normal hepatocyte, central vein and sinusoids while liver section in Group D also shows normal central vein, hepatocyte and sinusoids.

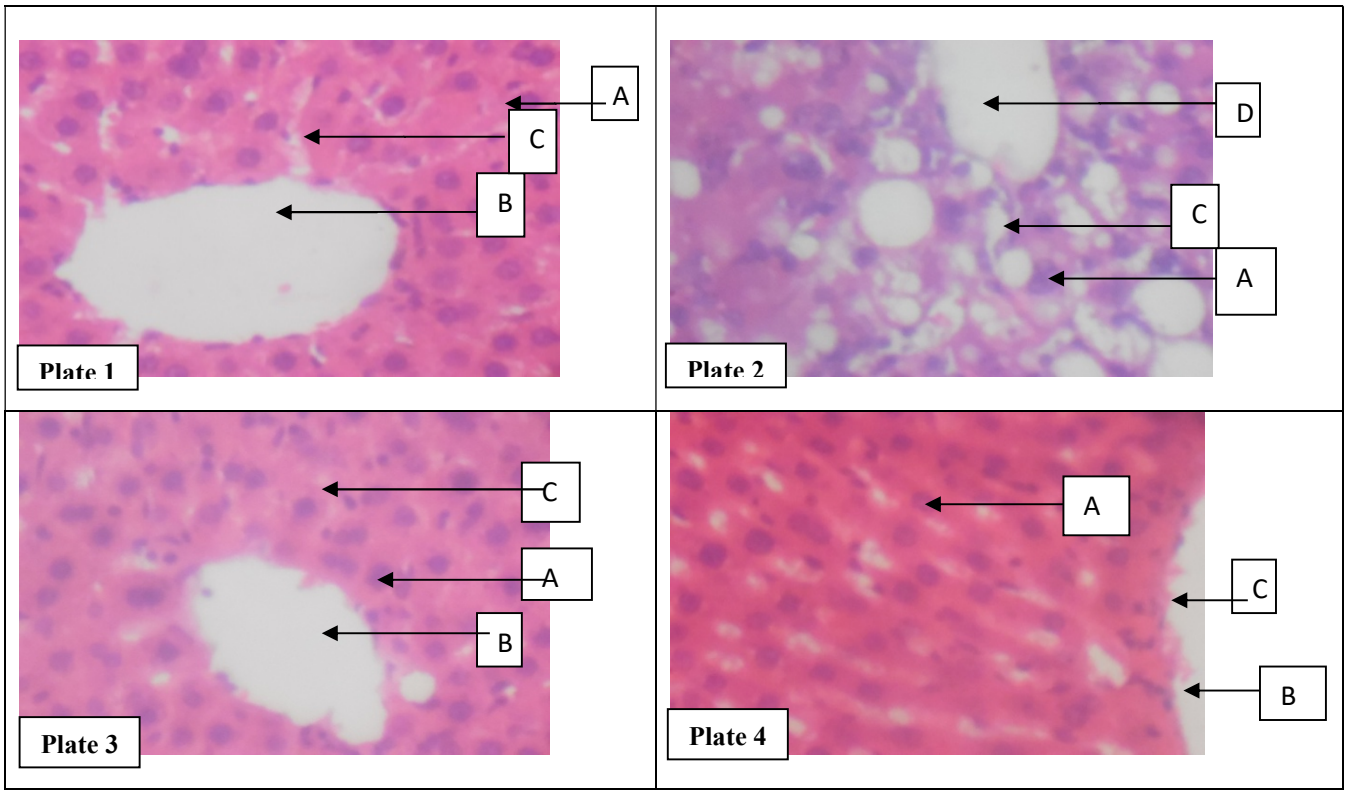

Plate 1-4: Microscopic evaluation of rat liver. **Plate 1**: Group A Normal control), **Plate 2**: Group B: Fed with 300 mg/kg body weight of egg yolk, **Plate 3:** Group C: treated with 300 mg/kg body weight of aqueous leaf extract of *Tectona grandis* only; **Plate 4:** Group D was given 300 mg/kg body weight of egg yolk concurrently with 300 mg/kg body weight of the extract.
Legend: A: hepatocyte B: central vein C: sinusoid D: hepatic seatosis (H&E x 100).

Discussion

Animal models of NAFLD are essential tools for studying the pathogenesis and treatment of NAFLD (Takahashi *et al*., 2012)**.** NAFLD is considered to cover a spectrum of disease activity (Mahan *et al*., 2012). This spectrum begins as fatty accumulation in the liver (hepatic steatosis). A liver can remain fatty without disturbing liver function, but continued insults to the liver will progress to non-alcoholic steato-hepatitis (NASH), a state in which steatosis is combined with inflammation and fibrosis(steato-hepatitis)(Drury *et al*., 1980). Non-alcoholic Fatty liver is classified as either macrovesicular or microvesicular steatosis depending on the size of the lipid vacuoles (Amacher, 2011). Macrovesicular steatosis are more abundant than the microvesicular steatosis(Sharma *et al*., 2010).

Histological assessment of the liver showed no evidence of fat deposition in the sections of liver tissue obtained from control group (Figure A), however severe steatosis (fat deposition) was seen in the group fed with high fat diet (Figure B). Similar histopathological changes was seen in work done by kucera *et al*., (2011), Rosenstengel *et al*., (2011), and Stoppeler *et al*., (2013). High fat diet also led to histopathological hepatic changes similar to human NASH in several other experiments (Zou *et al*., 2006 and Baumgardner *et al*., 2008). Treatment with the extracts of *Tectona grandis* attenuated the effects as seen in the histology of the groups treated with high fat diet which shows little or no hepatic steatosis. This agrees with the findings that medicinal plants as well as some food supplements ameliorated or reversed hepatic steatosis in animal models or patients with non-alcoholic fatty liver disease (Takayam *et al.,* 2009; Zheng *et al*., 2008)

Significant ($P<0.05$) reduction of total cholesterol was also observed in the treated groups (group C and D) when compared to the high fat fed group (group B). The lipid lowering effect of *Tectona grandis* observed in this study was similar to the result of Davoodi *et al*., (2017) who evaluated the promising effect of Rosa damascena extract on high- fat diet induced nonalcoholic fatty liver.

High cholesterol diet has been reported to induce oxidative stress in various organs such as the Liver,heart, and aorta (Dutta *et al*., 2009). Antioxidant enzymes such as Superoxide dismutase catalyses the conversion of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and O_2 (Oyedemi *et al*., 2010) and Catalase further detoxifies the H_2O_2 into H_2O and O_2 (Fridovich, 1986). However, imbalance between the formation of reactive oxygen species and their elimination occasioned by hypercholesterolemia has been implicated in oxidative-induced diseases.

In this study, serum superoxide dismutase and catalase activities significantly ($P<0.05$) increased in rats fed egg yolk only (group B) when compared with the control (group A) as well as the treated groups (groups C and D). Fardet *et al.* (2008) suggested that rats in diet-induced obesity models showed an increase in the levels of oxidative stress in their liver and that oxidative stress can result from the excessive production of reactive oxygen species and/or deficient anti-oxidant capacity(Roberts *et al*., 2006). Significant($P<0.05$) decrease was observed in the activities of these enzymes (superoxide dismutase and catalase) in the treated group(Group D) and this could be attributed to the utilization of these enzymes in inactivating the free radicals generated due to the high fat diet (Ma *et al*., 2011). This finding seems to be in accordance with the results by Cui *et al*., (2011) who reported a decrease in antioxidant enzyme (SOD) levels in rats fed a high-fat diet and treated with aqueous and ethanol extracts of *Lycium barbarum*.

The liver is the main organ responsible for detoxification processes occurring in the body. Besides the liver, there are extra-hepatic sites of metabolism which include organs such as lungs, kidney, skin, epithelial cells of gastrointestinal tract, adrenals and placenta (Coleman, 2010; Iyanagi, 2007). Liver injury in response to various chemicals results in the leakage of serum enzymes such as alanine transferase into the blood circulation, thus causing increase in their level in the serum (Drotman *et al*., 1978). The result of serum ALT analysis shows a significant increase in the levels of this enzyme in groups fed high fat diet (Group B) when compared to the control and treated groups. In the case of hepatocellular injury or death, release of ALT from damaged liver cells increases and so there is increase level in serum. Also the decreased level of this enzyme in the treated groups shows the protective property of *Tectona grandis* against injury to the liver caused by the high fat diet as higher activities of these enzymes in serum have been found in response to oxidative stress induced by high fat diets (Demori *et al*., 2006; Yang *et al*., 2003)

Conclusion

On the basis of the result obtained in this study we conclude that aqueous extract of *T. grandis* can act as a protective agent against oxidative stress induced by high fat diet as evidenced by the reduction in antioxidant enzymes seen in the treated groups.

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