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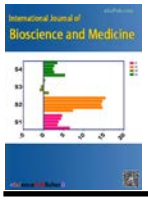

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Thaumatococcus daniellii Benn. (Marantaceae) Leaf Methanol Extract Possessed Hepatoprotective Effect Against Acetaminophen-induced Liver Injury in Albino Rats

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ABSTRACT

[Background]Acetaminophen or Paracetamol is widely used as analgesic and antipyretic or antimalarial drug, but at high dose it leads to undesirable side effects, such as hepatotoxicity. [Objective]This present study demonstrates the in vivo hepatoprotective effect of Thaumatococcus daniellii against acetaminophen induced liver toxicity. [Methods] Acetaminophen- induced liver injury was evaluated by an increase in serum AST (or SGOT), ALT (or SGPT), ALP activity and bilirubin level accompanied by significant decrease in albumin level. [Results] Acetaminophen hepatoxicity was manifested by an increase in lipid peroxidation, depletion of reduced glutathione (GSH) and catalase activity in liver tissue. Oral administration of the plant extract protects the rats against acetaminophen induced liver injury by increased lipid peroxidation, restored altered serum marker enzymes and antioxidant level to normal liver morphology.[Conclusion]The results showed that T. daniellii leaf extract displayed significant liver healing efficacy against acetaminophen induced hepatotoxicity in rats. The result further affirm the use of the plant as an ethno-medicinal prescription for liver related injury.

Keywords: Acetaminophen, hepatoprotective, Thaumatococcus daniellii, liver injury.

Abbreviations Used:
ALT = Alanine aminotransaminase or serum glutamic pyruvic transaminase, AST = Aspartate aminotransaminase or serum glutamic oxaloacetic transaminase, ALP = alkaline phosphatase

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Introduction

The liver has multiple functions. It makes many of the chemicals required by the body to function normally, it breaks down and detoxifies substances in the body, and it also acts as a storage unit. When the liver is damaged from injuries, medication, alcohol, or other factors, the individual with this problem may have symptoms of liver disease such as: nausea and vomiting, jaundice (yellowing of the skin), fatigue, weakness, shortness of breath, excessive bruising or bleeding, and leg swelling. Liver injury has become one of the major causes of morbidity all over the world. Drug-induced liver injury (DILC) is one of the most common causative factors that pose major clinical and regulatory challenges [1]. The manifestation of drug-induced hepatoxicity is highly variable, ranging from asymptomatic elevation of liver enzyme to fulminant hepatic failure [2].

Acetaminophen popularly known as Paracetamol (PCM), taken in overdose can cause severe liver injury and nephrotoxicity [3]. PCM is active and converted by cytochrome P450 enzymes to toxic metabolites NAPQI (N- acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GSH) depletion, and it is normally produced only in small amounts, and then almost immediately detoxified in the liver. Regardless of the tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver form damaged or help in regeneration of hepatic cell.

Many active plant extracts are frequently utilized to treat a wide variety of clinical disease involving liver disease [4]. Hence, searching for effective and safe drugs for liver injuries have gained popularity by researchers in the field of Pharmacognosy and ethnobiology as well as other related fields in recent times. Thaumatococcus daniellii is a member of the family Marantaceae is formerly called Donas daniellii (Benn).

In Nigeria, it is locally called ''Iwee'' in Igala language. It exhibits contraceptive, hypoglycaemic activity [5], cardio-protective effects and antibacterial activity [6]. It is a rhizomatous perennial herb up to 3.5 m high. The ovate-elliptic leaves up to 60 cm long and 40 cm wide arise singly from each node of the rhizome. Inflorescences are single or simply branched spikes and emerge from the lowest node. The fruit is freshly trigonal in shape and matures to dark red-brown colour when fully ripe. At maturity each fruit contains three black, extremely hard seeds. The seeds are enveloped by sticky thin, pale yellow basal aril, which contains the sweetening protein - thaumatin [7].

In West Africa, T. daniellii leaves are mostly cultivated for the leaves. The lamina of the leaves is used for wrapping foods especially ''Agidi'' in Eastern Nigeria. The petiole is used to weave mats and as tools for building materials. The entire leaf is also used for roofing. In traditional medicine the leaf sap is used as antidote against venom, stings and bites. Leaf and sap are used as sedative and for treating insanity [8].

In Nigeria, the fruits are used as sweetener. The aril of flowers contain a non-toxic intensely sweet protein compound thaumatin, which is at least 3000 times as sweet as sucrose. Since the mid-1990's, thaumatin is used as sweetener and flavor enhancer by the food and confectionary industry. Substituting synthetic sweetener, Thaumatin is not a carbohydrate thus, it is an ideal sweetener for diabetics [9]. The fruit is used as a laxative and the seed as an emetic and for pulmonary problems [10]. This present study was carried out in order to evaluate the hepatoprotective effects of T. daniellii leaf methanol extract against acetaminophen-induced liver injury in Swiss albino rats.

Materials and methods

Materials

25 Swiss albino rats of both sexes, T. daniellii powder leaf, plain tubes, normal saline, Whatman No 1 filter paper 9 cm wide, weighing balance, reflotron plus machine (Roche), centrifuge, bovine albumin, methanol, sucrose solution, Silymarin USP, acetaminophen, Soxhlet apparatus, muslin cloth, rotary evaporator, desiccator, etc.

Plant collection, identification and preparation

Thaumatococcus daniellii plant was collected from Mbamnga ward, Sardauna Local Government Area, Taraba State, Nigeria. The plant was identified by a taxonomist in the Department of Science Laboratory Technology, Federal Poly-

technic Bali, where a voucher number MAR001 was deposited for the plant. The leaves were air-dried under shade and good ventilation for two weeks. They were then grind into fine powder using an electronic blender and weighed on an electronic balance. The powdered leaves were then extracted in 800 mL of methanol (analytical grade) using Soxhlet apparatus for 12 hours, and filtered by vacuum liquid chromatography (VLC). After this, the filtrate was concentrated in vacuo using rotary evaporator to get a dark paste-like extract. This was then weighed to get the final yield and stored in the desiccator for further use.

Experimental design

Twenty five well fed albino rats were randomly grouped into five groups of five animals each. The animals were allowed to acclimatize with laboratory condition for 24 hours prior to treatment orally. The animals were kept in polypropylene cages and maintained at 25 ± 5 °C under 12 h light/dark cycles. The animals were provided with water ad libitum. All the experimental procedures were carried out in accordance with the guidelines of the National Institute of Health. The rats were observed daily for any signs of mortality. Body weights of each group were recorded at regular intervals throughout the experimental periods [11].

Extract preparation for experiment

10 g of dark plant methanol extract and acetaminophen 2000 mg were dissolved in 0.5 % dimethyl sulphoxide (DMSO) in 10 mL distilled water and used as the stock solution for evaluating the hepatoprotective effects of the plant extract against acetaminophen-induced liver injury in albino rats.

Hepatoprotective activity

Hepatoprotective effect of T. daniellii leaf methanol extract was evaluated according to the methods previously described by Lowry et al. [12], with slight modifications. Briefly, after acclimatization period of 24 h, the animals were grouped into five groups of five rats each and treated orally as designed below for four weeks (1 month).

Group I: received 10 mL distilled water and served as negative control group

Group II: received silymarin 280 mg/kg PO + 500 mg acetaminophen as positive control group

Group III: received 500 mg acetaminophen + TDE 50 mg/kg body weight

Group IV: received 500 mg acetaminophen + TDE 100 mg/kg body weight

Group V: received 500 mg acetaminophen + TDE 400 mg/kg body weight

After four weeks of the experiment, the animals were sacrificed under ether anesthesia 24 hours after the last dose. Blood was collected by cardiac puncture in plain tubes and liver was removed, rinsed in cold normal saline, blotted with filter paper and weighted. 10 % (w/v) liver homogenate was prepared in 0.25M sucrose solution, and centrifuged at 3000 rpm for 10 min at 4 °C.

The supernatant was used for various biochemical assays relating to liver problems and healing such as aspartate aminotransferase (APT), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, etc., while protein concentration was estimated using bovine serum albumin (BSA) as a standard. The experiments were repeated for each group three times and mean values were recorded.

Statistical analysis

Data obtained were expressed as mean ± SD of three replicate readings. Statistical significance among groups were compared using one-way analysis of variance (one-way ANOVA) by SPSS statistical software version 22.0. Difference between untreated and treated groups with a p-value < 0.05 was considered statistically significant.

Results and discussion

Thaumatococcus daniellii is a well-known medicinal plant in South-eastern Nigeria, which is widely used in traditional medicine. In the present study, distilled water was used orderly for crude extraction of T. daniellii leaf powder. To justify the ethno-medicinal claims of the use of T. daniellii extract in treating liver injury, we made an efficient attempt in evaluating the hepatoprotective effects of the plant. Acetaminophen commonly called Paracetamol (PCM) is a common analgesic and antipyretic drug produced by most pharmaceutical industries. Several studies have

shown the induction of hepatocellular damage or necrosis by acetaminophen at higher doses in experimental animals [13].

In evaluating hepatoprotective agents, acetaminophen- induced hepatoxicity assay has been used as a reliable method. Acetaminophen (AMP) is metabolized primarily in the liver and eliminated by conjugation with sulphate and glucuronide, and then excreted by the kidney [14]. Moreover AMP hepatoxicity has been attributed to the formation of toxic metabolites, when a part of it is activated by hepatic cytochrome P-450 to a higher reactive metabolite N-acetyl-p-benzo-quinoneimine [15]. This was achieved when the animals were induced with AMP 500 mg/kg body weight (b.w) in all the groups.

Toxic metabolites (N-acetyl-p-benzoquinoneimine) can alkylate and oxidize intracellular glutathione depletion subsequently leading to an increased lipid peroxidation removing hydrogen from a polyunsaturated fatty acid and ultimately causing liver damage due to higher doses of acetaminophen (AMP). From our study, at higher dose of 500 mg/kg Thaumatococcus daniellii leaf extract displayed significant healing of liver damage induced by over dose of AMP. Bilirubin is as a molecule, is a bye-product of the routine destruction of red blood cells occurring in the liver. It is normally released as bile in the feces. Elevation of the bilirubin may suggest liver dysfunction. However, other conditions with increased destruction of red blood cells also can cause elevated bilirubin levels despite normal liver function. Normal values are about 0.1 to 1.0 mg/dL. The value 0.80 ± 0.13 obtained in group v showed that the plant extract displayed significant liver healing potential with reduced bilirubin levels, these values increased in dose-dependent manner (Table 1).

Albumin is a very common protein found in the blood with a variety of functions. It also is produced only in the liver, and if its levels are lower than normal it can be suggestive of chronic liver disease or liver cirrhosis. Of note, many conditions other than liver disease also may cause low albumin levels. Normal values are about 3.5 to 5 g/dL. From our results, the values fall within the normal range, and were comparable to that of the positive control silymarin at $p \leq 0.05$ and $p \leq 0.01$ (one-way ANOVA). It has been reported that reactive metabolites can exert initial cell stresses through a wide range of mechanisms including depletion of, bilirubin, albumin, glutathione, lipids, nucleic acids and other cell structures [16]. However, T. daniellii extract was able to alter the metabolic pathways of most of the liver functioning parameters and enzymes either by decreasing or increasing their activities so as to achieve healing of liver injury. Aspartate aminotransaminase or serum glutamic oxaloacetic transaminase (AST) is predominantly found in mitochondria of hepatocytes. AST (SGOT) occurs normally in a variety of tissues such as liver, heart, muscle, kidney, and the brain. It is released into the serum when any one of these tissues is damaged. For example, AST level in serum is elevated in heart attacks or with muscle injury. It is therefore, not a highly specific indicator of liver injury as its elevation can occur as a result of other injured tissues. Elevated liver enzymes are a marker of inflammation or damage to liver cells. Injured liver cells cause the liver enzymes alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) to flow into the bloodstream. Mild elevations of ALT and AST are commonly discovered in individuals with no symptoms during routine blood work. In general, normal ranges for ALT are 7 to 56-units per liter, while normal ranges for AST are 10 to 40-units per liter. Mild elevations of both liver enzymes are 2 to 3-times higher than normal range. The values for these enzymes obtained from our study showed dose dependent values, hence T. daniellii contained metabolites which are capable of healing liver injury or disease in vivo as seen in the rats treated with T. daniellii extract for one month.

However, since ALT is more specific to liver, and a better parameter for diagnosing liver injury, serum alkaline phosphatase (ALP) and bilirubin are also good diagnostic parameters assessing liver cell damage, hence the values of these parameters in Table 1 revealed that the plant is capable of healing liver injury.

Moreover, the precise levels of these liver enzyme tests do not correlate well with the extent of liver problems or the prognosis. Thus, the exact levels of AST (SGOT) and ALT (SGPT) cannot be used to determine the degree of liver disease or predict the future prognosis for liver function. For example, individuals with acute viral hepati-

tis A may develop very high AST and ALT levels (up to thousands of units/liter range), but most patients with acute viral hepatitis A recover fully without residual liver disease. Conversely, people with chronic hepatitis C infection typically have only a little elevation in their AST and ALT levels while having substantial liver injury and even advanced scarring of the liver (cirrhosis) from ongoing minor inflammation of the liver [17]. Reduction of serum albumin in AMP treated group may be due to formation of protein adjunct in the animals. Our study therefore showed that T. daniellii leaf extract exhibited excellent hepatoprotective effects as indicated by maximum prevention of increased serum biochemical parameters on acetaminophen- induced liver injury.

Conclusion

Our study showed that T. daniellii leaf extract can correct liver injury by decreasing liver enzymes while it increased significantly within the range values the levels of bilirubin and total albumin in Swiss albino rats. The fact that the plant displayed higher degree of healing of liver injury justifies its used as an ethnomedicinal prescription for liver injuries and other liver associated problems. Thus, the plant represents a source for new drug discovering. The results obtained from the study can then be incorporated in the aspect of pharmacology and ethnobiology for drug discovery and clinical trials for liver problems.

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Table 1: Effect of T. daniellii leaf extract on some liver function parameters of albino rats

Group/ dose	Bilirubin (mg /dL)	Total Albumin (g/ dL)	ALT (u/L)	AST (u/L)	ALP (u/L)
AMP+ distilled water	2.13±0.62*	2.2±0.60*	64.99±4.00*	68.49±3.92*	322.11±25.82*
AMP+ silymarin PO	0.19±0.12^{+}	3.32±0.21*$^{+}$	36.75±6.85*$^{+}$	41.67±3.05*$^{+}$	153.30±25.82^{+}
50mg/kg TDE + AMP	0.26±0.63*	3.61±0.15*$^{+}$	35.75±3.75*$^{+}$	41.39±3.22*$^{+}$	148.73±18.92^{+}
100mg/kg TDE+ AMP	0.30±0.16*	4.74±0.08* $^{+}$	34.22±4.78* $^{+}$	36.13±6.24^{+}	125.42±22.62^{+}
400mg/kg TDE+ AMP	0.80±0.13	4.87±0.11	22.93±6.21	28.9±3.51	115.65±2.00

Data are expressed as mean ± SD for five rats in each group. Value is represented in percentage restoration of the activity to normal values *P<0.05 denote value significantly different from control, + P< 0.05 experimental groups compared with acetaminophen group, AMP = acetaminophen, PO= periocular, TDE = Thaumatococcus daniellii extract, ALT = alanine amino transferase, AST = aspartate aminotransferase, ALT = serum alkaline phosphatase