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Biochemical and Histological effects of Aqueous extract of *Cyperus Esculentus* in Triton Wr-1339 Induced Hyperglycemic Rats.

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ABSTRACT: Researchers and physicians look forward to effective therapeutic agents possibly from medicinal plants following the ineffectiveness of modern drugs used in the treatment of fatty liver and its related diseases. To this ends, we evaluated the effect of *Cyperus esculentus* on the liver in experimentally induced hyperlipidemia in rats.Aqueous extract of the plant was administered orally to rats at the doses of 300,400 and 600mg/kg body weight(b.wt) for a period of twenty nine days. Fatty liver was induced by twice intraperitoneal injection of TritonWR-1339 at a dose of 300mg/kg b.wt. to wistar rats . The result shows that administration of triton led to significant ($P<0.05$) serum increase in the activities of alanine and aspartate aminotransferases (ALT and AST) of the rats.There was no significant ($p>0.05$) change in the activities of serum alkaline phosphatase following the administration of triton.The study also shows that there were significant($p<0.05$) reduction in the serum level of total protein , albumin and globulin and increase in serum level of total and conjugated bilirubin though not statistically significant ($p>0.05$) following the administration of triton to rats.The observed effects are suggestive of mild toxicity of the liver by triton and this is further supported by several histopathological manifestations such as hepatocellular degeneration, macrovesicular vacuolation,periportal necrosis, congestion ,severe intimal ulceration and stenosis in the blood vessels . Treatment with the aqueous extract of *Cyperus esculentus* attenuated both the biochemical effects and histopathological manifestations.In conclusion, the results from the serum liver enzymes biochemical parameters and histopathological analyses suggested the toxicity of triton and also showed the effectiveness of the aqueous extract of *Cyperus esculentus* in attenuating the liver toxicity .©JASEM

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Key Words: *Cyperus esculentus*,Triton,macrovesicular vacuolation,periportal necrosis.

Tiger nut (*Cyperus esculentus var. sativus),* belongs to the *Cyperaceae* family and is also found to be a perennial crop of the same genus as the papyrus plant .It is widely distributed in the temperature zones within South Europe and also grows naturally in Ghana, Nigeria and Sierra Leone (Anon, 1992;Rita,2009). Tiger nuts are under-utilized due to lack of information on their therapeutic potential (Addy and Eteshola 1984; Adejweitan *et al*.2009). A lot of people eat the tiger nut without knowing the nutritional benefits and products that can be obtained.

Studies have shown that tiger nuts have a high energy nutrient such as glucose oleic acid, starch, fats, sugars and proteins (Kim *et al.,* 2007). They are rich in minerals such as phosphorous, potassium, calcium, magnesium and iron as well as vitamins E and C. Tiger nut milk is suitable for diabetic individuals and also helps in weight control because of its high fiber content(Emple *et al.,*1998).The presence of digestive enzymes like the catalase, lipase and amylase in tiger nuts makes it useful in the treatment of indigestion, constipation, flatulence and diarrhoea. The high content of oleic acid has positive effect on cholesterol, thereby preventing heart attacks, thrombosis and activates blood content of soluble glucose. Tiger nut reduces the risk of colon cancer. Tiger nut contains a good quantity of vitamin B1, which assists in balancing the central nervous system and helps to support the body to adapt to stress (Martinez 2003). The black species of the tiger nut is an excellent medicine for breast lumps and cancer. The tubers have a relatively high total antioxidant capacity, because they contain considerable amounts of water-soluble flavonoid glycosides (Belawu, 1996).The oil from tiger nut reduces low density lipoprotein-cholesterol (LDL-C) and increases high density lipoprotein-cholesterol (HDL-C) , reduces levels of triglycerides in blood and the risk of forming bloody clots, thereby preventing arteriosclerosis(Oladele, *et al.,*2009;Okafor *et al.,* 2003). It also stimulates the absorption of calcium in bones and the production of new bony material, due to short and medium chain fatty acids, oleic acid and

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essential fatty acids (Mokady and Dolev, 1970). It is also recommended for infants and the elderly because of its high content in Vitamin E and its antioxidant benefits in the cell membrane (Umerie and Enebeli,1997; Mohamed,*et al.,*2005).The waste residue after oil extraction could be further modified to produce syrups, flours, or livestock feeds(Oladele, *et al.,*2009;Okafor *et al.,* 2003;Mokady SH and A Dolev,1970).

Triton WR-1339 is used in quite a lot of studies to induce hypercholesterolemia in animals (Hamafi*et al.*, 2008; Bertges *et al.,* 2011). Its mechanism of actions are related to the inhibition of the activity of the enzyme lipoprotein lipase and the stimulation of the activity of the enzyme HMG-CoA reductase (Janicki and Aron 1962).These result in the increase in hepatic cholesterol biosynthesis and the accumulation of triglycerides and VLDL in plasma. Epidemiologic information demonstrates that the pathogenesis of fatty liver is related to high circulating cholesterol (Lankarani *et al.,* 2013). The risk factors comprise of high fat and protein diet, male gender, the presence of metabolic syndrome characteristics, extra meals before sleep, and sedentary lifestyle.Although the mechanism is complex and poorly understood, it is believed to involve an imbalance of fatty acid metabolism that leads to hepatic triglyceride accumulation (Shi *et al.,*2012).It is an important progressing liver damage and the high incidence of fatty liver indicated that there is a need for the new management of fatty liver and its related diseases (Gu *et al,.*2007;Assy *et al.,*2009;Tsuruta *et al.,*2011). The fatty liver can also lead to fibrotic change and cirrhosis, which have no potential drugs for the treatment (Zelber-Sagi,*et al*., 2011;Li *et al*.,2010;Luper *et al*.,1998). The use of modern drugs such as colchicine, interferon, corticosteroids, and penicillamine for the treatment of fatty liver, chronic hepatitis, and cirrhosis is not giving much satisfactory outcome. Researchers and physicians look forward to effective therapeutic agents possibly from medicinal plants (Tsuruta *et al.,* 2011; Zelber-Sagi,*et al*., 2011;Li*et al*.,2010;Luper *et al*.,1998.)

The purpose of this present study is to evaluate the biochemical and histological effects of triton WR-1339 and aqueous extract of *Cyperus esculentus on* the liver.

MATERIALS AND METHODS

*Collection, Identification and Preparation of Plant Material***:** Dried Cyperus esculentus tubers were procured from a local market in Benin City, Edo State, Nigeria. It was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin. The tubers were selected to remove the bad ones there after they were crushed into fine powder. The powdered form was soaked in 8L of water, stirred vigorously and left undisturbed for 48 hours at room temperature. The crude aqueous extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was then concentrated to dryness under reduced temperature and pressure using Rotary evaporator.The extract was stored in an air-tight container and kept in the refrigerator at 4°C until use.

*Experimental Animals***:** Wistar rats weighing 180-220 g were procured from the Animal House, Department of Anatomy,University of Benin. Food and water were provided *ad libitum*. Animals were exposed to controlled environmental temperature (28± 2°C), relative humidity (50± 5%) and 12 hour light or dark.

Drugs Preparation: Hyperlipidemic drug, Triton WR-1339 (Sigma–Aldrich) that was used for this study was dissolved in normal saline (pH 7.4) and administered intraperitoneally to rats at a dose of 300 mg/kg b.wt..The plant extract was administered orally to rats at the doses of 300, 400 and 600 mg/kg body weight.

Animal Groupings: Rats were randomly divided into five groups of five animals each.

Group I: (Control) received only 1ml of distilled water daily for a period of 29days.

Group II: (Triton WR-1339 –induced) received 1ml of distilled water daily for a period of 29 days and Triton WR-1339 at a dose of 300mg/kg body weight intraperitoneally on the 14th and 27th day.

Group III: received aqueous tuber extract of Cyperus esculentus at a dose of 300 mg/kg body weight orally for a period of 29 days and Triton WR-1339 at a dose of 300mg/kg body weight intraperitoneally on the 14th and 27th day.

Group IV: received aqueous tuber extract of Cyperus esculentus at a dose of 400 mg/kg body weight orally for a period of 29 days and Triton WR-1339 at a dose of 300mg/kg body weight intraperitoneally on the 14th and 27thday.

Group V: received aqueous tuber extract of Cyperus esculentus at a dose of 600 mg/kg body weight orally for a period of 29 days and Triton WR-1339 at a dose of 300mg/kg body weight intraperitoneally on the

14^{th} and 27^{th} day.
To ensure accuracy of treatment, administration of the extract was done using orogastic tube for 29 consecutive days .All the animals were then sacrificed the next day by anaesthesizing them with chloroform followed by incision on the abdomen and blood was collected via cardiac and aortic puncture.The blood was put into plain sample bottles and sera were obtained from it by allowing it to stand for 2hrs at room temperature followed by centrifuging at 2000rpm . The serum was used for estimation of various biochemical parameters. The liver was dissected and immediately fixed in formal-saline for tissue processing and histopathological analysis.

*Histopathological analysis***:** The liver was examined grossly in all the dissected rats and was preserved in 10 % buffered formalin, dehydrated in ethanol (70 to 100 %), cleared in xylene and embedded in paraffin. All tissue sections were examined under a light microscope after staining with hematoxyline and eosin (Drury, 1980).

Assessment of liver function: Serum Aspartate and Alanine Transferase(AST and ALT) The activities of these enzymes were estimated by the method of Reitman and Frankel (1957). 0.2 ml of serum was added to 1 ml of phosphate buffer containing substrate, mixed and incubated for 30 min for ALT and 60 min for AST at 37°C, then 1 ml of dinitrophenylhydrazine was added and incubated for 20 min at room temperature and 10 ml of 0.4% sodium hydroxide was added, mixed well and after five minutes read at 550 nm against sample blank .The value of AST and ALT were calculated from a series of standard curves.

Alkaline Phosphatase (ALP): Serum alkaline phosphatase activity was measured following the method of King and Armstrong using disodium phenyl phosphate as substrate.The colour developed was read at 510 nm. Activities are expressed as U/L King and Armstrong 1934.

Albumin (ALB): Serum total bilirubin was estimated following the method of Doumas *et al*.In brief, 1 ml of serum was mixed with 0.5 ml of diazo reagent, followed by 0.5 ml $(NH_4)_2SO4$ .The volume was made up to 10 ml with 85% ethanol.The contents were mixed well and allowed to stand for 30 mins for even distribution of the precipitate (Doumas*et al.,*1979).

*Protein Content (TP),Bilirubin***:** Protein content of serum was determined by Biuret method, (Peters, 1968),

bilirubin was estimated using assay kits (Randox Laboratories LTD. United Kingdom BT294QY)by the method described by Jendrassik and Grof (1938) ,Peters,*et al.,*1968;Jendrassik, and Grof,1993.

Statistical Analysis: Data were expressed as (Mean ± SD) of six replicates and were subjected to one way analysis of variance(ANOVA) using SPSS version 10.0.and the individual comparisons were obtained by the Duncan multiple range test (DMRT).A value of $P<0.05$ was considered to indicate a significant difference between groups.

RESULTS AND DISCUSSION

The results show that administration of triton to rats led to significant ($P<0.05$) increase in the activities of Aminotransferases (ALT and AST) in the serum of rats.There was no significant ($p>0.05$) change in the activities of serum alkaline phosphatase following the administration of triton.Treatment with aqueous extract of *Cyperus esculentus* at doses of 400 and 600 mg/kg significantly decreased serum activities of Aminotransferases and alkaline phosphatase to normal activities (table 1).The study also shows that there was significant($p<0.05$) reduction in the serum level of total protein , albumin and globulin and increase in serum level of total and conjugated bilirubin though not statistically significant ($p>0.05$), following the administration of triton to rats.

The observed effects are suggestive of mild toxicity of the liver by triton and this is further supported by several histopathological manifestations such as hepatocellular degeneration, macrovesicular vacuolation,periportal necrosis, congestion and severe intimal ulceration and stenosis in the blood vessels . Treatment with the extract *Cyperus esculentus* attenuated both the biochemical effects and histopathological manifestations as seen in figures II to V below:

Table 1: Effect of Ethanolic Extract of *Cyperus esculentus* on Liver Function Serum Markers

Parameter	Group A	Group B	Group C	Group D	Group E
ALP (U/l)	48.80±8.61	57.0±9.75	53.0±8.32	31.80±8.47[b]	41.6±7.16
AST (IU/l)	156.0±49.9	269.2±64.06[a]	160.2±53.7[b]	148.6±21.4[b]	132.8±22.8[b]
ALT (U/l)	78.2±33.24	110.2±18.7[a]	102.8±20.49	81.4±8.27[b]	98.8±6.61[b]
TBIL(mg/dL)	0.54±0.09	0.62±0.13	0.05±0.07	0.46±0.13	0.54±0.11
DBIL(mg/dL)	0.22±0.84	0.28±0.07	0.26±0.89	0.18±0.08	0.22±0.08
TP (mg/dL)	8.68±0.40	4.88±0.85[a]	7.38±0.34[b]	8.22±0.46[b]	8.02±0.27[b]
ALB(mg/dL)	3.56±0.40	2.90±0.14[a]	2.76±0.55[b]	3.94±0.21[b]	3.94±0.15[b]
GLO(mg/dL)	5.12±0.76	1.98±0.33[a]	4.62±0.36[b]	4.28±0.37[b]	4.08±0.24[b]

Data is expressed as Mean ± SEM n=6
[a]p<0.05 Compared with control group (group A)
[b]p<0.05 Compared with triton induced group(group B)

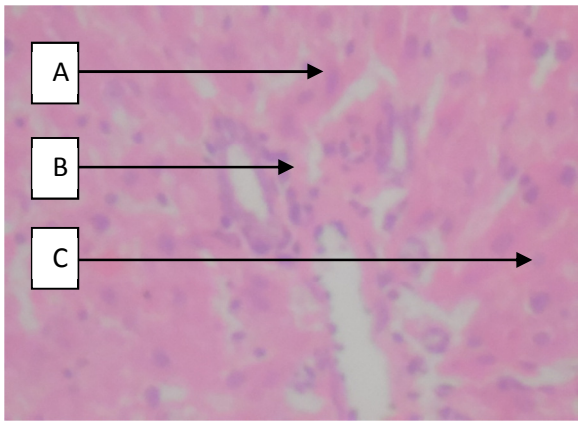

Fig. 1: Control: Rat liver composed of A, hepatocytes, B, sinusoids and C, bile duct (H&E x 100)

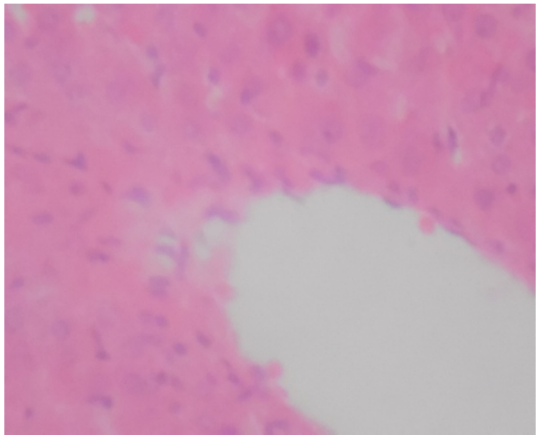

Fig. 2: Rat liver given Triton only A, focal macrovesicular vacuolation (H&E x 100)

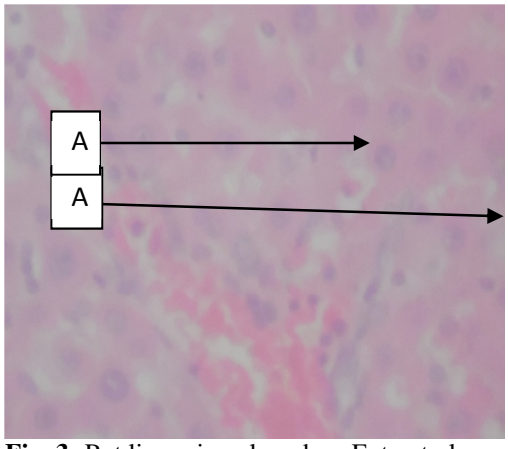

Fig. 3: Rat liver given low dose Extract plus Triton showing A, moderate vascular congestion (H&E x 100)

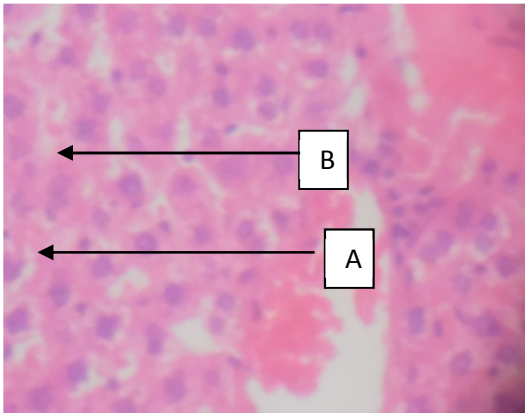

Fig. 4: Rat liver given moderate dose Extract plus Triton showing A, mild vascular congestion and B, mild kupffer cell activation (H&E x 100)

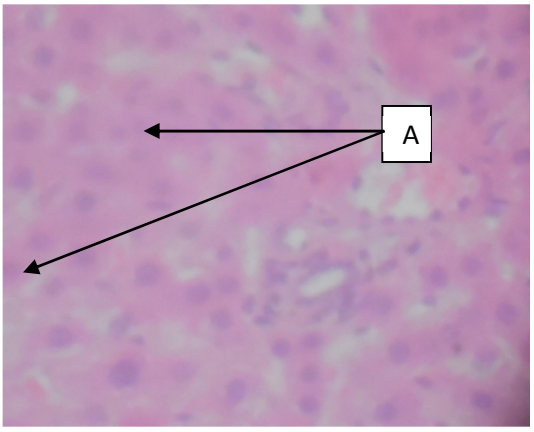

Fig. 5: Rat liver given high dose Extract plus Triton showing A, normal hepatocyte architecture (H&E x 100)

Elevated activities of various enzymes in the serum serve as useful markers in the diagnosis of liver diseases and toxicity Alanine and aspartate aminotransferases (ALT and AST) are produced in the liver while Alkaline phosphatase is produced in the outer layer of plasma membrane. These enzymes are used for assessing liver and plasma membrane damage (Raghuramu *et al.*1993 ;Sarawat *et al.,*1993) respectively since their high activities in the serum may indicate cellular leakage and loss of functional integrity of cell membrane (Shahjahan *et al.,* 1993;Talwar and Srivastera, 2004).

Administration of triton to rats led to significant ($P<0.05$) increase in the activities of Aminotransferases (ALT and AST). This increase might indicate hepatocellular injury induced by triton injection.This resulted in an increase in hepatic cholesterol biosynthesis and accumulation of triglycerides and VLDL in plasma.Induction of cellular linkages by high lipid accumulation in the hepatocytes of the liver parenchyma might be the cause of the observed increase.AST is less specific for liver disease whereas ALT is known to increase in hepatic necrosis (Toro and Ackemann, 1975).Treatment with ethanolic extract of *Cyperus esculentus* at doses of 400 and 600 mg/kg significantly decreased serum activities of aminotransferases to normal levels.There was no significant ($p>0.05$) change in the activities of serum alkaline phosphatase following the administration of triton.This could imply that triton was toxic to the liver cell because , in hepatocyte injury, ALP is often normal or marginally elevated and this feature is usually used as a guide to differentiate liver parenchymal disease from biliary dysfunction.

The study also shows that there were significant($p<0.05$) reduction in the serum level of total protein, albumin and globulin and increase in serum level of total and conjugated bilirubin though not statistically significant ($p>0.05$), following the administration of triton to rats. Albumin and globulin are synthesized in the liver and the ability of the liver to biosynthesize these proteins is affected in some types of liver disorder.This may be suggestive of mild toxicity of the liver. Alteration in total plasma protein is usually due to a decrease in the quantity of albumin synthesized in the liver .Bilirubin is elevated in animals with a haemolytic anaemia and as a result of increased rate of red cell catabolism coupled with the capacity of the liver to excrete bilirubin (Naganna,1989). The above effects caused by administration of triton indicated mild liver damage (Nagaha *et al.,*1989).Histopathological examinations of the liver of normal rat (control group) reveals that hepatocytes ,sinusoids and bile duct appear to have normal cellular architecture whereas the liver of triton induced groups were characterized with several deformations such as hepatocellular degeneration,macrovesicular vacuolation,periportal necrosis, congestion and severe intimal ulceration and stenosis in the blood vessels. Treatment with the extract *Cyperus esculentus* attenuated both the biochemical effects and histopathological manifestations as only mild congestion were seen (figures 1-5). Among the different doses administered 400mg/kg b.wt. showed the highest activities and this may be suggestive of the extract's effective dose.

Aqueous extract of *Cyperus esculentus* mitigated triton-induced toxicities in rat liver as judged from both biochemical and histopathological findings. The exact mechanism of action of *Cyperus esculentus* in ameliorating liver diseases is not well understood but it is believed to be related to its effectiveness in decreasing plasma lipid (Ubhenin *et al.,* 2016).

In conclusion, the results of the serum liver markers enzymes, biochemical parameters and

histopathological analyses suggest that administration of triton to rats induced liver toxicity. However, treatment with the aqueous extract of *Cyperus esculentus* attenuated both the biochemical effects and histopathological manifestations.

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