Therapeutic efficacy of *Tapinanthus globiferus* on acetaminophen induced nephrotoxicity, inflammatory reactions and oxidative stress in albino rats.

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ABSTRACT: Nephrotoxicity due to acetaminophen toxicity has been reported in many studies. This study was therefore designed to access the nephroprotective effect of *Tapinanthus globiferus* on possible acetaminophen induced nephrotoxicity. There were elevations in markers of inflammation namely tumor necrotic factor alpha and interleukin 2 and activities of gamma glutamyl transferase in groups given different doses of acetaminophen when compared with control. Serum concentrations of creatinine and urea were elevated in rats given acetaminophen. Serum concentrations of total proteins and albumin were reduced in groups given different doses of acetaminophen. However, all the side effects were ameliorated in rats given *T. globiferus* alongside acetaminophen. The study showed that administration of *T. globiferus* reduced damages due to acetaminophen administrations either at normal or higher doses.

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KEY WORDS: Acetaminophen, nephrotoxicity, tapinanthus globiferus, inflammation, oxidative stress.

1. INTRODUCTION

Tapinanthus globiferus is a semi-parasitic woody perennial plant commonly found growing on oaks and other deciduous trees. It is widespread in Europe and has been known to be very common in North Central Namibia and the tropical rain forest of Nigeria. Historically, it has been used to treat hypertension, epilepsy, exhaustion, anxiety, arthritis, vertigo and degenerative inflammation of the joints. It has equally been described to possess antispasmodic, cardiotonic, diuretic, emetic and hypotensive properties. Cook, et. Al., (1998), in their research to estimate antioxidative potentials of series of plants observed that tapinanthus globiferus possesses strong antioxidative property.

Acetaminophen (N-acetyl-p-aminophenol (APAP)) is one of the most widely used analgesic drugs worldwide. It has been described to be a major cause of liver injury. APAP is the most commonly reported toxic ingestion in the United States with 165,000 exposures reported (American Association of Poison, 2005). Studies have shown that renal insufficiency occurs in approximately 1-2% of patients with APAP overdose (Prescott, 1983). Boutis and Shannon, (2001), in a retrospective case series of pediatrics patients with acetaminophen poisoning suggested that associated nephrotoxicity may be more common in children and adolscents. Although nephrotoxicity is less common than hepatotoxicity in APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury (Carpenter, et al., 1981). Suresh, et. al., (2006) and Trumper et. al., (1998) reported nephrotoxicity due to APAP. Traditional medicine is undoubtedly a reliable alternative approach to health care delivery in the metropolis because it is cheap, easy accessible and efficacious (Odugbemi, 2006). These herbs had been used to treat drug side effects and to treat several ailments in form of antibiotics, antimalaria etc. Presently, attention is focused on search for newer drug that will be more effective and exert little or no side effect in humans. For instance, Ghosh et al., (2006) had reported effectiveness of cajanus indicus against acetaminophen-induced hepatotoxicity and nephrotoxicity. This study was designed to assess possible chemoprotective potentials of aqueous extract of *T. globiferus* on nephrotoxicity, oxidative stress and inflammatory response due to administration of APAP in albino rats.

2.0 METHODOLOGY

2.1 Experimental animals

Twenty 16-week old albino rats with an average weight of 200 g were purchased from a commercial breeder in Ilorin, Kwara State, Nigeria. They were kept in a well ventilated animal house of the department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria with conducive atmospheric pressure and temperature. The animals were separated into 4 groups with each group having five rats. The rats were housed in plastic cages and had access to feed and pipe-borne water.

2.2 Plant Materials and Extract Preparation

Leaves of tapinanthus globiferus parasitic on azadirhacta indica tree were harvested from neighboring bushes within Ogbomoso and were

verified by a botanist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Aqueous extract of the leaves were prepared by blending 672.6 g of the wet leaves in 1500 ml of distilled water to give 0.45g/ml extract. Commercial paracetamol (500 mg) soluble tablets were obtained from Jopats Pharmacy, Ogbomoso. An oral method of administration was adopted.

2.3 Experimental design and administration of acetaminophen and *Tapinanthus globiferus*

Group 1 consisted of 5 rats that were not given *T. globiferus*, and APAP to serve as control. Group 2 consisted of 5 rats that were given 2.7 mg/kg of APAP 4 times per day. Group 3 consisted of 5 rats given 3.5 mg/kg body weight of APAP 4 times per day. Group 4 consisted of 5 rats given of 3.5 mg/kg of APAP. Rats in group 4 were further given 0.45 mg/ml of *T. globiferus* 5 minutes later. All administrations were given 4 times (4-hour interval) daily for fourteen (14) days.

2.4 Sample collection

On the 15th day, the rats were sacrificed and blood samples obtained through cardiac puncture. The blood was collected into appropriately labeled sample bottles and spinned at 4000rev/sec for 5 minutes. The supernatants were decanted and stored at -2^{0C} for analyses of biochemical parameters.

2.5 Homogenization of kidney tissue

The kidney tissue were excised and immediately placed on a blotting paper to remove the blood. It was rinsed in 1.15% of poattasium chloride solution to remove the hemoglobin and homogenized in phosphate buffer using Teflon homogenizer. The resultant homogenates were centrifuged at 10,000g for 20 minutes to obtain the post mitochondria supernatant fraction which was used to assay for malondialdehyde.

2.6 Determination of biochemical parameters

The biochemical parameters determined included total cholesterol, triglyceride, high density lipoprotein, albumin, urea, total protein, tumor necrotic alpha, interleukin-2, alanine transaminase, aspartate transaminase, gamma glutamine transferase (GGT). Total protein was determined using enzymatic colorimetric method of Weichselbaum, (1946). The bromocresol green method of Doumas and Watson, (1971) was used for spectrophotometric determination of albumin. Urea was determined by the method of Hallet and Cook, (1971). Serum creatinine was determined by method of Bonsnes and Taussky, (1945). Ray BioR Rat TNF-alpha Elisa Kit and RayBioR RatIL-2 Elisa kit were used for determination of concentrations of tumour necrotic factor-alpha and interleukin-2 and were products of RayBiotech Incorporation, Italy. Gamma glutamyl transferase (γ-GT) was determined using enzymatic colorimetric method according to Bergmeyer et. al., (1986).

2.7 Statistical Analysis

Quantitative data are described as mean ±SD. Pairwise comparisons were used to determine statistical difference between the groups. A value of $p \leq 0.05$ was considered to be statistically significant.

3.0 Results

3.1 Table I: Effects of acetaminophen and aqueous extract of tapinanthus globiferus on serum urea and creatinine concentrations in white albino rats.

Parameters	Control	2.30mg/kg (ACT)	2.80mg/kg (ACT)	2.80mk/kg ACT + 0.45mg/ml of T. globiferus
Urea	80.05±13.22	88.22±21.22	98.67±12.32	82.42±6.69
Creatinine	1.12±0.12	1.28±0.30	1.35±0.33	0.78±0.22

Administration of different doses of acetaminophen caused significant ($p \leq 0.05$) increase in serum concentrations of urea and creatinine when compared with corresponding concentration in control group. Administration of 0.45mg/ml of tapinanthus globiferus moderate effects of acetaminophen by reducing serum concentrations of urea and creatinine

3.2 Table 2: Effects of acetaminophen and aqueous extract of T. globiferus on serum total proteins and albumin in rats.

Parameters	Control	2.30mg/kg (ACT)	2.80mg/kg (ACT)	2.80mk/kg ACT + 0.45mg/ml of T. globiferus
Total proteins (g/dl)	14.62±8.77	6.45±1.77	5.49±2.88	11.13±3.03
Albumin (g/dl)	8.56±3.39	3.22±1.22	2.67±1.32	7.15±2.16

Serum concentrations of total proteins, and albumin were significantly ($p \leq 0.05$) reduced in groups given different doses of acetaminophen when compared with corresponding concentrations in control

group, however, the extent of reductions were more in group given 2.8mg/kg bw when compared with group given 2.3mg/kg bw. Administration of 0.45mg/ml of aqueous extract of tapinanthus globiferus five minutes after administration of 2.8mg/kg bw of acetaminophen caused elevations in serum concentrations of albumin and total proteins when compared with groups given acetaminophen alone.

3.3 Table 3: Effects of acetaminophen and aqueous extract of T. globiferus on markers of inflammatory reactions (TNF- IL-2) in rats.

Parameters	Control	2.30mg/kg (ACT)	2.80mg/kg (ACT)	2.80mg/kg ACT + 0.45mg/ml T. globiferus
TNF-alpha(pg/ml)	879.75±766.13	1204.76±1104.22	1245.88±1132.53	142.10±134.65
Interleukin-2 (µmol/l)	799.11±708.23	956.23±709.66	1120.89±1105.41	76.60±15.22

Concentrations of tumour necrotic factor-alpha and interleukin-2 (markers of inflammation) were significantly increased ($p \leq 0.05$) in groups given different doses of acetaminophen when compared with corresponding concentrations in control group. The extent of elevation in concentration of TNF-α and IL-2 were more in group given 2.8mg/kg b when compared with group given 2.3mg/kg bw of acetaminophen. Administration of 0.45mg/ml of tapinanthus globiferus five minutes after administration of 2.8mg/kg bw of acetaminophen caused significant ($p \leq 0.05$) reduction in inflammatory reactions. This is shown by marked reductions in the serum concentrations of tumour necrotic factor-alpha and interleukin-2 in group given tapinanthus globiferus when compared with group given different doses of acetaminophen alone.

3.4 Table 4: Effects of acetaminophen and aqueous extract of T. globiferus on markers of lipid peroxidation (malondialdehyde) in rats.

Parameters	Control	2.30mg/kg (ACT)	2.80mg/kg (ACT)	2.80mg/kg ACT + 0.45mg/ml T. globiferus
MDA (µmol/gram tissue)	1346.46±89.88	19454.65±99.97	21311.48±101.23	1287.74±98.77

Administration of different doses of acetaminophen induced lipid peroxidation as shown by significant ($p \geq 0.05$) elevation of markers of lipid peroxidation i.e. malondialdehyde when compared with concentrations in control group. Lipid peroxidation was reduced in group given 0.45mg/ml bw of aqueous extract of tapinanthus globiferus when compared with group given 2.3mg/kg and 2.8mg/kg bw of acetaminophen alone.

3.5 Table 5: Effects of acetaminophen and aqueous extract of T. globiferus on activities of gamma glutamyl transferase in rats.

Parameters	Control	2.30mg/kg (ACT)	2.80mg/kg (ACT)	2.80mg/kg ACT + 0.45mg/ml T. globiferus
Gamma glutamyl transferase (iu/l)	0.29±0.12	0.42±0.16	0.49±0.22	0.34±0.11

Activity of gamma glutamyl transferase was elevated by administrations of 2.7mg/kg and 3.5mg/kg of acetaminophen when compared with control and group given tapinanthus globiferus. Activity of gamma glutamyl transferase was reduced by administration of tapinanthus globiferus as compared with activities in groups given 2.7mg/kg and 3.5mg/kg of acetaminophen.

4.0 DISCUSSION

In the present study, administration of APAP induced nephrotoxicity manifested biochemically by a significant increase in serum urea and creatinine and by a significant decrease in total proteins and albumin concentrations after 14 days. Furthermore, this result was supported by significant increase in malondialdehyde concentrations, a marker of peroxidation of cellular lipids, in renal cells after administration of APAP. This was further supported by increased activities of gamma glutamyl transferase in the rats given different doses of APAP alone. These results were consistent with the reports of previous investigators such as Palani et al., 2010; Boutis and Shannon, 2001) who reported APAP-induced nephrotoxicity. Administration of aqueous extract of *T. globiferus* 5 minutes after ingestion of 3.5mg/kg of APAP protected the kidney from damage induced by APAP. This was clearly shown by reductions in the

serum concentrations of urea and creatinine, and by increase in the concentrations of total proteins and albumin in the group given 0.45mg/ml of *T. globiferus*. Furthermore, administration of *T. globiferus* reduced the extent of lipid peroxidation as shown by reduction in concentration of malondialdehyde in the renal tissues.

Increased concentrations of malondialdehyde in the renal cells further supported the believe that APAP induced oxidative stress due to generation of free radicals leading to peroxidation of cellular lipids. Several reports have proposed that mechanism by which APAP induced nephrotoxicity may involve oxidative stress. Thus, the findings in this study were consistent with previous investigators such as Palani et. al., 2010; Khandkar, et. al., 1996. Reduction in the concentration of malondialdehyde by *T. globiferus* may be an indication that it possesses anti-oxidative property. Blood urea nitrogen in the liver protein that is derived from diet or tissue sources is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne, 1994). Elevation of urea and creatinine levels in the serum is an index of nephrotoxicity (Anwar, et. al., 1999). In the present study, administration of APAP induced oxidative stress in renal tissues as shown by elevated concentration renal tissue malondialdehyde. Here, APAP-induced nephrotoxicity showed a significant ($p<0.05$) increase in the serum urea and creatinine concentrations in rats given different doses of APAP when compared with control. However, oral administration of 0.45mg/ml of *T. globiferus* caused significant ($p<0.05$) decrease in the serum concentrations of urea and creatinine when compared with groups given only APAP. Oxidative stress and lipid peroxidation have been proposed to be early events related to radicals generated during hepatotoxicity (Palani, et. al., 2010). Furthermore, generation of reactive oxygen species had been proposed as a mechanism by which many chemicals induced nephrotoxicity (Somani, et. al.,). Studies have shown that administration of acute overdose of APAP induced lipid peroxidation and repress antioxidant defense system of the renal cells (Abdel, et. al., 2007: Ghosh and Sil, 2007). This is consistent with the findings in this study where administration of different doses of APAP induced oxidative stress and lipid peroxidation as indicated by increased concentrations of malondialdehyde. Increased activities of gamma glutamyl transferase alongside elevated concentrations in markers of oxidative stress i.e. malondialdehyde, may indicate association between gamma glutamyl transferase and oxidative stress. A series of epidemiological studies have suggested serum gamma glutamyl transferase within its normal range might be an early marker of oxidative stress (Duk et. al., 2004). However, administration of *T. globiferus* significantly ($p<0.05$) reduced oxidative stress and the peroxidation of tissue lipids as indicated by reduction in concentration of malondialdehyde when compared with groups given APAP alone. APAP induced inflammatory reactions as shown by significant elevation of concentrations of tumour necrotic factor alpha and interleukin-2. However, administration of *T. globiferus* reduced inflammatory responses as indicated by reduction in concentrations of the markers of inflammatory responses. Renal disease is associated with a graded increase in oxidative stress markers even in early chronic kidney disease. This could be consequence of an increase in reactive oxygen species as well as a decrease in antioxidant defence. This oxidative stress can accelerate renal injury progression. Inflammatory markers increase with renal function deterioration suggesting that kidney disease is a low-grade and infact facilitator of inflammatory process. Administration of *T. globiferus* reduced inflammatory processes thus preventing deterioration of the kidney cells.

In this study, *T. globiferus* exhibited nephroprotective, antiinflammatory and antioxidative properties. Adekunle Adeniran Sanmi, Afolabi Olusegun Kayode, Oyewo Bukoye Emmanuel

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