# Increased Inflammatory Activities and Oxidative Stress in Hepatotoxicity due to Acetaminophen Administration: Ameliorative Effects of *Tapinanthus Globiferus.*

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**ABSTRACT:** Acetaminophen (APAP) overdose has been reported to cause severe liver damage. This study was therefore designed to access the modulatory effect of *Tapinanthus globiferus* on possible side effects due to acetaminophen overdose. APAP induced hepatotoxicity which was indicated by elevated gamma glutamyl transferase (GGT) and aspartate aminotransferase activities (AST); reduced serum total proteins and albumin; elevated tumour necrotic factor-alpha (TNF-α) and interleukin-2 (IL-2); and increased lipid peroxidation as evidenced by elevated malondialdehyde in hepatic tissues. However, administration of T. globiferus caused reduction in activities of GGT and AST; hepatocyte malondialdehyde, TNF-α and IL-2 concentrations and increased total proteins and albumin when compared with groups given only APAP. Determination of biocomposition showed APAP contain high concentration of phenolic contents. The observation implied that T. globiferus possess antioxidative and anti-inflammatory activities and its administration may reduce damages due to acetaminophen administrations in hepatocytes.

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**KEY WORDS**: Acetaminophen, Hepatotoxicity, tapinanthus globiferus, inflammation, oxidative stress.

## 1. INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol (APAP)) is a very effective analgesic and antipyretic agent. It is also a very safe drug as long as the recommended dosage is not exceeded. However, when the therapeutic range is exceeded, it becomes toxic. Its poisoning is one of the most common causes of poisoning worldwide and this may lead to acute liver failure, non specific symptoms such as abdominal pain and nausea (Larson et al., 2005; Ryder and Beckingham, 2001).

More than 200 million persons take APAP each year and of these, about 200 persons (approximately half of which is intentional) die of hepatic failure from APAP overdosage yearly (Park, 2006; Rowden et al, 2006). The severity of the overdosage problem stems from the fact that APAP is an extremely common medication often used casually. It is found in many combination drugs for cough and cold remedies and also in opioid medications for severe pain.

Controversial reports have been received regarding possibilities of APAP causing liver injury at therapeutic dose. In the 1970's, studies were published with reports describing the occurrence of chronic liver disease that was associated with the long term use of APAP in recommended doses. Today, series of reports have described occurrence of significant liver damage due to be very rare or that does not occur when the patient's liver is normal (Park, 2006; Rowden et al, 2006).

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). Medicinal plants have been recipes for the treatment of common ailments which are reported almost on daily basis such as hypertension, dysentery, low sperm count, and weak erection, coated tongue, pile, menstrual disorder, leucorrhoea and fevers. Although orthodox medicine has improved worldwide, however, it is noteworthy that modern medicine compliment traditional practices in industrialize societies such as China and India (Odugbemi, 2006). This study is therefore designed to assess hepatotoxic effects of administrations of APAP and the ability of aqueous extract of *T. globiferus in* attenuating these drug induced effects using experimental animals model.

## 2. METHODOLOGY

### 2.1 Experimental animals.

Sixteen 14-week old albino rats with an average weight of 170g 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 condusive atmospheric pressure and temperature. The animals

were separated into 4 groups with each group having four rats. The rats had access to feed and pipe-borne water. All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals.

**2.2 Plant Materials and Extract Preparation**

Leaves of *T. 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. The plant with voucher number LHO 214 was deported at the Department of Pure and appliedBiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. In this environment, *T. globiferus* concoction for drinking is prepared by manual maceration of the wet leaves in cold water until the fluid in the leaves is completely extracted. This traditional method was adopted in this study. As maceration continues, the green liquid formed was continually removed into another clean bowl and replaced with distilled water until when the maceration of the leaves remnant can no longer produce green-coloured liquid. To achieve this, a volume of 1500ml of distilled water was used to macerate 672.6g of the wet leaves.

**2.3 Acetaminophen**

Commercial paracetamol (500mg) soluble tablets were obtained from Jopats Pharmacy, Ogbomoso. An oral method of administration was adopted.

**2.4 Experimental design and administration of acetaminophen and tapinanthus globiferus.**

Group 1 consisted of 4 rats that were not given *T. globiferus*, and APAP (control). Group 2 consisted of 4 rats that were given 2.3mg/kg body weight of APAP 4 times per day to serve as group with normal dose. Group 3 consisted of 4 rats given 2.8mg/kg body weight of APAP 4 times per day to serve as overdose. Group 4 consisted of 4 rats given of 2.8mg/kg bw of APAP and 0.45mg/ml of *T. globiferus* 5 minutes later. All administrations were given 4 times (4-hour interval) daily for fourteen (14) days.

**2.5 Sample collection**

On the 15$^{th}$ day, the rats were sacrificed and blood samples obtained through cardiac puncture. The blood was collected into appropriately labeled sample bottles and centrifuged at 4000rev/sec for 5 minutes to obtain serum. The supernatants were decanted and stored at -2$^{0C}$ for analyses of biochemical parameters. The liver was quickly removed, washed with washing buffer and homogenized in phosphate buffer (pH 7.4). The homogenate was kept frozen for analysis.

**2.6 Determination of biochemical parameters**

The biochemical parameters determined included total cholesterol, triglyceride, high density lipoprotein, albumin, urea, total protein, tumor necrotic factor-alpha, interleukin-2, aspartate aminotransferase, gamma glutamine transferase (GGT).

**2.6.1 Determination of total cholesterol**

Total cholesterol was determined using enzymatic method described by Allain et al., 1974.

**2.6.2 Determination of triglycerides**

Triglyceride was determined using enzymatic method described by Buccolo and David, 1973.

**2.6.3 Determination of high density lipoprotein.**

The precipitation method by Assmann et al., 1983 and total cholesterol determination method by Allain, et al., 1974 were used to determine HDL-cholesterol.

**2.6.4 Determination of total protein**

Total protein was determined using enzymatic colorimetric method and measurement was done at 540nm.

**2.6.5 Determination of Albumin**

The bromocresol green method of Doumas and Watson, 1971 was used for determination of albumin.

**2.6.6 Determination of urea**

Urea in the sample was determined by the method described by Orsonneau et al., 1982.

**2.6.7 Determination of GGT**

Gamma glutamyl transferase (γ-GT) was determined using enzymatic colorimetric method measured at 405nm and matched cuvette 1.0cm light path.

**2.6.8 Determination of aspartate aminotransferase**

The method of Bergmeyer et al., 1985 based on IFCC recommendation was used to determine activities of aspartate aminotransferase.

**2.6.9 Determination of Tumour necrotic factor-alpha and Interleukin-2**

Ray BioR Rat TNF-alpha Elisa Kit and RayBioR RatIL-2 Elisa kit used for determining concentrations of tumour necrotic factor-alpha and interleukin-2 were purchased from RayBiotech Incorporation.

**2.6.1.0 Determination of total phenolic content**

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent according to the method of Singleton *et al*., (1999). Briefly, the plant extract (0.1 mL) was mixed with 0.75 mL of FC reagent (previously diluted 1000-fold with distilled water) and incubated for 5 min at 22°C, then 0.06% Na2CO3

solution was added. After incubation at 22°C for 90 min, the absorbance was measured at 725 nm. The mean of three readings was used and the total phenol content was expressed in milligram of gallic acid equivalents/g extract.

**2.7 Statistical Analysis.**

Values were expressed as mean ± SEM (standard error of mean). Statistical analyses were performed by one way ANOVA, followed by Duncan's multiple range tests. The results were considered statistically significant for $p < 0.05$.

**3.0 Results**

**3.1 Table I: Effects of APAP and aqueous extract of *T. globiferus* on lipid profile in white albino rats.**

| Parameters | Control | 2.30mg/kg (APAP) | 2.80mg/kg (APAP) | 2.80mg/kg(APAP) and .045mg/ml of *T. globiferus* |
|---|---|---|---|---|
| T.cholesterol(mg/dl) | 69.03± 23.59[b] | 230.13±47.01[a] | 230.59±48.48[a] | 203.43±26.31[a] |
| Triglyceride(mg/dl) | 89.91±24.76[b] | 423.84±379.18[a] | 193.84±39.99[a] | 243.08±125.18[a] |
| HDL-C (mg/dl) | 221.70±190.17[b] | 130.20±20.70[a] | 133.78±45.91[a] | 145.64±43.49[a] |

Results are expressed as mean ±SD. Mean that does not share the same letters in the same line are significantly different (Duncan test at $p \leq 0.05$).

Administration of different doses of APAP caused significant ($p \leq 0.05$) elevations in serum total cholesterol and triglyceride when compared with concentrations of corresponding parameters in control rats; however, administration of *T. globiferus* alongside APAP in group 4 caused a reduction in the effects of APAP. Administration of different doses of APAP caused significant reduction ($p \leq 0.05$) in the serum concentrations of HDL-C when compared with control group, however, this effect was reduced in geoup 4 when *T. globiferus* was administered alongside APAP.

**3.2 Table 2: Effects of APAP and aqueous extract of *T. globiferus* on liver marker enzymes in white albino rats.**

| Parameters | Control | 2.30mg/kg (APAP) | 2.80mg/kg (APAP) | 2.80mg/kg(APAP) and .045mg/ml of *T. globiferus* |
|---|---|---|---|---|
| AST (iu/l) | 131.25±17.75[a] | 138.66±5.69[a] | 146.50±6.56[a] | 130.33±6.51[a] |
| GGT (iu/l) | 0.35±0.12[a] | 0.40±0.23[b] | 0.46±0.27[b] | 0.31±0.15[a] |

Results are expressed as mean ±SD. Mean that does not share the same letters in the same line are significantly different (Duncan test at $p \leq 0.05$).

Administration of different doses of APAP caused dose-dependent elevation in the activities of AST and GGT when compared with control group. Administration of *T. globiferus* 5 minutes after administration of APAP in group 4 reduced the extent of effects of APAP on enzyme activities.

**3.3 Table 3: Effects of APAP and aqueous extract of *T. globiferus* on serum total protein, albumin and urea in white albino rats.**

| Parameters | Control | 2.30mg/kg (APAP) | 2.80mg/kg (APAP) | 2.80mg/kg(APAP) and .045mg/ml of *T. globiferus* |
|---|---|---|---|---|
| Total proteins (g/dl) | 12.82±9.03[a] | 11.79±1.23[a] | 11.36±2.37[a] | 12.41±5.03[a] |
| Albumin (g/dl) | 7.37±15.83[a] | 5.00±1.88[b] | 4.85±1.14[b] | 6.94±425[a] |
| Urea (mmol/dl) | 204.11±15.62[b] | 87.15±22.53[a] | 96.66±15.27[a] | 77.05±7.39[a] |

Results are expressed as mean ±SD. Mean that does not share the same letters in the same line are significantly different (Duncan test at $p \leq 0.05$).

Serum concentrations of total proteins, albumin and urea were reduced in groups given different doses of APAP when compared with corresponding concentrations in control group. Administration of *T. globiferus* 5 minutes after administration of APAP in group 4 caused reduction in the effects of APAP on total proteins, albumin and urea.

**3.4 Table 4: Effects of APAP and aqueous extract of *T. globiferus* on serum inflammatory reaction.**

| Parameters | Control | 2.30mg/kg | 2.80mg/kg | 2.80mg/kg(APAP) |
|---|---|---|---|---|

| | | (APAP) | (APAP) | and .045mg/ml of *T. globiferus* |
|---|---|---|---|---|
| **TNF-alpha(pg/ml)** | **805.66±633.99[c]** | **1178.87±1262.33[b]** | **862.08±1249.43[b]** | **120.03±155.94[a]** |
| **Interleukin-2 (µmol/l)** | **807.17±695.54[c]** | **944.54±838.58[b]** | **613.67±941.32[b]** | **51.50±22.39[a]** |

Results are expressed as mean ±SD. Mean that does not share the same letters in the same line are significantly different (Duncan test at p≤ 0.05).

Concentrations of TNF-α and IL-2 (markers of inflammation) were significantly increased (p≤0.05) in groups given different doses of APAP when compared with corresponding concentrations in control group. Administration of 0.45mg/ml of *T. globiferus* 5 minutes after administration of 2.8mg/kg of APAP in group 4 caused significant (p≤0.05) reduction in concentrations of TNF-α and IL-2.

**3.5 Table 5: Effects of APAP and *T.globiferus* on markers of oxidative stress.**

| **Parameters** | **Control** | **2.30mg/kg (APAP)** | **2.80mg/kg (APAP)** | **2.80mg/kg(APAP) and .045mg/ml of *T. globiferus*** |
|---|---|---|---|---|
| **MDA (µmol/gram tissue)** | **7151.11±607.12[b]** | **11879.17±125.33[b]** | **33717.45±833.34[b]** | **3912.23±131.05[a]** |

Results are expressed as mean ±SD. Mean that does not share the same letters in the same line are significantly different (Duncan test at p≤ 0.05).

Administration of different doses of APAP induced lipid peroxidation as shown by significant (p≥0.05) elevation of malondialdehyde when compared with concentrations in control group. Administration of *T. globiferus* alongside APAP caused reduction in concentrations of malondialdehyde.

### 3.6 Amount of total phenolic compound

The amount of total phenolic of extract of leaves of *T. globiferus* estimated by Folin-Ciocalteau method was 3.22 ± 1.14 mgGAE/g of dry extract.

## 4.0 DISCUSSION

Acetaminophen toxicity like many other conditions is widely believed to be involved in hepatotoxicity and further studies have shown that phytochemical products including plant herbs and extracts have been used for centuries to modulate liver damage. Although the exact mechanism behind this protection is uncertain, many theories have been proposed (Suresh et al., 2006). APAP is being used extensively to investigate hepatoprotective activity of different treatments on various experimental animals. It is selected as hepatotoxicant in inducing injury to the liver as is it known to cause hepatotoxicity in experimental animals when taken overdose (Ahmed and Khater, 2001).

In this study, administration of APAP induced elevations in serum concentrations of total cholesterol and triglyceride but caused reduction in serum concentration of high density lipoprotein cholesterol. This may be suggestive of atherogenic tendency of the drug in these rats.

Increased concentrations of total cholesterol and triglyceride coupled with reduction in HDL-C concentration are predictive indices of atherogenicity. The HDL-C mediates transportation of cholesterol from extra-hepatic tissues to the liver for excretion as bile. However, in rats given *T. globiferus* 5 minutes after administration of APAP, there was reduction in effects of APAP as indicated by reduction in concentrations of total cholesterol and triglyceride and increase in HDL-C concentration when compared with group given APAP alone. This may be suggestive of chemoprotective property of *T. globiferus* against atherogenicity. The mechanism for this may not be well understood, but it may be suggestive that *T. globiferus* induce "mopping" up of cholesterol via reverse transportation by HDL-C for excretion as bile.

Administration of APAP induced hepatotoxic effect in the rats as shown by the results of the study. Administration of APAP induced increased activities of AST and GGT, strong enzymic markers of hepatotoxicity. This was coupled with decrease in serum concentrations of albumin and total protein when compared with corresponding values in control group. Increased activity of gamma glutamyl transferase coupled with reduction in concentrations of total proteins and albumin are indices of hepatotoxicity. Administration of *T. globiferus* alongside APAP in group 4 reduced the side effects as compared with groups 2 and 3, a demonstration of protective effect of the plant against hepatotoxicity. Gamma glutamyl transferase is an intracellular enzyme with the plasma having a reference range. Damage to the host cell may cause leakage of the enzyme thereby leading to its increased activity in the plasma. Furthermore, damage to the liver may affect

its functions one of which is its ability to synthesize protein. The side effect of APAP on the liver may have been shown by the reduced concentrations of total protein and albumin.

The reduced concentration of serum urea in group given APAP may be connected to reduced serum concentrations of protein within the same group. Urea cycle is strongly linked to protein metabolism. This further supported hypothesis that APAP may have hepatotoxic effect. However, administration of aqueous extract of *T. globiferus* ameliorates this effect.

This study equally showed that APAP induced increased inflammation as evidenced by increased in markers of inflammatory reactions including TNF-α and IL-2. This inflammation induces acute-phase responses characterized by changes in the concentrations of several plasma proteins secondary to altered hepatic transcription and synthesis of excretory proteins. Among the negative acute-phase proteins, of which the synthesis and secretion are decreased, are albumin, transferin and transthyretin (Trey and Kushner, 1995). The results of this study equally showed decreased concentrations of albumin and total protein in group given APAP alone when compared with control animals. However, administration of *T. globiferus* ameliorated the effect. This is inversely proportional to the level of the cytokines which are observed to be higher in group given APAP compared with control.

These results also showed positive association between oxidative stress as implicated by elevated malondialdehyde concentrations and imflammatory responses as indicated by elevated concentrations of TNF-alpha and IL-2 in groups given different doses of APAP. Oxidative stress is one of the several risk factors involved in triggering the inflammatory process. This observation may be an indication that APAP triggers inflammatory responses mediated through TNF-alpha and IL-2 by inducing oxidative stress. This may account for increased lipid peroxidation as marked by elevation of malondialdehyde in groups given different doses of APAP as compared with control group. The pattern, that is elevated markers of oxidative stress and imflammatory responses are consistent with risk factors predisposing to cardiovascular disorders. Alteration in redox status triggers oxidative stress which consequently increased inflammatory activities in the liver. Akbulut, et, al., (2005) showed correlation between renal tissue oxidative stress parameters and TNF-alpha levels in an experimental model of ischemia-reperfusion injury in mice.

The group given *T. globiferus* with APAP showed a better redox status as shown by reduced level of lipid peroxidation (low concentration of malondialdehyde) and reduced inflammatory processes. This further express that the plant *T. globiferus* may possess antioxidative and anti-inflammatory properties. The ability of aqueous extract of *T. globiferus* to reduce inflammation and lipid peroxidation due to APAP may be attributed to the presence of high content of phenolics which are well recognized as potential antioxidants and free radical scavengers and inhibit lipid peroxidation via the scavenging of radicals and metal chelation (Dorman *et al*., 2003; Majid *et al*., 2004). Phenolic compounds are also very important plant constituents because their hydroxyl group confers scavenging ability (Wattenberg *et al*., 1989; Zheng *et al*., 1992; Dorman *et al*., 2003).

On the basis of our results, it is revealed that *T. globiferus* extract possess free radical scavenging activity which could exert a beneficial action against liver damage induced by APAP. Since the preliminary phytochemical analysis of the extract shows the known presence of phenolic contents, we can suggest that it may be responsible for the observed effects. In conclusion, aqueous extract of *T. globiferus* exhibited a liver protective effect against APAP induced hepatotoxicity and possessed anti-lipid peroxidative and free radical scavenging activities

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