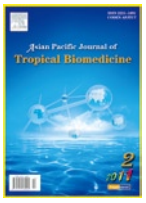
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



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The protective role of *Gongronema latifolium* in acetaminophen induced hepatic toxicity in Wistar rats

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ARTICLE INFO

Article history:
Received 5 August 2011
Received in revised form 8 September 2011
Accepted 2 October 2011
Available online 15 October 2011

Keywords:
Acetaminophen
Caffeine
AST
ALT
ALP
Protein
Gongronema latifolium
Hepatic toxicity

ABSTRACT

Objective: To evaluate the protective effect of leaf extract of *Gongronema latifolium* (*G. latifolium*) against acute acetaminophen induced hepatic toxicity in Wistar rats. **Methods:** Thirty six Wistar rats were divided into 6 groups with 6 rats in each group. Animals in group 1 and 2 were administered with 600 mg/kg b.w. of acetaminophen only and acetaminophen plus 100 mg/kg b.w. of caffeine by oral gavages, respectively. Experimental groups 3 and 4 were treated as in group 1 but in addition received 200 and 400 mg/kg b.w., respectively of the leaf extract of *G. latifolium* by oral gavages. The experimental groups 5 and 6 were treated as in group 2 and in addition received 200 and 400 mg/kg b.w. of leaf extract of *G. latifolium*, respectively. The treatment lasted for 14 days. **Results:** The results obtained showed that the serum glutamic-oxalacetic transaminease (AST), glutamic-pyruvic transaminase (ALT) and alkaline phosphatase (ALP) levels had a greater increase in group 2 than in group 1 but dropped marginally in groups 3 and 4. However, in groups 5 and 6, AST, ALT and ALP were significantly reduced ($P<0.05$). Similarly, serum protein levels were significantly increased in groups 3, 4, 5 and 6 when compared with group 1 and 2. **Conclusions:** It can be concluded that extract of *G. latifolium* offers protection against acetaminophen and caffeinated acetaminophen toxicity in Wistar rats.

1. Introduction

Acetaminophen or paracetamol is non-narcotic analgesic and antipyretic[1]. It is as potent as aspirin especially in the central nervous system. Acetaminophen is normally well tolerated, side effects and interactions with other drugs are usually rare in a normal dosage. However, over dosage and prolonged use of acetaminophen are known to cause liver impairment[2]. The overdose of acetaminophen due to prescription is not common, though the case of acquisition of acetaminophen over the counter for self medication without prescription has led to damage to liver[3]. It has come to our knowledge that the combination of acetaminophen with caffeine preparations is now available with various trade names such as, Boska, Pentax, Caffeinated Paracetamol, *etc*. The administration of acetaminophen and caffeine has been shown to cause more inducement in the hepatic toxicity[2]. The first reports of acetaminophen poisoning in humans describing hepatic necrosis provoked a series of animal studies which demonstrated that acute centrilobular hepatic necrosis with collapse of the reticulum frame work could be produced in some species[4].

Lipid peroxidation in which malondialdehyde is generated has been linked to the impairment of kidney and liver[5] and antioxidants have been reported to play prominent beneficial roles in the prevention of lipid peroxidation and generation of free radicals[6]. Many minor components of foods, such as minerals and antioxidant vitamins have been shown to reduce the risk of chronic diseases[7]. The antioxidant vitamins A, C and E have been linked to play a major role in the protection against diabetes[8]. These antioxidants are mostly found in edible vegetables, fruits and other herbal plants particularly *Gongrenema latifolum* (*G. latifolum*)[9-13]. *G. latifolum* (Asclepiadaceae) is a herbaceous shrub with yellow flowers and the stem yields milky exudates when cut[14-17]. It is locally called "Utazi" by Igbos and "Arokeke" by the Yorubas in Nigeria. The Igbos in the South Eastern Nigeria use the *G. latifolium* crude leaf extract in the treatment of diabetes, malaria,

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hypertension and as laxative in folk medicine. It is also used as spices and vegetable[10].

Scientific studies have established the hypoglycaemic, hypolipidaemic and antioxidant effects of extract of *G. latifolium*[9,14]. The ease with which acetaminophen is bought from the market in Owerri for self medication with the possibility of over dosage and prolonged use warrants research into the possible therapeutic intervention. Hence, in this study, the protective effect of *G. latifolium* of leaf extract against acute acetaminophen overdose was investigated.

2. Materials and methods

2.1. Drug

Acetaminophen was purchased from a standard pharmacy shop in Owerri Imo State. The tablets were dissolved in distilled water according to the requirement for administration to the Wistar rats on basis of their body weight.

2.2. Plant materials and extraction

The *G. latifolium* leaves were obtained from Ekeonunwa market in Owerri Nigeria. The botanical identification and authentication was confirmed by Dr. C Okere (Head of Department of Plant Science and Biotechnology, Imo State University,Owerri). The plant material was sun dried for seven days. The dried leaves of *G. latifolium* were milled to get a fine powder. The appropriate concentrations of the extract were made in distilled water for the experiment. Hence, the following concentrations: 200 and 400 mg were prepared.

2.3. Experimental animals

Wistar albino rats weighing (160-220 g), aged 8-10 weeks were used in the study. These animals were obtained from the Animal House of College of Medicine and Health Science, Imo State University, Owerri Nigeria. They were kept under standard laboratory conditions, and fed with commercial growers mash, product of Tops Feeds Ltd, Sapele Nigeria. Water and feed were provided *ad libitum*. The animals were left for two weeks to acclimatize and then divided into groups for experimentation.

2.4. Experimental design

The animals were randomly assigned to 6 groups with 6 rats in each group. Group 1 received acetaminophen only (600 mg/kg b.w.); group 2 received acetaminophen (600 mg/kg b.w.) plus caffeine (100 mg/kg b.w.); group 3 received acetaminophen (600 mg/kg b.w.) and 200 mg/kg of *G. latifolium* extract; group 4 received acetaminophen (600 mg/kg b.w.) plus 400 mg/kg of *G. latifolium* extract; group 5 received acetaminophen (600 mg/kg b.w.) plus caffeine (100 mg/kg b.w.) and 200 mg/kg of *G. latifolium* extract; group 6 received acetaminophen (600 mg /kg b.w.) plus caffeine (100 mg/kg b.w.) and 400 mg/kg of *G. latifolium* extract.

In all groups the drug was administered through oral route using a feeding tube attached to a 5 mL syringe. All animals were allowed free access to food and water throughout the experiment which lasted for 14 days.

2.5. Blood collection

Twenty four hours after the last dose was administered, the animals were anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into dry test tubes. The blood was allowed to stand for about 15 minutes to clot and further spun in a westerfuge centrifuge (Model 11384) at 10 000 g for 5 minutes. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of the biochemical parameters.

2.6. Biochemical analysis

The serum glutamic-oxalacetic transaminease (AST) and glutamic-pyruvic transaminase (ALT) were assayed by the method of Reitman and Frankel[15]. Alkaline phosphatase (ALP) was determined by the method of King and King[19].

2.7. Statistical analysis

The result were expressed as mean ± standard deviation. The statistical evaluation of data was performed by using one-way ANOVA (analysis of variance) followed by Duncan's Multiple range test[20]. $P<0.05$ was considered significant.

3. Results

The liver enzymes and total protein in serum were

Table 1
Liver enzyme activities and serum protein levels in the experimental rats.

Treatments	AST (μ/L)	ALT (μ/L)	ALP (μ/L)	Protein (g/100 mL)
Acetaminophen only	19.12±0.67	26.13±0.91	20.10±2.00	86.71±0.94
Acetaminophen + caffeine	28.32±1.90	30.96±1.21	23.00±1.60	85.20±1.22
Acetaminophen + 200 mg/kg *G. latifolium*	18.94±0.60	13.14±2.64	20.40±2.20	91.92±1.40
Acetaminophen + 400 mg/kg *G. latifolium*	20.11±1.90	3.00±2.02	19.90±2.00	91.30±1.00
Acetaminophen + caffeine + 200 mg/kg *G. latifolium*	22.41±2.18	18.51±0.62	20.30±1.50	94.62±2.81
Acetaminophen + caffeine + 400 mg/kg *G. latifolium*	20.10±1.30	18.93±0.84	20.00±2.20	91.40±1.94

presented in Table 1. The concentration of AST in group 2 (28.32±1.90 μ/L) treated with acetaminophen and caffeine was significantly higher (P<0.05) than group 1 (19.12±0.67 μ/L) which received acetaminophen only. The change in serum AST concentration observed in groups 3 and 4 treated with acetaminophen plus 200 and 400 mg/kg of *G. latifolium*, respectively showed significant effect of the extract. The concentrations of AST in group 5 treated with acetaminophen, caffeine and 200 mg/kg of *G. latifolium* (22.41±2.18 μ/L) and in group 6 treated with acetaminophen plus caffeine and 400 mg/kg of *G. latifolium* (20.10±1.30 μ/L) were significant lower than that of group 2 treated with acetaminophen and caffeine.

The ALT activity in group 2 (30.96±1.21 μ/L) treated with acetaminophen plus caffeine was higher than that of group 1 (26.13±0.91 μ/L) treated with acetoaminophen alone. The levels of ALT in groups 3 and 4, treated with acetaminophen plus 200 and 400 mg/kg of *G. latifolium*, respectively were significantly reduced than that of group 1 treated with only acetaminophen (P<0.05). In the same vein, the total serum proteins in groups 5 and 6 treated with acetaminophen plus caffeine and *G. latifolium* extract were (94.62±2.81 g/100 mL) and (91.40±1.94 g/100 mL), respectively when compared with groups 1 and 2 (86.71±0.94 g/100 mL and 85.20±1.22 g/100 mL), respectively. The serum ALP level followed the same trend as in ALT.

4. Discussion

The commonest enzymes in the diagnosis of hepatocellular damage are the transaminase enzymes which include AST, ALT and ALP[5,20]. The impairment in the liver results in their increased activities. The increases in serum enzyme activities are roughly proportional to the extent of tissue damage[4,21,22]. Acetaminophen toxicity like many other disease conditions is widely believed to involve the generation of reactive oxygen species (ROS) oxidative stress resulting from the acetaminophen toxicity which plays an important role in the liver damage. Free radicals induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological conditions[23,24]. Antioxidants have been linked with the prevention of ROS[25-27] and hence offer protection against acetaminophen toxicity[2]. This leads to the evaluation of medicinal plants with free radical scavenging potentials for protective roles against drug induced toxicity. Therefore, *G. latifolium* has been reported to have antioxidant effect[9].

In this study, serum enzyme activities such as AST, ALT, and ALP were increased following acetaminophen and caffeine administration.

The increase in liver enzymes following acetaminophen administration has earlier been reported[2,28]. It has been reported that acetaminophen could be bioactivated enzymatically by cytochrome P4502EI in both liver and kidney. The metabolic activation by reactive intermediate, N-acetyl parabenzoquinoneimine is believed to play an important role in acetaminophen mediated toxicity[29]. The proinflammatory cytokines such as tumor necrosis factor (TNF-a) and interleukin-la, that are released in response to acetaminophen intoxication are thought to be responsible for some pathological manifestations of acetaminophen induced toxicity[2]. However, the simultaneous administration of acetaminophen, caffeine and extract of *G. latifolium* significantly lowered AST, ALT and ALP concentrations when compared with those that received acetaminophen only and acetaminophen and caffeine. This is in line with the work of Etim *et al*[30] and kumarapp *et al*[31]. The mechanism by which *G. latifolium* lowered liver enzymes may be attributed to their ability to maintain liver cell integrity[32-35]. In the same vein, acetaminophen administration produced a decrease in total protein while on the coadministration with *G. latifolium* results in an increase in total protein. This is in line with the works of Ekam and Ebong[4,36-39].

The hepatoprotective effect of *G. latifolium* extract was evidenced by the reduction of biochemical indicators of liver impairment caused by acetaminophen induced toxicity. It could be concluded that the leaf extract of *G. latifolium* protects the liver from oxidative damage. Hence, it could be used as an effective protector of acetaminophen induced damage.

Conflict of interest statement

We declare that we have no conflict of interest.

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