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# Acetaminophen Induced Hepatic Toxicity: Protective Role of *Ageratum conyzoides*

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**Abstract:** The preventive potentials of ethanol leaf extract of *Ageratum conyzoides* (ACE) against acute acetaminophen and caffeinated acetaminophen over dose in Wister rats were investigated. Thirty Wister rats of both sexes were divided into 6 groups of 5 rats per group. There were two control groups. Animals in control group 1 were administered 600 mg/kg body weight of acetaminophen intraperitoneally (ip) whereas, animals in control group 2 in addition to acetaminophen were administered 100 mg/kg body weight of caffeine by oral gavage. Experimental groups 3 and 4 were treated with acetaminophen as in group 1 but in addition received 250 and 500 mg/kg body weight, respectively of ACE by oral gavage, The experimental groups 5 and 6 were treated as in control group 2 and in addition received 250 and 500 mg/kg body weight, respectively of ACE. The treatment lasted 14 days. Serum Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALP) levels (U/L) significantly increased ($p<0.001$ and $p<0.01$, respectively) in group 2 than group 1 but dropped marginally in groups 3 and 4. Comparing group 2 with group 5, ALT, AST and Akaline Phospotase (ALP) (U/L) activities reduced significantly ($p<0.01$) in group 5 treated with 250 mg/kg of ACE. Similar significant reductions were observed in group 6 treated with 500 mg/kg of ACE, ALT activity ($p<0.01$), AST and ALP activities ($p<0.001$). Total serum protein level (g/100mL) was marginally increased in group 3 (acetaminophen plus 250 mg/kg ACE) than group 1 (acetaminophen only). Total serum protein was however increased significantly ($p<0.01$) in group 5 (acetaminophen plus caffeine plus 250 mg/kg ACE) and ($p<0.05$) group 6 (acetaminophen plus caffeine plus 500 mg/kg ACE) more than group 2 (acetaminophen plus caffeine). It is concluded from these findings that ACE offered protection against acetaminophen and caffeinated acetaminophen toxicity in rats.

**Key words:** Acetaminophen, caffeine, enzymes, serum protein, rats, *Ageratum conyzoides*

## INTRODUCTION

Paracetamol or acetaminophen is the principal active metabolite of phenacetin, a very popular analgesic and antipyretic drug (Dahlin *et al*., 1984). The drug is as potent as aspirin particularly in the central nervous system. In therapeutic doses, acetaminophen is normally well tolerated, side effects and interaction with other drugs are usually not observed. An overdose of acetaminophen is known to cause liver damage (Boyd and Bereczky, 1966). However, overdose of acetaminophen due to prescription rarely occurs, but its ready availability as well as ease of acquisition over the counter for self medication without prescription has led to some reported cases of toxicity. Acetaminophen has been reported to cause acute centrilobular hepatic necrosis in animals exposed to its overdose (Mitchell *et al*., 1973; Zhang *et al*., 2000). Acetaminophen has a narrow therapeutic index which means that its therapeutic dose is close to the overdose, making it a relatively dangerous substance particularly for people who indulge in self medication. Single dose of 10-15 g can potentially cause major hepatotoxicity in adult human being (Bioka *et al*., 1993).

Combination of acetaminophen with other drugs is gaining popularity, acetaminophen with caffeine preparations are now available with various trade names such as, caffeinated paracetamol, Boska etc. Despite the benefit of rapid analgesic effect derived from this combination and the possible decrease in toxicity, such combinations are still a cause for concern (Laurence *et al*., 1997). Simultaneous administration of caffeine has been shown to potentiate acetaminophen-induced hepatotoxicity in the rat and depletion of hepatic glutathione is more pronounced than with acetaminophen alone (Sato *et al*.,1985; Sato and Izumi, 1989).

Most disease conditions have also been linked to the generation of Reactive Oxygen Species (ROS) (Valko *et al*., 2007) and antioxidants have been reported to play prominent roles in the prevention of ROS generation (Valko *et al*., 2006). Antioxidant vitamins A, C and E are reported to play a role in the protection against cardiovascular and malignant diseases (Haendeler *et al*., 1996). These antioxidants can be obtained over the counter, most importantly, they are readily found in edible vegetables, fruits (Lachance and Langseth, 1994)

and other herbal plants. One of such herbal plants with such great potentials is *Ageratum conyzoides*. This plant is widely utilized in traditional medicine wherever it grows, (Durodola, 1977; Bioka *et al*., 1993). It has been reported to contain biological ingredients that are very important in pharmacological industries (Ming, 1999). Various pharmacological investigations have verified its efficacy as an antibiotic (Durodola, 1977), as analgesic agents in rats (Menut *et al*., 1993) and as a blood booster (Ita *et al*., 2007). Jagetia *et al*. (2003) actually reported that *in vitro, Ageratum conyzoides* Extract (ACE) was found to scavenge 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radicals in a concentration-dependent manner. These authors suggested that radioprotection may be due to the scavenging of reactive oxygen species induced by ionizing radiation.

The ease with which acetaminophen is obtained over the counter for self medication with the possibility of over dose and attendant consequences on health, warrants research into the possible therapeutic intervention procedures. In this study, the preventive potentials of *Ageratum conyzoides* leave Extract (ACE) against acute acetaminophen over-dose was investigated.

## MATERIALS AND METHODS

A total of thirty rats of Wister strain of both sexes weighing 120-200 g were randomly assigned to six groups of five animals each. Acetaminophen was administered intraperitoneally (i.p). Caffeine was administered by oral gavage. Ethanol extract of *Ageratum conyzoides* leaves (ACE) dissolved in 20% tween 80 was also administered by oral gavage.

The animals in their respective groups were treated as follows:

**Group 1:** Acetaminophen only (ip) 600 mg/kg body weight (control 1).

**Group 2:** Acetaminophen 600 mg/kg body weight plus caffeine 100 mg/kg body weight (control 2).

**Group 3:** Acetaminophen 600 mg/kg body weight plus 250 mg/kg of ACE.

**Group 4:** Acetaminophen 600 mg/kg body weight plus 500 mg/kg of ACE.

**Group 5:** Acetaminophen 600 mg/kg body weight plus caffeine 100 mg/kg body weight, plus 250 mg/kg of ACE.

**Group 6:** Acetaminophen 600 mg/kg body weight plus caffeine 100 mg/kg body weight, plus 500 mg/kg of ACE.

All animals were weighed before the commencement of treatment. While, the treatment lasted all animals had free access to drinking water and rat chow (Guinea feeds Nigeria limited, Benin Nigeria). At the end of 14 days treatment, all experimental animals were denied their feed and water for at least 18 h before they were again weighed and anesthetized with chloroform. Blood samples were collected by cardiac puncture, allowed to clot and centrifuged at 1000 rpm for 5 min to obtain the serum. Serum protein was estimated using the Biuret method of Donninger *et al*. (1972). Alanine amino Transferase (ALT) and Aspartate Aminotransferase (AST) activities were determined by the method of Mathieu *et al*. (1982). Alkaline phosphatase activity in serum was estimated by kit method of Tietz *et al*. (1984).

**Statistical analysis:** Statistical analysis were carried out using Window SPSS. One way analysis of variance was adopted for comparison and results were subjected to post hoc test using Least Square Deviation (LSD). The data were expressed as mean±standard error. $p<0.05$ were considered significant.

## RESULTS

The total serum protein as well as the concentration of some liver enzymes in serum were measure and presented in Table 1. The total serum protein (g/100) in groups 5 and 6 treated with acetaminophen plus caffeine plus ACE extract were 91.26±1.65 and 87.8±0.58, respectively. These values were significantly ($p<0.01$ and $p<0.05$, respectively), higher than the value in group 2 (acetaminophen plus caffeine), which was 82.24±0.98.

The concentration of ALT (U/L) in group 2 (29.74±2.08 U/L) treated with acetaminophen plus caffeine was marginally higher than that of group 1 (24.68±1.00) treated with acetaminophen only. The concentration of ALT (U/L) in groups 3 and 4 treated with acetaminophen plus 250 and 500 mg/kg of ACE, respectively were significantly lower ($p<0.05$) than that of the control group 1 treated with acetaminophen only . A similar comparison between groups 5 and 6 treated with acetaminophen plus caffeine plus 250 mg/kg (17.00±1.00 U/L) and 500 mg/kg (17.21±2.19 U/L) ACE, respectively against group 2 treated with acetaminophen and caffeine. The results as shown in Table 1, indicates that the ALT concentration in groups 5 and 6 was significantly ($p<0.01$) lower than group 2 (which were treated with acetaminophen and caffeine).

The serum AST concentration (U/L) in group 2 treated with acetaminophen and caffeine (30.00±1.00 U/L) was significantly higher ($p<0.001$)than the control group 1 (17.50±0.50 U/L) which received acetaminophen alone. The change in serum AST concentration observed in groups 3 and 4 treated with acetaminophen plus 250 mg/Kg and 500 mg/Kg of ACE, respectively showed remarkable effect of the extract. Group 5 treated with acetaminophen, caffeine and 250 mg/Kg of the ACE (19.50 ± 1.50 U/L) and group 6 treated with

Table 1: The total serum protein and serum enzyme activities in the experimental animals (Mean±SEM)

| Group | Treatment | Total protein (g/100 mL) | ALT(U/L) | AST(U/L) | ALP (U/L) |
|---|---|---|---|---|---|
| 1 (n = 5) | Acetaminophen only | 83.11±0.41 | 24.68±1.00 | 17.50±0.50 | 19.37±0.78 |
| 2 (n = 5) | Acetaminophen plus caffeine | 82.24±0.98 | 29.74±2.08 | 30.00±1.00*** | 27.28±1.78** |
| 3 (n = 5) | Acetaminophen plus 250 mg/kg of ACE | 88.17±1.73 | 11.67±2.91* | 17.47±1.53 | 19.04±0.32 |
| 4 (n = 5) | Acetaminophen plus 500 mg/kg of ACE | 86. 34±1.10 | 11.04±2.50* | 18.00±2.00 | 18.92±0.13 |
| 5 (n = 5 | Acetaminophen plus caffeine plus 250 mg/kg of ACE | 91.26±1.65a | 17.00±1.00a | 19.50±2.50a | 19.41±0.45a |
| 6 (n = 5) | Acetaminophen plus caffeine plus 500 mg/kg of ACE | 87.81±0.58b | 17.21±2.19a | 18.00±0.88c | 19.12±0.19c |

* = Significantly different from group 1 (p<0.05), ** = Significantly different from group 1 (p<0.01), *** = Significantly different from group 1 (p<0.001), a = Significantly different from group 2 (p<0.01), b = Significantly different from group 2 (p<0.05), c = Significantly different from group 2 (P<0.001)

acetaminophen plus caffeine plus 500 mg/kg of ACE (18.0±0.88 U/L) were significantly (p<.0.01 and p<0.001, respectively) lower than the control group 2 treated with acetaminophen and caffeine as shown in Table 1.

Serum ALP concentration followed the same trend as in AST. The control group 2 with acetaminophen and caffeine (27.28±1.78 U/L) was significantly (p<0.01) higher than the control group 1 treated with acetaminophen only (19.37±0.78 U/L). Again as in AST, the reduction in ALP concentration in groups 3 and 4 treated with acetaminophen and 250 and 500 mg/kg ACE, respectively were only marginally different from the control group 1 and were not considered significant. Comparison between control group 2 (acetaminophen plus caffeine) and groups 5 and 6 (acetaminophen plus caffeine plus ACE) showed remarkable effect of the extract. Group 5 (acetaminophen plus caffeine plus 250 mg/kg ACE) (19.4±0.45 U/L) as well as group 6 (acetaminophen plus caffeine plus 500 mg/kg ACE) (19.12±0.19 U/L) were significantly lower (p<0.01 and p<0.001, respectively) than group 2 as shown in Table 1.

## DISCUSSION

Acetaminophen toxicity like many other disease conditions is widely believed to involve the generation of Reactive Oxygen Species (ROS). Antioxidants have been reported to play prominent roles in prevention of ROS generation (Valko *et al*., 2006) and by extension may offer protection against acetaminophen toxicity. These antioxidants are most readily available in edible vegetables and other herbal plants. Hence, the evaluation of medicinal plants or herbs with free radical scavenging potentials for protective roles against drug induced toxicity becomes relevant. Several plant extracts, including *Ageratum conyzoides* have been reported to possess free radical scavenging potentials (Jagetia, 2007).

Hepatic damage is usually associated with elevated serum ALT, AST and bilirubin concentration (Reichling and Kaplan, 1988; Kew, 2000; Green and Flamm, 2002; Collier and Bassendine, 2002). In this study, ACE significantly lowered serum ALT concentration. When, acetaminophen was administered, alone (group 1), the ALT level obtained was 24.68±1.00 U/L, whereas, in group 3 where ACE was simultaneously administered with acetaminophen ALT level significantly dropped to 11.67±2.91 and 11.04±2.50 U/L when the extract was administered at doses of 250 and 500 mg/kg, respectively. Out of the three enzymes analyzed in this study, there was a marginal increase in ALT activity but significant increases in AST and ALP activity when, the group treated with acetaminophen plus caffeine was compared with group 1 (acetaminophen only). This potentiating effect of simultaneous administration of caffeine and acetaminophen to cause hepatotoxicity in rats had earlier been reported by Sato *et al*. (1985) and Sato and Izumi (1989). The simultaneous administration of acetaminophen, caffeine and ACE significantly reduced the combined effect of acetaminophen and caffeine by lowering ALT, AST and ALP concentrations as well as significantly increasing total serum protein concentration.

The protective effect of ethanol leaf extract of *Ageratum conyzoides* may be mediated through several mechanisms since the extract itself is a complex mixture of many chemicals. *Ageratum conyzoides* contain chromenes, benzofurans, sterols and terpenoids (Wiedenfeld and Roder, 1991; Sur *et al*., 1997), wide range of amino acids (Tyagi *et al*., 1994; Mondal *et al*., 1998), protein, fructose, ribose, glucose (Tyagi, *et al*., 1995). The particular mechanism by which, the protective effect is enforced was not investigated in this study. Galati *et al*. (2001) in their research on the anti-inflammatory effect of methanolic extract of *Ageratum conyzoides* reported that its anti-inflammatory action depends on its flavonoid fraction. The authors suggested that this could produce a protection against free radical mediated damage to cells and tissues. The ability of ACE to scavenge reactive oxygen species has also been reported (Jagetia *et al*., 2003), following the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in a dose dependent manner (Jagetia *et al*., 2003). The present study shows that ACE offers protection against acetaminophen toxicity through a yet to be established mechanism. These findings may have far reaching implications for the a readily available therapeutic intervention in cases of acetaminophen toxicity.

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