

Antioxidant Properties and Glutathione S-Transferases Inhibitory Activity of *Alchornea cordifolia* Leaf Extract in Acetaminophen-Induced Liver Injury

MARY T. OLALEYE, AYODELE O. KOLAWOLE and JOSHUA O. AJELE

For author affiliations, see end of text.

Received October 27, 2006; Revised May 11, 2007; Accepted June 11, 2007

This paper is available online at <http://ijpt.iums.ac.ir>

ABSTRACT

Paracetamol (acetaminophen, PCM) is a widely used over-the-counter analgesic and antipyretic drug. Intake of a large dose of PCM may result in severe hepatic necrosis. Oxidative stress is mediated by oxidative capacities of the PCM metabolite (N-acetyl-para-benzo quinoneimine, NAPQI), which covalently binds to proteins and other macromolecules to cause cellular damage. At low doses, NAPQI is considered as the main cause of PCM toxicity. This work was therefore designed to evaluate the antioxidant potential of *Alchornea cordifolia* and to study the effect of its ethanol extract pretreatment (100-500mg/kg) for two weeks before paracetamol intoxication (2g/kg) on glutathione S-transferases activity. The antioxidant property revealed total phenol level of 0.22mg/ml and reducing power of 0.062mg/ml which was higher than the vitamin E reducing value of 0.042 mg/ml. Paracetamol toxicity produced a significant decrease in the hepatic levels of glutathione S-transferases when compared with the control. *Alchornea cordifolia* significantly reduced the level of hepatic glutathione S-transferase however, it produced a dose dependent significant increase in the levels of glutathione S-transferases in the presence of the toxicant. The results suggest that the *Alchornea cordifolia* is a potent antioxidant since the detoxification of paracetamol can be mediated by glutathione S-transferase (GST) catalyzed conjugation with glutathione (GSH) in the liver. The plant extract can ameliorate the effect of PCM intoxication. The increased hepatic glutathione S-transferases (GSTs) levels induced by the extract treatment can reduce the acute paracetamol toxicity.

Keywords: Glutathione S-transferases, *Alchornea cordifolia*, Antioxidant properties, Acetaminophen, intoxication

Alchornea cordifolia is an erect and bushy perennial shrub or small tree up to 4 meters high reproducing from seeds [1]. *Alchornea cordifolia* has been subjected to intensive phytochemical screening to determine its medicinal usage. The plant has been shown to possess antibacterial, antifungal and spasmolytic properties [2]. Osadebe and Okoye (2003)[3] have shown that methanolic extract of *Alchornea cordifolia* leaves possesses anti-inflammatory activity, when given by intraperitoneal injection in the egg albumin induced rat paw oedema test (inhibition of 68.25% for 50mg/kg) and the topical inflammatory activity was inhibited by the plant extract [4]. The hepatoprotective activity of its ethanol extract against paracetamol induced toxicity has also been reported [5]

At therapeutic doses, PCM is considered a safe drug. However, it can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions, and even death in humans and experimental animals when taken in overdoses [6]. Among several other hypotheses, is that oxidative stress mediated by the oxidative capacities of NAPQI (a metabolite of PCM) is the main cause of hepatotoxicity of Amyloid Precursor Protein (APP). Lipid peroxidation, resulting from oxidative stress contributes to the initiation and progress of liver damage [7, 8]. At low doses, NAPQI is efficiently detoxified, principally by conjugation with glutathione, a reaction catalyzed in part by the glutathione S-transferases (GSTs).

Glutathione S-transferases (GSTs) are a family of intracellular isoenzymes with broad substrate specificities that catalyze the conjugation of the tripeptide and glu-

Table 1. Antioxidant indices of ethanolic extract of *Alchornea cordifolia*

| Indices | Level (mg/ml) |
|--|---------------|
| Total phenol | 0.22 |
| Reducing property of <i>Alchornea cordifolia</i> leaf extracts | 0.0625 |
| Reducing property of Vitamin E | 0.042 |

tathione (GSH) to many compounds bearing a sufficiently electrophilic center. The electrophilic compounds are mainly xenobiotics, some endogenously generated toxic compounds and many environmental pollutants [9].

GSTs play an important role in the detoxification of many substances, organic pollutants and plant secondary metabolites [10]. Many of the xenobiotics from plant have been shown to induce GST in liver of vertebrates that feed on these herbivores [11].

Paracetamol, an over the counter drug, is grossly abused in Nigeria and this work aims to evaluate the antioxidant properties of *Alchornea cordifolia* and to study its effect on glutathione S-transferases activity in paracetamol toxicity.

MATERIALS AND METHODS

Acetaminophen (Aspar Pharmaceutical, London), vitamin E and curcumin standards were gifts from Chemiron International limited, Lagos, Nigeria. Other reagents used were of analytical grade and water used was glass distilled.

Plant / Sample preparation

Fresh leaves of *Alchornea cordifolia* were collected from a piece of land at the Federal University of Technology, Akure, Nigeria. The leaves were later taken to the Crop Production Department of the Federal University of Technology, Akure for botanical identification.

Extracts from Plant Materials

Five hundred (500) grams of ground leaves were soaked in 2000 ml of 98% ethanol for 48 hours. The suspension was then filtered. The filtrate was concentrated under reduced pressure, using a vacuum evaporator, to dryness. The lyophilized aqueous extract was screened for phytochemicals [5].

Animals

Adult albino rats (bred in the animal house of Ani-

mal Production and Health Department, Federal University of Technology, Akure) weighing 150-200g were acclimatized to laboratory conditions for 10 days and were fed on commercial pelleted rat chow and water *ad libitum*.

Bioassay

Doses of acetaminophen greater than 1g/kg are considered toxic to rats. Based on our previous work *Alchornea cordifolia* is capable of ameliorating PCM toxicity up to 2g/kg. The animals were divided into nine groups of five animals each as follows:

Group I: Control; not induced but had free access to water. Group II: Intoxicated control induced with 2g/kg acetaminophen (Paracetamol). Group III: Induced with 2g/kg paracetamol and treated with 100mg/kg vitamin E standard. Group IV: Induced with 2g/kg paracetamol and treated with 100mg/kg curcumin standard. Group V: Treated with 300mg/kg plant extract only. Group VI: Induced with 2g/kg paracetamol and treated with 200mg/kg plant extract. Group VII: Induced with 2g/kg paracetamol and treated with 300mg/kg plant extract. Group VIII: Induced with 2g/kg paracetamol and treated with 400mg/kg plant extract. Group IX: Induced with 2g/kg paracetamol and treated with 500mg/kg plant extract. Hepatotoxin (acetaminophen 2g/kg) was administered to all the groups except control followed by the plant extract of varying dosages (200-500mg/kg) to all the groups except the intoxicated control (acetaminophen only) and those that were given the standards; vitamin E and curcumin (100mg/kg) for a period of 14 days. All these were prepared in 1% DMSO (Dimethylsulfoxide) and administered to animals orally. The animals were sacrificed 24 hours after the last administration by cervical decapitation. The livers were removed and homogenized in ice-cold isotonic sucrose, blood was obtained by cardiac puncture and centrifuged to obtain the serum. The doses of the drugs and the toxicants were selected based on the previous results in our laboratory [5].

Preparation of serum and post mitochondrial fraction from the liver homogenates

All procedures were carried out at 0-4°C unless otherwise stated. The livers were homogenized (20% w/v) in a 10 mM Tris-HCl pH 7.4 buffer containing 250mM sucrose, 1mM phenylmethanesulfonyl fluoride and

Table 2. Effects of *Alchornea cordifolia* leaf extracts on liver GST activity of rats given 2 g/kg acetaminophen

| Treatment | GST activity μM/min/ml | Total protein (mg/ml) |
|--|---------------------------|--------------------------|
| Control | 0.33 ^c ±0.08 | 1.74 ^a ±0.18 |
| Toxicant | 0.25 ^b ±0.03 | 3.96 ^{cd} ±0.19 |
| Toxicant + Vit. E | 0.64 ^e ±0.05 | 1.94 ^{ab} ±0.29 |
| Toxicant + curcumin | 0.50 ^d ±0.04 | 1.86 ^{ab} ±0.29 |
| 300 mg/kg Plant extract (Plant Extract) only | 0.14 ^a ±0.01 | 2.24 ^b ±0.41 |
| Toxicant + 200 mg/kg (Plant Extract) only | 0.28 ^{bc} ±0.06 | 4.24 ^d ±0.09 |
| Toxicant + 300 mg/kg (Plant Extract) only | 0.46 ^d ±0.08 | 2.20 ^b ±0.45 |
| Toxicant + 400 mg/kg (Plant Extract) only | 0.81 ^e ±0.06 | 3.93 ^c ±0.31 |
| Toxicant + 500 mg/kg PE | 0.89 ^e ±0.04 | 3.70 ^c ±0.32 |

The data were recorded as means ± standard deviation (n = 5). One way analysis of variance (ANOVA) and Duncan test were carried out to test any significant differences between the means. P-value < 0.05 is considered significant.

Table 3. Effects of *Alchornea cordifolia* leaf extracts on serum GST of rats given 2 g/kg acetaminophen

| Treatment | GST activity $\mu\text{M}/\text{min}/\text{ml}$ | Total protein (mg/ml) |
|--|--|--------------------------|
| Control | $0.8^a \pm 0.02$ | $1.36^a \pm 0.28$ |
| Toxicant | $0.40^b \pm 0.04$ | $1.04^b \pm 0.36$ |
| Toxicant + Vit. E | $0.29^a \pm 0.02$ | $1.48^a \pm 0.13$ |
| Toxicant + curcumin | $0.31^a \pm 0.02$ | $1.83^b \pm 0.57$ |
| 300 mg/kg Plant extract (Plant extract) only | $0.17^a \pm 0.01$ | $1.36^a \pm 0.39$ |
| Toxicant + 200 mg/kg (Plant extract) only | $0.81^a \pm 0.02$ | $1.78^b \pm 0.23$ |
| Toxicant + 300 mg/kg (Plant extract) only | $0.72^a \pm 4.15$ | $1.38^c \pm 0.25$ |
| Toxicant + 400 mg/kg (Plant extract) only | $0.36^a \pm 0.04$ | $2.23^c \pm 0.21$ |
| Toxicant + 500 mg/kg (Plant extract) only | $0.23^a \pm 0.04$ | $1.66^{ab} \pm 0.09$ |

The data were recorded as means \pm standard deviation ($n = 5$). One way analysis of variance (ANOVA) and Duncan test were carried out to test any significant differences between the means. P-value < 0.05 is considered significant.

1mM dithiothreitol using an ultra-Turrax homogenizer. It was centrifuged at 19,000 g for 40min to remove cell debris, nuclei and mitochondria.

All analyses were performed on the supernatants which were used immediately or stored at -8°C . The blood was centrifuged at 5000 g for 15 minutes and the serum was refrigerated until use for GST activity.

Determination of protein concentration and glutathione S- transferases activity assay GST activity was determined according to Habig et al (1974) [11] as modified by Ajele and Afolayan (1992) [12]. For a typical assay, the reaction mixture of 3 ml contained a final concentration of 100mM potassium phosphate buffer pH 6.5 and 1mM of Glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) each, together with an appropriate amount of enzyme. Three replicates were used for each measurement. The protein concentration of homogenates was determined by the method of Lowry et al (1951) [13]. Two replicates were used for each experiment using bovine serum albumin as a standard. A Cecil CE 595 double beam digital spectrophotometer was used for absorbance measurement.

Statistical Analysis

All analyses were performed in triplicate. The data were recorded as means \pm standard deviation and analysed by SPSS (version 11 for windows SPSS Inc.). One way analysis of variance (ANOVA) and Duncan test were carried out to test any significant differences between the means. P-value less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The yield of *Alchornea cordifolia* leaf extract was 75%. The ethanolic extract was screened for phytochemicals and our preliminary results showed that it contained alkaloids, saponins, tannins, flavonoids and steroidal rings. The ethanolic extract has an antioxidant level of 0.0625 mg/ml compared to reducing property of vitamin E of 0.042 mg/ml. The phenolic content of the leaf extract was 0.22mg/ml. Table 2 shows the effects of *Alchornea cordifolia* leaf extracts on some liver glutathione S-transferases indices of rats given 2 g/kg acetaminophen.

Alchornea cordifolia ethanolic leaf extract has a potential to exert a considerable antioxidant activity *in vitro* as shown in the total phenolic content of ethanolic leaf extract determined by using tannin as standard as in

the method of Singleton *et al*, (1995) [14] and DPPH radical scavenging capabilities in Table 1. The high antioxidant activity of *Alchornea cordifolia* ethanolic leaf extract could be attributed to its constituents (alkaloids, flavonoids terpenoids) [15]. Phenolic content of therapeutic plants has been extensively studied because it is an important contributor to the antioxidants activity of the plants [15]. Total phenolic content of plant extract has been demonstrated to correlate well with its antioxidant activity [16]. Our results show that *Alchornea cordifolia* ethanolic leaf extract has high antioxidant activity and high reducing property of 0.062 mg/ml compared to 0.042 mg/ml of Vitamin E. It is plausible to suggest, also, that it might have a high lipid peroxidation inhibitory capacity. There might be a strong correlation between the *Alchornea cordifolia* intake and degenerative disease prevention. This study has shown that *Alchornea cordifolia* is a good source of antioxidant.

A dose of 300 mg/kg of the extract of *Alchornea cordifolia* was effective as an inhibitor of both liver and serum GSTs in the absence of acetaminophen. The GST activity decreased from $0.33 \pm 0.08 \mu\text{M}/\text{min}/\text{ml}$ in control to $0.14 \pm 0.01 \mu\text{M}/\text{min}/\text{ml}$ for the liver GST (Table 2) while it decreased from $0.8 \pm 0.02 \mu\text{M}/\text{min}/\text{ml}$ (Control) to $0.17 \pm 0.01 \mu\text{M}/\text{min}/\text{ml}$ for the serum GST (Table 3). In the presence of the toxicant (acetaminophen), the inhibitory effect of the extract on the liver GST decreased with increasing concentration of the extract (Table 2) while for the serum GST the inhibitory effect was noticeable at higher doses of 400 mg/kg and 500 mg/kg of the plant extract (Table 3). There is a general increase in the protein concentration of both liver and serum with increase in the administration of plant extract (Table 1 & 2).

It appears that the extract of *Alchornea cordifolia* acting in the absence of any other interacting substances has a strong inhibitory effect on the activity of both liver and serum GSTs. However, this inhibitory property is altered in the presence of substances such as the toxicants (acetaminophen). The plausible explanation for this phenomenon is that the phenolic compounds of the *Alchornea cordifolia* ethanolic extract are directly involved in the reduction of the toxic intermediate, NAPQI, of the Acetaminophen (PCM), to a non-toxic aminophenol without involvement of GST and its conjugating substrate, glutathione. This increase in liver and serum GST activity in the presence of acetaminophen (the toxicant) is due to competitive reduction of

the toxic intermediate by the phenolic components of the extract with less or no involvement of GST. There is less availability of the phenolic components to inhibit GST resulting in dose-dependent rise in GST activity.

The toxic effect of NAPQI is the addition reaction with liver proteins and other macromolecules to form adduct which cause cellular damage. The increase in liver and serum proteins with the increase in administration of *Alchornea cordifolia* ethanolic extract is a clear demonstration that NAPQI is well detoxified without causing cellular damage by decreasing the protein concentration.

Inhibitory effects of naturally occurring plant polyphenols such as tannins, ellagic acid, ferulic acid, caffeic acid, stilbene, quercetin, curcumin and chlorogenic acid against glutathione S-transferases have been demonstrated [10]. Plant extracts that are high in polyphenols are known to have important inhibitory effect on glutathione S-transferases [17,18]. Glutathione S-transferases play a very important role in the detoxification of many potential carcinogenic compounds such as electrophilic agents and alkylating agents that are potentially harmful to DNA [19]. They also play an important role in the elimination of many cellular alkylating agents through glutathione conjugation and subsequent mercapturic acid formation [9]. *Alchornea cordifolia* ethanolic leaf extract which has high phenolic content and reducing property is expected to be effective in glutathione S-transferases inhibition because the search for novel antioxidants with GST inhibitory activity has become an important issue due to their role in multi-drug resistance and cancer cell treatment [18]. The present result might justify the rationale for the use of *Alchornea cordifolia* extract for clinical trials in such conditions as Alzheimer's disease, Parkinson, cancer, viral and cardiovascular diseases where free radicals and glutathione S-transferases are implicated.

The present investigation shows that paracetamol caused changes in the levels of liver GST. The ability of *Alchornea cordifolia* to abolish the negative effect of paracetamol on the antioxidant defense enzyme (GST) coupled with the antioxidants suggested that it possesses antioxidant effects. These antioxidant effects may contribute to its protective action against paracetamol-induced toxicity.

REFERENCES

- Lamikanra A, Ogundain AO, Ogungbamila FO. Antibacterial constituents of *Alchornea Cardifolia* leaves. *Phytother Res* 1990; 4: 198-200.
- Tona L, Kimble K, Nimbi N, Messiah K, Panged O, Lusakibanza M, Cimanga K, DeBruyne T, Apers S, Totte J, Pitters L, Vlietinck AJ. Antiamoebic and antiplasmodic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine* 2000, 7; 31-38.
- Osadebe PO, Okoye FBC. Anti inflammatory effects of crude methanol extract and fractions of *Anchomen cordipolia* leave. *Journal of ethnopharmacology* 2003, 89; 19-24.
- Mangar HM, Brkic D, Marie DE, Quetin Reclercq J. In vivo anti-inflammatory ear oedema. *J. Ethnopharmacol* 2001, 92; 209-214.
- Olaleye MT, Adegboye OO, Akindahunsi AA. *Alchornea cordifolia* extract protects wistar albino rats against acetaminophen-induced liver damage. *African Journal of Biotechnology* 2006, 5; 2439-2445.
- Ray SD, Mumaw VR, Raje RR, Fariss MW. Protection of acetaminophen-induced hepatocellular apoptosis and necrosis by cholesteryl hemisuccinate pretreatment. *Journal of Pharmacology Experimental Therapeutics* 1996, 279; 1470-1483.
- Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. Mechanisms of N-acetyl-p-benzoquinone-imine cytotoxicity. *Mol. Pharmacol* 1985, 28; 306-311.
- Kyle ME, Miccadei S, Nakae D, Farber JL. Superoxide dismutase and catalase protect cultured hepatocytes from the cytotoxicity of acetaminophen. *Biochem. Biophys. Res. Commun.* 1987, 149, 889.
- Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprevention and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology* 1995, 30; 445-600.
- Gyamfi AM, Ohtani II, Shinno E, Aniya Y. Inhibition of glutathione S transferases by thoningianin A, isolated from the African medicinal herb, *honningia sanguinea*, in vitro. *Food and Chemical Toxicology* 2004, 42; 1401-1408.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry* 1974, 249; 7130-7139.
- Ajele JO, Afolayan A. Purification and characterization of glutathione transferase from Giant African land snail *Arachachatina marginata*. *Comp. Biochem. Physiol. B* 1992, 103; 47-55.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 1951, 193; 265-275.
- Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999, 299; 152-177.
- Skerget M, Kotnik P, Hadolin M, Hras A, Simoncic M, Knez Z. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food chemistry* 2005, 89; 191-198.
- Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences* 2004, 74; 2157-2184.
- Cnubben NHP, Rietjens IMCM, Wortelboer H, Van Zanden J, Van Bladeren PJ. The interplay of glutathione-related processes in antioxidant defense. *Environmental Toxicology and Pharmacology* 2001, 10; 141-152.
- Coruh N, Sagdic,og'lu Celep AG, zgo'kc FO. Antioxidant properties of Prangosferulacea (L.) Lindl., Chaerophyllum macropodium Boiss. and Heracleum persicum Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. *Food Chemistry* 2007, 100; 1237-1242.
- Bongers V, Snow GB, Braakhuis BJM. The role of glutathione S-transferases in head and neck squamous cell carcinogenesis. *Oral Oncol Eur J Cancer B* 1995, 31; 349-54.

CURRENT AUTHOR ADDRESSES

Ayodele O. Kolawole, Department of biochemistry, Federal University of Technology, Akure, Nigeria. E-mail: maok210@yahoo.com (Corresponding author)

Mary T. Olaleye, Department of biochemistry, Federal University of Technology, Akure, Nigeria.

Joshua O. Ajele, Department of biochemistry, Federal University of Technology, Akure, Nigeria.