Ameliorating potentials of aqueous extract of *Talinum triangulare* (Waterleave) against acetaminophen–induced hepatotoxicity in Wistar albino rats

Afolabi OB, Obafemi TO, Awe JO, Jaiyesimi KF, Anadozie SO, Olayide II, Afolabi BA

Afolabi OB
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Obafemi TO
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Awe JO
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Jaiyesimi KF
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Anadozie SO
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Olayide II
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Afolabi BA
Department of Biochemistry, Bowen University Iwo, Nigeria.

Abstract
The antagonistic potentials of aqueous extract of *Talinum triangulare (Tt)* against Acetaminophen-induced damage in the hepatic cells were concisely studied in randomly grouped female Wistar albino rats. The dosage of the extract received by the various groups was based on the average weight of each group. Negative control group received normal animal feed and water *ad libitum*, the Control group (Positive) received 200 mg/ml Acetaminophen without *Tt* while group 1 received 200 mg/ml Acetaminophen with 100 mg/kg *Tt*. On the other hand, based on average weight, group 2 received 200 mg/kg *Tt* with 200 mg/ml Acetaminophen and group 3 received 200 mg/ml Acetaminophen alongside with the counter dose of 300 mg/kg of the extract. The levels of liver marker enzymes, malondialdehyde (MDA) and reduced glutathione (GSH) in the tissue of the animals (both the treated and untreated) were estimated after the experiment. The result showed a significant ($p < 0.05$) increase in the level of ALT, ALP and AST in both the liver and serum. Also, a significant ($p < 0.05$) decrease was observed in the GSH level in the serum of the tested groups when compared to the control group (positive). Severe liver impairment was observed in the Control group (Negative) based on histophotographic analysis and ameliorating effects were observed in the *Tt* treated groups significantly.

Keywords: Detoxification, antioxidant, phytochemical, bioactive compounds

1. Introduction
Liver is a vital organ of vertebrates and some other animals with wide range of functions which include detoxification of various metabolites, production of biochemicals necessary for digestion etc [1]. Damage to the liver or hepatotoxicity is as a result of many factors [2], but Drug-induced liver injury accounts for approximately 10 percent of all cases of acute liver diseases chronicled and it is the most common cause of acute liver failure [3]. Hepatotoxicity induced by excess acetaminophen is the most common cause of death by acute liver failure [4]. Paracetamol structurally figured below (Fig. 1) was firstly introduced as an analgesic by von Mering in 1893 [5]. Paracetamol chemically named *N*-acetyl-p-aminophenol is a widely used pain reliever and antipyretic, it's a principally abused drug that causes hepatotoxicity when used in overdose, this was first established by Davidson and Eastham, 1966 [6, 7, 8]. Also, studies have suggested the possibility of a moderately increased risk of upper gastrointestinal complications such as stomach bleeding when high doses are taken chronically [9, 10].
Avalanche of factors also influence the risk of liver injury e.g. dose ingested, dietary habit among others [11, 12]. In case of overdose, the toxic dose of paracetamol is highly variable, higher doses that are not attended to cause increasing risk of toxicity and death within days [13]. In adults, singular dose above 10 g or 200 mg/kg of bodyweight, which however is lower have a reasonable likelihood of inducing toxicity [14]. Following a regular dose of one gram paracetamol four times a day for two consecutive weeks, patients can always expect an increase in the activities of alanine transaminase in the liver to typically about three times the normal value [9]. Increased oxidative stress that is traced to calcium homeostasis changes and initiation of signal transduction responses, leading to mitochondrial permeability and loss of the ability of the mitochondria to produce ATP which subsequently leads to necrosis has been associated with acetaminophen-induced hepatotoxicity.
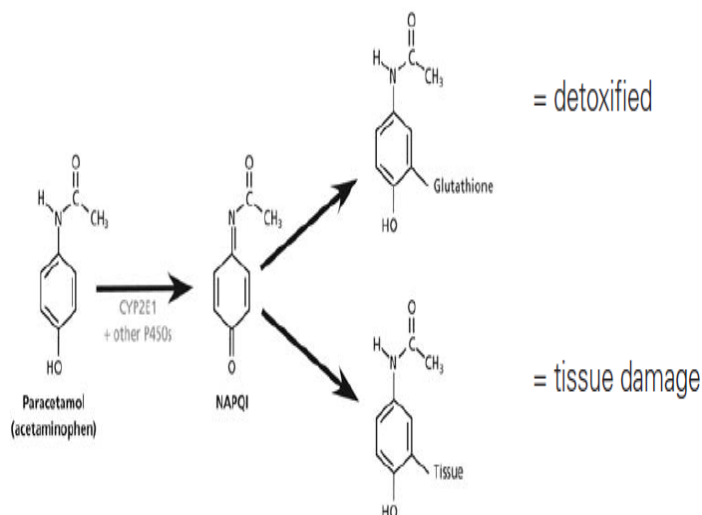
Correspondence:
Afolabi OB
Chemical Sciences Department, College of Science, Afe-Babalola University, Ado-Ekiti, Nigeria.

Fig 1: Paracetamol metabolism (acetaminophen or Tylenol)

Studies have shown that potentially acetaminophen-induced hepatic damage is not directly caused by the drug but its metabolic product- N-acetyl-pbenzoquinoneimine (NAPQI) also known as N-acetyliminoquinone (NAIQ) in the primary glucuronidation metabolic pathway in adult (Fig 1). It is normally produced only in small amounts by Cytochrome P$_{450}$ (CYP450) and then almost immediately detoxified in the liver and subsequently converted to an inactive product through additional metabolic processes involving phase II detoxifying enzymes, which eliminate toxic metabolites [15]. The isoforms of CYP450 importantly involved in the acetaminophen metabolism have been shown to include: CYP2E1, CYP1A2, CYP3A4, and CYP2D6 [16, 17]. Under normal circumstances, N-acetyl-p-benzoquinoneimine is detoxified by conjugating with glutathione in phase 2 reaction, however, under some conditions in which NAPQI is not effectively detoxified, usually in case of paracetamol overdose or where a large amount of NAPQI is generated thereby overwhelming the detoxification process, N-acetyl-p-benzoquinoneimine, the electrophile interferes with the liver's natural antioxidant integrity by depleting glutathione where the reactive N-acetylimidoquinone specie will directly react with nucleophilic thiol (-SH) groups that are present on hepatic proteins by conjugation to form 3-glutathion-S-yl-acetaminophen and by reduction to acetaminophen [18, 19]. This results in the formation of covalent adducts which produce hepatic necrosis and in the end damages the cells in the liver leading to liver impairment and subsequently leads to acute liver failure (ALF) [20, 21]. However, N-acetylcysteine, a precursor of GSH, is still the leading and well known therapeutic treatment for acetaminophen overdose [22], a low therapeutic window and critical timing of N-acetylcysteine involvement is a limiting factor for the treatment acetaminophen poisoning because the GSH level alone is not enough to protect against it [23]. Currently, treatment with N-acetylcysteine (NAC) has been the normal therapy used for acetaminophen overdose [24]. NAC is actively transported into hepatocytes, being the precursor of glutathione, against the potential pathological reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI), it also helps in replenishing the lost glutathione and itself react with the N-acetylimidoquinone to neutralize the risk imposes and eliminate it safely from the body. It is also believed that the N-acetylcysteine can help reduce the formation of the N-acetylimidoquinone metabolite itself [20, 25].

Waterleaf (*Talinum triangulare*) is predominantly one of the commonest nutritious annual vegetable; a smooth herbaceous plant found in most Nigerian markets, it has succulent leaf and stem that are edible among the folks with a pink flower at fruition [26]. According to a research on its phytochemical composition, it was revealed that *Talinum triangulare* is rich in considerable amount of alkaloids, flavonoids, saponins and appreciable amount of bioactive compounds among others [27]. It has also been shown to have therapeutic potentials in traditional medicine due to its nutritional supplement with minimal level of oxalic acid compare to some other plants supplements with severe adverse effect [22, 28, 29]. Cognoscible research into its chemotherapeutic ability revealed that it can reduce injury to the liver that is associated with impaired liver function caused by paracetamol which at higher dose has extreme effect on the hepatic cells [30].

The present study was designed to show how aqueous extract of *Talinum triangulare* in different dosages can effectively ameliorate the effect of acetaminophen overdose in matured female albino rats.

2. Materials and Methods
Plant materials and preparation
Fresh water leaves of *Talinum triangulare* were bought in Ado-Ekiti, Ekiti State, Nigeria. A sample was taken to the Department of Plant Science in Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria for identification. It was identified by a taxonomist (Mr Omotayo) in the department and after proper taxonomic investigations from the data base with herbarium number *UHAE 2013/76*.

3. Chemical
All the chemicals used were of analytical grades and prepared using sterilized distilled water.

4. Preparation of Sample
Preparation of Aqueous extract of *Talinum triangulare*.
50 g of the powdered sample was extracted in 500 ml distilled water for 48 hrs. The extract was then re-concentrated and later subjected to bioassay analysis.

5. Experiment Design
Albino rats of weights ranging between 100-200 g were used for this experiment. The rats were randomly distributed into four treatment groups of four rats each while the experiment lasted for a period of 14 days. Positive control group received normal animal feed and water *ad libitum*, throughout the experiment window, whereas negative control group (untreated), group 1, 2 and 3 received 200 mg/ml acetaminophen for the first 7 days to establish hepatic damage in the rats. In the second phase of the experiment, negative control group (untreated) received normal animal feed and water *ad libitum*, for the rest of the experiment period while group 1, 2 and 3 received counter doses of 100, 200 and 300 mg/kg body weight aqueous extract of *Tt* respectively for another 7 days.

Preparation of animal serum
The animals were dissected after treatment with di-ethyl ether and the whole blood taken using cardiac puncture method. More so, blood samples were collected into plain sample bottles and were allowed to stand at room temperature for 30 mins and thereafter centrifuged at 3000 g (gravity) for 10

mins, after which the supernatant which is the serum was collected using a Pasteur's pipette.

Preparation of tissue homogenates
After the animals have been sacrificed and dissected, tissues of interest (i.e. liver) was removed and washed with 0.25 M sucrose solution. The isolated tissues were weighed and immediately stored on ice-cold 0.25 M sucrose solution. The tissues were cleaned and were homogenized using Teflon homogenizer in ice-cold 0.25 M sucrose solution (1:5 w/v). The supernatants were stored in the freezer at -5°C.

Lipid Peroxidation assay
Levels of lipid peroxidation were estimated by the thiobarbituric acid reaction as essentially described by Buege and Aust [31]. The determination of the values for thiobarbituric acid reactive substances (TBARS) was reported as malondialdehyde (MDA) equivalents [31]. MDA formation was quantified using a molar extinction coefficient of 1.56×10^5 cm M^{-1} and expressed as nmol/ml MDA.

Determination of enzyme activities
The activities of the enzymes in the serum and tissues were estimated from the standard calibration curves using standard Randox kits with spectrophotometric method as described by Reitman and Frankel [32].

Determination of Alanine Aminotransferase Activity
A standard calibration curve for ALT was first prepared by dispensing various volumes (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml) of 2 mM sodium pyruvate solution (as standard) into different test-tubes. The mixtures was shaken and incubated for 30 mins at 37 °C. 0.001M 2, 4-dinitrophenylhydrazine (DNPH) (1 ml) was added to each of the test-tubes. The mixture was allowed to stand for 20 mins at 25 °C after which 5ml of 0.4 N NaOH was added. The mixture was shaken and after 5 mins, the absorbance was read at 546 nm against a reagent blank. The values obtained were plotted against the corresponding concentration of pyruvate.
The ALT activity in the serum and tissue homogenate was determined using appropriate dilution factors. ALT buffered substrate solution (1 ml) was dispensed into test-tube followed by the 0.2 ml of the enzyme source (i.e. serum diluted tissue homogenate). This was mixed and incubated for 30 mins at 37 °C after which 1ml of 0.001 M 2, 4-dinitrophenylhydrazine solution was added and allowed to stand for 20 mins at 20- 25 °C. 0.4 N NaOH (5 ml) was thereafter added and the absorbance was read at 546 nm after 5 mins. The blank was constituted as described above except that distilled water was used to substitute for the enzyme source. The absorbance value obtained from the spectrophotometer was used to obtain the equivalent activity of ALT by extrapolation from the standard curve.

Determination of Aspartate Aminotransferase Activity
The procedure for the calibration of AST standard curve and the determination of AST activity in the serum and tissue homogenate of rats as described in ALT except that AST buffered substrate was used instead of ALT buffered substrate. The absorbance value obtained from the spectrophotometer was used to obtain the equivalent activity of AST by extrapolation from the standard curve.

Determination of Alkaline Phosphatase Activity
Alkaline phosphatase (ALP) was assayed using the method described by Wright et al., 1972 [33].
0.1 M carbonate buffer (pH 10.1) (2.2 ml) was dispensed into clean test-tube 0.1 ml of 0.1 M MgSO$_4$ and 0.2 ml of the enzyme source (serum or tissue homogenate) were added. The mixture was equilibrated in water bath for 10 mins. An aliquot (0.5 ml) of 19 mM ρ-nitrophenyl phosphate was added and the mixture incubated at 37 °C for 10 mins. The reaction was stopped with 2.0 ml of 1 N NaOH and the absorbance read at 400 nm. Blank was prepared by adding enzyme source to the test tube labelled blank immediately after stopping the reaction with NaOH. Enzyme activity was calculated using the following expression:

$$\text{Enzyme activity (nM/min/ml)} = \frac{\Delta OD/min \times 1000 \times TV \times F}{9.9 \times SV \times L}$$

Where:
ΔOD/min = Change in optical density of reaction mixture per minute, TV = Total volume of the reaction mixture, F = Total dilution factor, SV = Volume of enzyme source, L = Light path length (1cm), 9.9 = Extinction co-efficient of 1 μm of ρ-nitrophenol in an alkaline solution of 1 ml and 1 cm path length, 1000 =The factor introduced to enable the enzyme activity to be expressed in nM/min/mg protein.
Therefore, Specific activity (nM/min/mg protein) is given by

$$= \frac{\text{Enzyme activity (nM/min/ml)}}{\text{Protein concentration (mg/ml)}}$$

Determination of Reduced Glutathione (GSH)
The method of Beutler et al., 1963 [34], was adopted in the estimation of the level of reduced glutathione (GSH) in the liver homogenate. Enzyme source (0.2 ml) was added to 1.8 ml of distilled water and 3 ml of the precipitating agent, sulphosalicylic acid was mixed with the sample. This was centrifuged at 3,000 g for 4 mins. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman's reagent. A blank was prepared with 0.5 ml of the diluted precipitating agent and 4 ml of phosphate buffer and 0.5 ml of Ellman's reagent. The absorbance of the reaction mixture was taken within 30 mins of colour development at 412 nm against a reagent blank. The concentration of the GSH was extrapolated from the GSH standard curve.

Data analysis
One way analysis of variance was used to analyse the results and Duncan multiple tests was applied for the *post hoc* [35]. Statistical package for Social Science (SPSS) was used for the analysis. The p value < 0.05 was considered statistically significant in the analytical data.

6. Results

Table 1: Shows different mean results of assays carried out on the liver tissue homogenate and the serum of both the treated and untreated Albino Rats after the 14 days study, n= 4 with their different ± S.E

| Groups	MDA Liver	ALT		ALP		AST		GSH Liver
		Liver	Serum	Liver	Serum	Liver	Serum	
Control (+ve)	150.75±39.53ᵃ	37.08±3.11ᵃ	12.96±1.34ᵃ	44.85±8.76ᵃ	153.18±38.27ᵃ	81.71±4.49ᵃᵇ	9.123±2.82ᵃ	4.13±0.28ᶜ
Control (-ve)	190.35±24.04ᵃ	43.80±0.49ᵇ	13.80±1.00ᵃ	54.51±34.60ᵃ	220.11±19.33ᵃ	100.01±12.35ᵇ	3.51±2.13ᵃ	3.47±0.13ᵇ
Group 2	245.25±42.62ᵃ	39.07±1.76ᵇ	13.84±1.11ᵃ	31.74±11.30ᵃ	158.70±59.94ᵃ	86.84±5.77ᵃᵇ	5.53±3.44ᵃ	3.27±0.06ᵇ
Group 3	162.00±19.98ᵃ	38.75±0.70ᵃ	15.00±0.98ᵃ	42.09±16.42ᵃ	269.10±46.53ᵃ	87.76±10.40ᵃᵇ	11.05±2.40ᵃ	3.01±0.29ᵃᵇ
Group 4	172.58±38.89ᵃ	37.64±0.91ᵃ	13.01±1.08ᵃ	22.08±5.63ᵃ	188.37±7.72ᵃ	70.13±9.55ᵃ	6.05±2.08ᵃ	2.45±0.06ᵃ

7. Discussions

Lipid peroxidation produces a wide variety of oxidation products in which the primary ones are lipid hydroperoxides (LOOH) with malondialdehyde (MDA) being one of many different aldehydes which can be formed as secondary products during lipid peroxidation [36]. MDA which is a lipid peroxidation biomarker, appears to be the most mutagenic product of lipid peroxidation and its content has been researched to be unstable due to the presence of aldehyde oxidase which catalyses it in the tissue cells [37]. However, studies on hepatocytes suggested that acetaminophen toxicity may induce the formation of MDA through iron-mediated oxidative stress by Fenton reaction and other nitrosative-routes; also, the importance of iron in the toxicity of acetaminophen has been shown by researchers [38]. Considering table 1 above, the data showed a remarkable increase in MDA levels in the group treated with acetaminophen alone when compared with the normal group indicating an induced oxidative stress in the hepatic tissue, this suggests that, the increase observed in the level of MDA above the normal group could have followed the literature described by Adamson and Harman, 1993 [38]. Also, there was significant ($p < 0.05$) reduction observed in the levels of MDA within the treated groups compare to that of the untreated control (negative) group with noticeable increase in the MDA as mentioned above and observable reduction when compare to that of control group (positive).

The appearance of acetaminophen-protein adducts in serum correlated with increases of ALT and AST in serum [39]. These data indicated that the presence of acetaminophen-protein adducts in serum was a biomarker for the formation of hepatic acetaminophen-protein adducts and acetaminophen toxicity. In the table above, there were significant ($p > 0.05$) increase in the level of ALT and AST in the tissue homogenate signalling hepatic necrosis in correlation with Prescott *et al*, 1977 [40], that summarized the clinical and biochemical changes to be an intense increase in serum alanine aminotransferase (ALT) and asparatate aminotransferase (AST) levels just as observed in the above table 1.

In the presence of increasingly amount of MDA, the carbonyl group of MDA covalently binds with proteins and subsequently depletes glutathione, the amount of covalent binding correlated with the relative hepatotoxicity [41, 42]. Even though there is an excellent correlation between covalent binding of acetaminophen to protein and development of hepatotoxicity, there is significant evidence that suggest that covalent binding only is not the majorly mechanism of toxicity but subsequently depletes glutathione, nucleic acids and as well as amino groups of phospholipids [41]. Glutathione depletion may predictably lead to an increased intracellular peroxide levels and increased oxidative stress through a Fenton reaction [43]. This mechanism involves the reduction of peroxide by ferrous ions forming the highly reactive hydroxyl radical which may in turn oxidize lipids and initiate lipid peroxidation [44]. Loss of glutathione with an increased formation of reactive oxygen (ROS) in hepatocytes which induce necrotic changes in the hepatocytes has been implicated in hepatotoxicity [43]. In the table 1 above, there showed marked reduction in glutathione (GSH) levels in liver tissues, indicating oxidative stress has been induced with considerable amount of MDA being generated. Studies have shown that diethyl maleate also depletes hepatic glutathione without causing toxicity, It has been postulated that glutathione depletion ordinarily is not the mechanism of toxicity [43], studies has shown that drugs that deplete GSH or influence the activity of any of the GSH-dependent enzymes may result in toxic response(s), while the formation of 3-glutathion-*S*-yl-acetaminophen in the liver increases the risk of hepatotoxicity [38, 42].
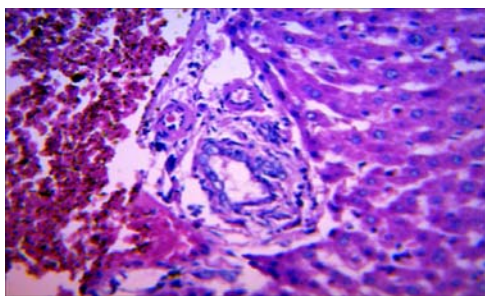
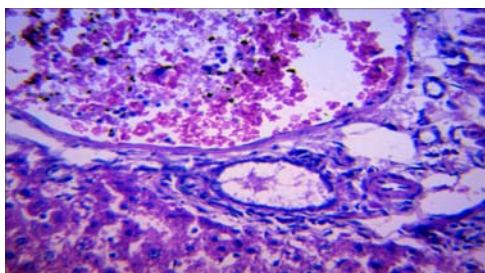## 8. Histological Pictures of the Liver Tissues
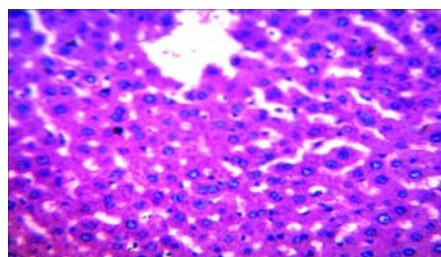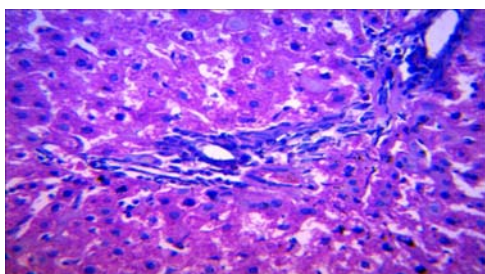

Control group: Positive
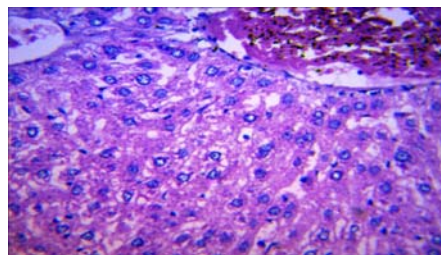
Control group: Negative without *Tt*

Group 1: Acetaminophen + 100 mg/kg *Tt*

Group 2: Acetaminophen + 200 mg/kg *Tt*

Group 3: Acetaminophen + 300 mg/kg *Tt*

Photographs of the Histological alterations in the liver after the administration of acetaminophen with and without aqueous extract of *Talinum triangulare* (*Tt*) (H&E 100×).

9. Histological analysis
Photomicrograph of a liver in the control group (positive) - where no visible lesions seen. Control group negative (Acetaminophen without *Tt*)- There is a severe portal congestion; the bile duct is hyperplasic. Group 1: (Acetaminophen + 100 mg/kg *Tt*)- There is a moderate to severe portal congestion; there is a moderate periportal cellular infiltration by mononuclear cells. Group 2: (Acetaminophen + 200 mg/kg *Tt*)- There is a mild portal congestion; there is a moderate diffuse vacuolar degeneration of hepatocytes. Few hepatocytes are necrotic. Group 3: (Acetaminophen + 300 mg/kg *Tt*)- There is a moderate to severe periportal cellular infiltration and periportal fibrosis; there is a mild diffuse vacuolar degeneration of hepatocytes.

10. Conclusion
In the present study, it has been clearly shown that aqueous extract of *Talinum triangulare* leave part demonstrated amelioration properties in Acetaminophen hepatotoxicity as implicated in the rat subjects used for the experiment. Hepatotoxicity induced by excess acetaminophen being the most common cause of death through acute liver failure (ALF), could therefore be ameliorated and managed in the future through dietary intake of *Talinum triangulare* in moderately engaged amount as it has been demonstrated in this work.

12. Conflict Of Interest
The authors hereby declare that, there are no conflicts of interest

13. References
1. Maton A, Jean H, Charles WM, Susan J, Maryanna QW, David L *et al*. Human Biology and Health. Englewood Cliffs, New Jersey, USA, Prentice Hall, ISBN 0-13-981176-1, 1993.
2. Davern TJ. Drug-induced liver disease. Clin Liver Dis 2012; 16:231.
3. Zimmerman HJ. Drug-induced liver disease. Clin Liver Dis 2000; 4:73.
4. Moore M, Thor H, Moore G, Nelson S, Moldéus P, Orrenius S. The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic Ca^{2+}. J Bio Chem 1985; 260(24):13035-13040.
5. Jack AH, Dean WR, Laura PJ. Mechanisms of Acetaminophen-Induced Liver Necrosis. Handb Exp Pharmacol 2010; 196:369-405.
6. Aghababian RV. Essentials of Emergency Medicine. Jones & Bartlett Publishers, 2010, 814.
7. Ahmad, Jawad. Hepatology and Transplant Hepatology: A Case Based Approach. Springer. ISBN 978-1-4419-7085-5, 2010, 194.
8. Davidson DG, Eastham WN. Acute liver necrosis following overdose of paracetamol. Br Med J 1966; 5512:497-499.
9. Daly FF, Fountain JS, Murray L, Graudins A, Buckley NA. Guidelines for the management of paracetamol poisoning in Australia and New Zealand—explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australasian poisons information centres. Med J Aust 2008; 188(5):296-301.
10. García Rodríguez LA, Hernández-DS. The risk of upper gastrointestinal complications associated with nonsteroidal anti-inflammatory drugs, glucocorticoids, acetaminophen, and combinations of these agents. Art ResTher 2000; 3(2):98-101.
11. Leung WTW, Busson F, Jardin C. Phytochemical composition of *Talinum triangulare* and food composition table for use in Africa. FAO, Rome, Italy, 1968, 306.
12. Hawkins LC, Edwards JN, Dargan PI. "Impact of restricting paracetamol pack sizes on paracetamol poisoning in the United Kingdom: a review of the literature". Drug Saf 2007; 30:465-79.
13. Larson AM, Polson J, Fontana RJ. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study". Hepatol 2005; 42:1364-72.
14. Dart RC, Erdman AR, Olson KR, Christianson G, Manoguerra AS, Chyka PA *et al*. American Association of Poison Control Centers. "Acetaminophen poisoning: an evidence-based consensus guideline for out-of-hospital management". Clin toxicol, 2006, 44.
15. Akiko E, Ken I, Eiko N, Junko H, Toyoe K, Tania O *et al*. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of

ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci 2001; 59:169-177.

16. Raucy JL, Lasker JM, Lieber CS, Black M. Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. Arch Biochem Biophys 1989; 271:270-283.

17. Dong H, Haining RL, Thummel KE, Rettie AE, Nelson SD. Involvement of human cytochrome P450 2D6 in the bioactivation of acetaminophen. Drug Metab Dispos 2000; 28:1397-1400.

18. Gutteridge JMC, Wilkins S. Copper-dependent hydroxyl radical damage to ascorbic acid. Formation of a thiobarbituric acid-reactive product. FEBS Letters 1982; 137(21): 327-330.

19. Hinson JA, Monks TJ, Hong M, Highet RJ, Pohl LR. 3-(glutathion-S-yl) acetaminophen: a biliary metabolite of acetaminophen. Drug Metab Dispos 1982; 10:47-50.

20. Borne RF. Nonsteroidal Anti-inflammatory Agents. In: Foye's Principles of Medicinal Chemistry 6th Edition, Williams DA, Lemke TL, Lippincott Williams & Wilkins Philadelphia, 2007, 751-793.

21. Prescott LF. Paracetamol (Acetaminophen): A Critical Bibliographic Review. CRC Press, 1996.

22. Michele ZK. Comparison of oral and i.v. acetylcysteine in the treatment of acetaminophen poisoning. Am J H Pharm 2006; 63(19):1821-1827.

23. Dawson AH, Henry DA, McEwen J. Adverse reactions to N-acetyl cysteine during treatment for paracetamol poisoning. Med J Aust 1989; 150(6):329-331.

24. Rumack BH, Peterson RC, Koch GG, Amara IA. Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. Arch Intern Med 1981; 141:380-385.

25. Corcoran GB, Racz WJ, Smith CV, Mitchell JRJ. Effects of N-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. Pharmacol Exp Ther 1985; 232(3):864-72.

26. Andarwulan N, Batari R, Sandrasari DA, Bolling B, Wijaya H. Flavonoid content and antioxidant activity of vegetables from Indonesia. Food Chem 2010; 121:1231-1235.

27. Eweka AO, Eweka A, Om'Iniabohs FAE. Histological studies of the effects of monosodium glutamate of the fallopian tubes of adult female Wistar rats. North Am J Med Sci 2010; 2:146-149.

28. Keeffe EB, Friedman LM. Handbook of liver diseases. Edinburgh, Churchill Livingstone, 2004, 104-123.

29. Aja PM, Okaka ANC, Onu PN, Ibiam U, Urako AJ. Phytochemical Composition of Talinum triangulare (Water Leaf) Leaves. Pak J Nutr 2010; 9:527-530.

30. Nwoha PU, Ojo GB, Ajayi SA, Ofusori DA, Oluwayinka OP, Odukoya SA *et al*. Garcinia kola diet provides slight protection to mice hippocampal neurons against neurotoxins. J Environ Neurosci Biomed 2007; 1(2):125-136.

31. Buege JA, Aust SD. Microsomal lipid, Peroxidation. In: Flesicher, S., Packer, L. (Eds.), Methods in Enzymology. Academic Press, New-York 1978; 52:302-310.

32. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56-63.

33. Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine: Alkaline phosphatase. Enzymol 1972; 42:459-468.

34. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882-890.

35. Zar JH. Biostatistical analysis: Prentice-Hall, Englewood Cliffs, Edn 2, 1984, 718.

36. Esterbauer H, Cheeseman KH, Dianzani MU, Poli G, Slater TF. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe 2+ in rat liver microsomes Bio J 1982; 208:129-140.

37. Chiu D, Kuypers F, Lubin B. Lipid Peroxidation in human red cells. Sem Hematol 1989; 26:257-276.

38. Adamson GM, Harman AW. Oxidative stress in cultured hepatocytes exposed to acetaminophen. Biochem Pharmacol 1993; 45:2289–2294.

39. Pumford NR, Roberts DW, Benson RW, Hinson JA. Immunochemical quantitation of 3-(cystein-S-yl) acetaminophen protein adducts in subcellular liver fractions following a hepatotoxic dose of acetaminophen. Biochem Pharmacol 1990; 40:573-579.

40. Prescott LF, Park J, Ballantyne A, Adriaenssens P, Proudfoot AT. Treatment of paracetamol (acetaminophen) poisoning with N-acetylcysteine. Lancet 1977; 2:432-434.

41. Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J Pharmcol Exp Ther 1973; 187:185-194.

42. Jack AH, Dean WR, Laura PJ. Mechanisms of Acetaminophen-Induced Liver Necrosis. Handb Exp Pharmacol 2010; 196:369-405.

43. Aust SD, Morehouse LA, Thomas CE. Role of metals in oxygen radical reactions. J Free Radic Biol Med 1985; 1:3-25.

44. Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. J Pharmacol Exp Ther 1973; 187:195-202.