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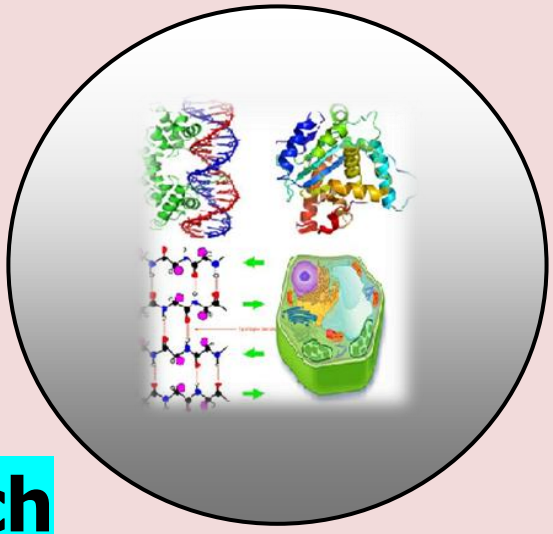

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RESEARCH PAPER

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Evaluation of the Effects of *Azadirachta Indica* Leaf Aqueous Extract on Liver Enzymes of Paracetamol - Induced Oxidative Stress in Wistar Rats

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ABSTRACT

One of the potent versatile medicinal plants, Azadirachta indica, used widely in India, Nigeria and other parts of the world has been discovered to inhibit paracetamol induced oxidative stress resulting to reduced production of malondialdehyde (MDA) and consequently preventing hepatotoxicity. Twenty four Wistar rats, placed into four groups, each containing six rats were used. Group A (control) was given only normal saline; group B was given paracetamol (800mg/kg body weight) to induced oxidative stress; groups C and D were given paracetamol (800mg/kg body weight) and graded doses of A. indica leaf aqueous extract (400mg and 800mg/kg body weight respectively). The animals were weighed before and after the experiment. The plasma and liver homogenate levels of MDA and liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatases (ALP) were estimated. There was significant increase (P<0.05) in the final weights of groups A and D rats, decrease in B and no significant change in C. The plasma and homogenate levels of MDA were significantly increased (P< 0.05) in group B against A, and groups C and D significantly decreased against B. ALT, AST, and ALP were significantly increased (P<0.05) in B compared to A and decreased in C and D compared to B.

These findings show that paracetamol-induced oxidative stress indicated by increased MDA and liver enzymes in B might have been effectively neutralized on treatment with the extract, suggesting the hepatoprotective property of A. indica.
Key words: Azadirachta indica, Oxidative Stress, Paracetamol, Malondialdehyde and Liver Enzymes.

INTRODUCTION

Plants have long been recognized as synthetic media capable of making diversity of organic molecules that have complex structures and variety of physical, chemical and biological properties (Mayunga, 1996). These phytochemicals have the promise of ever remaining an inexhaustible source of medicines for the relief of man's many diseases and pains. *Azadirachta indica* (*A. indica*) commonly known as Neem, an Indomalaysian plant is found also in Nigeria, most of the tropical and subtropical countries and is widely distributed in the world. The aqueous leaf extract of *Azadirachta indica* (*A. indica*), has been demonstrated to offer protection against paracetamol induced liver necrosis (Bhanwra *et al*, 2000). *Azadirachta Indica (neem)* leaves extracts have been recorded to prevent hepatocellular damage induced by paracetamol in rats (Bhanwra *et al*, 2000). It possesses potent immnostimulant activity which is evidenced by both humoral and cell mediated responses(Sen, 1992). The chemical constituents found in the leaves of neem are nimbin, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n- hexacosanol and amino acid, 7-sdesacetyl-7- benzoylazadiradione,7-sdesacetyl-7-benzoylgedunin, 17-hydroxy azadiradione and nimbiol(Kokate *et al*, 2010; Hossain *et al*,2013). It has been shown that levels of serum AST and ALT elevated in paracetamol induced oxidative stress, indicative of liver damage, were found to be significantly reduced on administration of aqueous leaf extract of *A. indica* (Kausik *et al*, 2002). Hepatocellular activity of aqueous leaf extract of *A. indica* has been demonstrated by the finding that it offers protection against paracetamol induced liver necrosis in rats (Bhanwra *et al*, 2000). It has also been discovered that fresh juice of tender leaves of *A. indica* inhibited paracetamol induced lipid peroxidation resulting to reduced production of MDA (Yanpallewar *et al*, 2003).

Oxidative stress is caused by the presence of reactive oxygen species (ROS) in excess of the available antioxidant buffering capacity (Momoh, *et al* 2015). Reactive oxygen species are highly reactive and in the absence of any protective mechanism can disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of cell including proteins, lipids and DNA, thus altering the structure and function of the cell, tissue, organ and system respectively. Oxidative stress from oxidative metabolism causes base damage as well as strand-breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species generated, e g. superoxide radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) (Chandra *et al,* 2015). Many studies have shown that ROS can damage lipids, proteins and DNA, thus altering the structure and function of the cell, tissue, organ and system respectively. SOD is an effective defence enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide (H_2O_2) (Momoh *et al* , 2015). The assaults by ROS lead to changes in membrane permeability, membrane lipid bilayer disruption and functional modification of various cellular proteins (Marian *et al*., 2007; Malavi and Mehta 2004). In addition to these damages, abnormalities in myocyte function due to increased oxidative stress are considered to be associated with the effects of ROS on subcellular organelles (Kaneko *et al.,* 1989). Increased ROS production lowers cellular antioxidant levels and enhances the oxidative stress in many tissues, especially the liver (Hye-Lin Ha *et al.,* 2010). Documented evidence has been reported that reactive oxygen species (ROS), including singlet oxygen, superoxide, and hydroxyl radicals, are known to play an important role in liver-disease pathology and progression (Vitaglione *et al.,* 2004). ROS have also been proven to be associated with paracetamol-induced hepatotoxicity (Momoh *et al.*, 2015).

Exposure to oxidative stress induces a series of antioxidant genes through the activation of the antioxidant response element (ARE) as a protective mechanism. ARE-containing gene expression is largely regulated by Nrf2 (Cichoż-Lach and Michalak 2014). Studies conducted on various animal models have indicated that the Nrf2-ARE loop counteracts alcoholic and nonalcoholic liver disease by activating gene expression. And studying Nrf2 has expanded the understanding of oxidative stress and may enable the design of new therapies against liver disorders connected with redox state (Shin *et al.,* 2013).

Paracetamol (acetaminophen) is a commonly and widely used analgesic and antipyretic drug. At the therapeutic levels, paracetamol is primarily metabolized in the liver by glucuronidation and sulphation; however, a small proportion reacts with cytochrome P450 -mediated bioactivation to N-acetyl-p-benzoquinoimine (NAPQI), which is rapidly quenched by glutathione (James *et al* 2003). Research has shown that at high doses, paracetamol leads to undesirable side effects, such as liver necrosis and hepatic injury, and production of reactive oxygen species (ROS) that lead to oxidative stress. Several researchers have reported elevations in serum transaminases following administration of toxic doses of paracetamol in rats (Randle *et al*.,2008; Rasool *et al*., 2010; Mahesh *et al*.,2009).

It is well documented that liver tissue contains relatively high content of polyunsaturated fatty acids (PUFAs), which are sensitive to peroxidative damage, and lead to an increase in lipid peroxidation in the group of animals intoxicated with paracetamol. Any oxidative insult to a cell induces lipid peroxidation of cell membrane lipids. The lipid peroxidation products cause widespread damage of macromolecules. The peroxidation of lipid components of cells by reactive oxygen species (ROS) generates toxic species like lipid peroxides, lipid hydroperoxides and aldehyde breakdown products (Sherlock 1995). Lipid peroxidation can be described generally as a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) producing malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) (Ayala *et al*., 2014). MDA can cross-link with amino group of proteins to form intra and inter molecular cross-links thereby inactivating several membrane bound enzymes (Kikhugawa *et al* 1984). MDA is widely used to assess lipid peroxidation.

Liver is the major organ for detoxification and removal of endogenous substances. It is continuously and widely exposed to hepatotoxins, xenobiotics and chemotherapeutic agents that lead to impairment of its functions. Hepatocytic proteins, lipids and DNA are among the cellular structures that are primarily affected by ROS and reactive nitrogen species (Cichoż-Lach and Michalak 2014).The liver is therefore equipped with a special defense mechanism to scavenge ROS, in which nuclear factor E2-related factor 2 (Nrf2) plays an important role. Elevated levels of ROS and electrophiles cause Nrf2 to release from sequestration and translocate to the nucleus, where it promotes the transcription of cytoprotective genes. Nrf2 assists in protecting the liver, through increased sensitivity to acetaminophen-induced centrilobular hepatocellular necrosis and hepatotoxicity (Thomson 2013). The commonest enzymes regarded as indicators of liver damage are aspartate transaminase(AST), alanine transaminase (ALT), and alkaline phosphatases (ALP). The damage to hepatocellular cells results in increase in these enzymes activities (Nnodim *et al*., 2012).

MATERIALS AND METHODS

Plant Materials

Procurement

Fresh matured Leaves of *A. indica* were collected from a local neem tree at Ihiala, Anambra State, Nigeria, and identified at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A voucher specimen was deposited at the herbarium for further references.

Extract preparation

The leaves were thoroughly washed and dried in carbonated moisture extraction drying oven (Grand instruments, Cambridge, England) at 45^0C - 50^0C for 3 hours. Grinding was done using Thomas contact Mills (Py Unicam, Cambridge, England), the powder was sieved through 1mm sieve and 200g and soaked in 1000mls of water and allowed to stand for 48hours. The extract was filtered and the filtrate dried using hot air oven (Arrant instrument, Cambridge, England) at 45^0C - 50^0C. The residue yield was 52g and appropriate concentrations made for the experimental design using distilled water (Nunomura *et al*., 2006).

Paracetamol

A commercially available brand of paracetamol tablets (Emzor) were purchased from a registered pharmaceutical shop at Ihiala, Anambra State, Nigeria. The tablets were dissolved in distilled water (w/v) according to the required concentration for the administration on the Wistar rats on the basis of body weight.

Experimental Animals

Wistar rats weighing 150 - 250g were procured from the Animal House of College of Medicine and Health Science, Imo State University, Owerri. They were maintained under controlled conditions of light (12/24 hours) and temperature. The animals were fed with standard pellet diet (product of Pfizer, Nigeria Ltd) and allowed free access to water *ad libitum* throughout the period of the experiment (Challopadhyay and Bandyopadhyay, 2005).

Experimental Design

Twenty four wistar rats were used in this study and randomly divided into four groups of six animals each as shown in table 1.

Table 1: Experimental design showing the groups of Wistar rats and the treatments they were given.

Group	*Treatment given*
Group A (Control)	Received only normal saline (0.9% NaCl w/v) 5ml/kg body weight.
Group B	Received only paracetamol (800mg/kg body weight) once daily.
Group C	Received paracetamol (800mg/kg body weight) and *A. indica* leaves aqueous extract (500mg/kg bodyweight) once daily.
Group D	Received paracetamol (800mg/kg body weight) and *A. indica* leaves aqueus extract (1000mg/Kg bodyweight) once daily.

Leaf extract, paracetamol and saline were administered with the aid of a feeding canula.

Sample collection

All the animals were treated for 28 days. After fasting for 16h following the last administration they were weighed and sacrificed via euthanasia using chloroform. Blood was collected by cardiac puncture, allowed to clot and then centrifuged at 10,000 revolutions per minute for 5 minutes using Wisperfuge model 1384 (Tamson, Holland). Serum was separated for various biochemical analysis and stored at -20° prior to use. The livers were dissected from all the animals, cleared of blood using normal saline and immediately transferred into blood ice-cold container of normal saline.

Acute Toxicity Testing

The acute toxicity of *A. indica* aqueous leaf extract was done using 30 mice divided into 5 groups of 6 mice each. Each group received graded doses (200 - 1000mg/kg body weight) of the extract and the animals observed for toxic effects after 48 hours of treatment. The toxicological effect was observed in terms of mortality expressed as LD_{50}. The number of animals that died during the period was noted. The LD_{50} of the extract was estimated from the graph of percentage (%) mortality, converted to probity, against log - dose of the extract, probit 5 being 50% (Litch Field and Wilcoxon, 1959).

Laboratory methods and procedures/biochemical analysis

The serum AST and ALT were determined using the Reitman and Frankel method (1957) while ALT was measured by King and King method (1954). Malondialydehyde (MDA) was determined by the method of Albro *et.al.* (Albro *et al.* 1986).

Statistical Analysis of Data

All values were expressed as mean ± SD and then subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago Illinois). Statistical significance was considered at ρ< 0.05.

RESULTS

Table 1. Mean weight changes (g) in Wistar rats treated with paracetamol.

Groups	Initial weight (g)	Final weight (g)	Change in weight (g)
A	167±8.4	208 ±8.3	41± 6.2*
B	184 ± 3.7	175 ± 4.4	- 9±3.1*
C	171 ±5.8	177±5.6	6 ±4.2
D	194 ±5.8	218 ±5.1	24 ±4.1*

*Significantly different (p<0.05) from initial weight.

Table 2 shows that there was a significant increase (p<0.05) in the body weight of rats in groups A and D, decrease in group B and no significant change (p<0.05) in the weights of Wistar rats in group C.

Table 2. Plasma and liver homogenate MDA levels of different groups of paracetamol treated Wistar rats treated with *A. indica*: aqueous leaf extracts and control.

Groups	MDA	
	Homogenat (nmol/ml)	Serum (nmol/ml)
A	13.3 ± 0.44	3.51 ± 0.16
B	24.1 ± 0.22*	7.79 ± 0.152*
C	13.6 ± 0.22***	4.87 ± 0.13*
D	12.7 ± 0.21***	3.97 ± 0.56***

Key

*Significantly different (*P*<0.05) from group A

** Significantly different (*P*<0.5) from group A and B

*** Significantly different (*P*<0.5) from group B

In table 3, it was observed that there was a significant increase (P<0.05) in the liver homogenate level of MDA in group B rats when compared with group A, while groups C and D did not show significant difference from group A. Group C and D showed significant decrease when compared with group B. This table also shows that the plasma MDA level in group B is significantly increased compared with group A while the plasma MDA levels in groups C and D indicate significantly decrease when compared with group B. Group C was significantly different while group D was not significantly different compared with group A.

Table 3. Plasma levels of hepatic marker enzymes of various groups of paracetamol treated Wistar rats with *A. indica* aqueous leaf extract, and control.

Groups	AST (μ/l)	ALT (μ/l)	ALP (μ/l)
A	60.6 ± 3.98	62.8 ± 3.25	80.4 ± 2.33
B	106.2± 2.56*	100 ± 4.1*	89.2 ± 3.12*
C	91.6 ± 3.44**	98.6 ±3.93*	86.2 ± 2.71
D	69.2 ± 2.48***	65.6 ± 2.8***	80.1 ± 3. 12***

Key
*significantly different ($p<0.05$) from control group A
**Significantly different ($p<0.5$) from group A and B
***Significantly different ($p<0.05$) from group B.

Table 4 shows that there was significantly area ($p<0.05$) in the plasma levels of the liver enzymes AST, ALT, and ALP in group B compared to those of group A. The plasma level of AST in group C Wistar rats was significantly increased ($p<0.5$) compared with group A but significantly decreased when compared with group B. the plasma ALT level in group C was significantly increased when compared with group A but no significant difference from group B. There was no significantly variation in plasma ALP level of group C from those of groups A and B rats. This table also shows that there was no significant difference in the plasma levels of AST, ALT and ALP in group D compared with group A. There was, however, significant decrease in plasma level of AST, ALT and ALP in group A compared with group B. It was evident from the table that there were significant difference in the plasma levels of ALP between group C and D.

DISCUSSION

In this present study, it appears that the overall effects of various treatments of the different groups, used in this study, were summarized in their respective weight changes presentation (Table 1). The significant weight loss in paracetamol treated Wister rats may be attributed to the negative biochemical effect engendered by the induced oxidative stress, while the no significant weight change observed in the ones treated with both paracetamol and *A. indica* aqueous leaf extract reflects the summary of the inhibitory effect of *A. indica* aqueous leaf on oxidative stress.

The observed increase in the hepatic marker enzymes, AST, ALT and ALP, in paracetamol treated Wister rats (table 2) agrees with its already documented hepatotoxic effect (Sen, *et., al.,* 1992). And the no significant change demonstrated by these enzymes in the Wister rats treated with both paracetamol and *A. indica* extract is also similar to documented reports on hepatoprotective activity of *A*. *indica* by Chattopadhyay and Bandyopadhyay (2005). The elevated levels of these hepatic enzymes in paracetamol treated Wister rats (group B) also agree with the alteration in the hepatocellular integrity. The significant increase in liver enzymes and MDA, which are indicators of oxidative stress and its resultant hepatotoxicity, in paracetamol intoxicated wister rats, as observed in this study supports the concept that paracetamol over-dose is a precursor of pro-oxidants, which mediate oxidative stress and cell death as reported by Gujral*, et.al.* (2002). The mild but significant effect on ALP may mean that though paracetamol intoxication causes hepatocellular damage (Hazai, *et. al*., 2002), cholestasis may not be primarily involved.

The increase in MDA implies increased lipid peroxidation as a result of oxidative stress which could have been induced by paracetamol, as shown in table 4. The no significant change in MDA of groups C and D compared with control (group A) animals and the significant decrease when compared with group B animals, may mean that *A. indica* aqueous leaf extract can offer protection against paracetamol induced free radical injury and thus prove to be a good antidote to oxidative stress and its resultant cell death (hepatotoxicity). This finding helps to potentiate the fact that the extract could be an effective antioxidant, hence its hepatoprotective potential.

Consequently, the observed variations in the elevated or reduced levels of biochemical parameters evaluated in this work, with regards to varied doses of *A. indica* extract used, could be an evidence that its effect in controlling oxidative stress and hepatotoxicity may be dose-dependent.

CONCLUSION

Based on the findings in this study, it could be inferred that *A. indica* aqueous leaf extract has antioxidative and consequent hepatoprotective effects on paracetamol-treated Wister rats. This could be achieved by either directly scavenging the reactive oxygen metabolites or enhancing the levels of antioxidant molecules. Considerably, having observed the invaluable role *A. indica* could play in attenuating or obliterating oxidative stress conditions including hepatocellular damage, it could be recommended that further studies be carried out to elucidate the molecular basis of both the biochemical and pharmacological involvement of this plant extract in achieving this.

REFERENCES

Albro, P.W., Corbelt, J.T. and Schroeder, J.L. (1986). Application of thiobarbituate assay to the measurement of lipid peroxidase product in microsomes. *Chem. Bio. Interact.* 86: 185 -196.

Ayala, A., Muñoz, M.F. and Argüelles, S. (2014). Review: Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Longev.* http://dx.doi.org/10.1155/2014/360438.

Bhanwra, S., Singh, P. and Koshla, P. (2000). The effect of *Azadirachta indica (Neem)* leaf aqueous extract on paracetamol induced liver damage in rats. *Indian Journal of Physiology and Pharmacology* 44: 64-69.

Chandra Kala, Syed Salman Ali, Abid Mohd, Sweety Rajpoot and Najam Ali Khan (2015). Protection Against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costus speciosus Rhizome Extract. *Int. J. Pharmacog. Phytochem. Res.* 7 (2): 383 - 389.

Chattopadhyay, R.R. and Bandyopadhyay, M. (2005). Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. *African J. Biomed. Res.* 8(2):101 - 104.

Esterbauer, H., Eckl, P. and Ortner, A. (1990). "Possible mutagens derived from lipids and lipid precursors," *Mut. Res.* 238 (3): 223 – 233.

Halina Cichoż-Lach and Agata Michalak (2014). Oxidative stress as a crucial factor in liver diseases. *World J. Gastroenterol.* 20(25): 8082–8091.

Hossain, M.A., Al-Toubi, W.A.S., Weli, A.M., Al-Riyami, Q.A. and Al-Sabahi, J.N. (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *J Taibah Univ Sci.,* 7(4): 181 - 188.

Hye-Lin, Ha, Hye-Jun Shin, Mark, A.F. and Dae-Yeul Yul (2010). Oxidative stress and antioxidants in pathogenesis. *World J gastroenterol.* 16 (48): 6035 - 6043.

James, L.P., Mayeux, P.R. and Hinson, J.A. (2003). Acetaminophen-induced hepatotoxicity. *Drug Metab Dispos.* 31: 1499 -1506.

Kaneko, M., Elimban, V. and Dalla, N.S. (1989). Mechanism of depression of heart sarcolema Ca2+ pump by oxygen free radicals. *Am. J. Physiol.* 254: H804 - H811.

Kausik, B., Ishita, C., Ranajit, K.B. and Uday, B., (2002). Biological Activities and Medicinal Properties of *Neem (Azadirachta indica). Current Science* 82(11):1336-1345.

Kikhugawa, K., Kosuge and Asakura, T. (1984). Effects of MDA, a product of lipid peroxidation on the function and stability of haemoglobin. *Arch. Biochem, Biophy.* 7: 229 - 232.

King, E.J. and King, P.R. (1954). Estimation of plasma phosphates by determination of hydrolysed phenol with amino antipyrene. *J. Chem. Path.* 7: 322 - 326.

Kokate, C., Purohit, A.P. and Gokhale, S.B. (2010). Pharmacognosy. *Nirali Prakashan India,* 10: 28 – 29.

Litch Field, J.T. and Wilcoxon, F. (1959). Determination of acute toxicity tests. *J. pharmacol. Exp. Ther*. 96: 99 – 113.

Mahesh, A., Shaheetha, J., Thangadurai, D. and Rao, D.M. (2009). Protective effect of Indian honey on acetaminophen-induced oxidative stress and liver toxicity in rat. *Biologia* 64: 1225-1231.

Malavi, B. and Mehta, J.L. (2004). Oxidative stress in cardiovascular disease: molecular basis of its deleterious effects, its delection and therapeutic considerations. *Curr Opin Cardiol,*19: 488 – 493.

Marian Valko, Dietar, L., Ja M., Mark, T.D., Cronin Milan M. and Joshu, T. (2007). Free radicals and antioxidants in normal physiological function and human disease. *Int. J. Biochem. cell Biol* 39: 44 - 84.

Marnett, L.J. (1999). Chemistry and biology of DNA damage by malondialdehyde. *IARC Sci. Publ.* (36) 17-27.

Mayunga, H. (1996). Natural products research development: theTanzanian experience. In: Science in Africa, (utilizing Africa' Genetic affluence through natural products research and development)sub-Saharan Africa Program, Washington. USA. 35.

Momoh, J.L., Adeteju, O., Damazio, O.A. and Eleyowo, O.O. (2015). Hepatoprotective Effect of Ethanolic Leaf Extract of *Vernonia amygdalina* and *Azadirachta indica* against Acetaminophen-Induced Hepatotoxicity inSprague-Dawley Male Albino Rats**.** *Am. J. Pharmacol. Sci.* 3(3):79-86. doi: 10.12691/ajps-3-3-5.

Nnodim, J.K., Ujowundu, C.O., Udujih, H.I., Okorie, H., Nwobodo, E.I. and Nwadike, C.N. (2012). Hepatocellular enzyme activities and protein level following administration of vitamins during chloroquine induced hepatotoxicity in wistar rasts. *Indian J. Innovations Dev.* 1 (4): 299 - 302.

Nunomura, A., Castellani, R.J., Zhu, X., Moreira, P.I., Perry, G. and Smith, M.A. (2006). Involvement of oxidative stress in Alzheimer disease. J. Neuro. Exp. Neuro. 65 (7): 631 - 41.

Randle, L.E., Sathish, J.G., Kitteringham, N.R., Macdonald, I., Williams, D.P. and Park, B.K. (2008). Alpha(1)-adrenoceptor antagonists prevent paracetamol-induced hepatotoxicity in mice. *Br J Pharmacol.* 153: 820-830.

Rasool, M.K., Sabina, E.P., Ramya, S.R., Preety, P., Patel, S., Mandal, N., *et al.* (2010). Hepatoprotective and antioxidant effects of gallic acid in paracetamol-induced liver damage in mice. *J Pharm Pharmacol.* 62: 638-643.

Sen, P., Medinata, P.K. and Ray, A. (1992). Immunostimulant activities of *A. indica. Ind. J. Exp. Biol.* 12:1170- 1175.

Sherlock, S. (1995). Alcohol and the liver, In: Sherlock S, ed. Diseases of the liver and the Billiary System, 6th ed. Blackwell Publication, London, 385 - 403.

Shin, S.M., Yang, J.H. and Ki, S.H. (2013). Role of the Nrf2-ARE pathway in liver diseases. *Oxid. Med. Cell Longev.* 763257.

Thomson Glen (2013). Review: Nrf2, a multi-organ protector. NRF2.com on May 3, 2013 http://www.fasebj.org/content/19/9/1061.long.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamate oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT). *Am. J. clin. Path,* 1957; 28: 56 – 63.

Vitaglione, P., Morisco, F., Caporaso, N. and Fogliano, V. (2004). Dietary antioxidant compounds and liver health. *Crit. Rev. Food Sci. Nutr*. 44: 575-586.

Yanpallewar, S.U., Sen S., Kumar, M., Raju, S.S. and Achary, S.B. (2003). Effect of *Azadirachta indica* on paracetamol induced hepatic damage in albino rat. *Phytomedicine* 10:391-396.

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