



COMPARATIVE STUDIES ON THE EFFECT OF TURMERIC AND GINGER AGAINST PARACETAMOL INDUCED LIVER DAMAGE IN FEMALE ALBINO RATS

Olushola Ladeji, Sangodele J. O, Chinenye Egwuonwu, Ijeoma B. Eluwa

Department of Biochemistry, Bingham University, Karu, Nigeria.

* Corresponding Author: Olushola Ladeji

Department of Biochemistry, Bingham University, Karu, Nigeria.

Article Received on 02/04/2016

Article Revised on 22/04/2016

Article Accepted on 12/05/2016

ABSTRACT

The present study was conducted to evaluate and compare the effect of the ethanol extract of *Curcuma longa* and *Zingiber officinale* against paracetamol induced liver damage in female albino rats. The rats were grouped into seven groups (A, B, C, D, E, F and G), with five rats in each group. Group A served as the normal control and was given normal saline orally (1ml/kg). Group B served as the positive control (paracetamol treated group) in which the rats were treated orally with paracetamol at dose of 400mg/kg body weight for 7 days. Group C was pretreated with a standard drug, silymarin -200mg/kg plus paracetamol for 7 days. Group D and E were pretreated with turmeric extract (200mg/kg and 400mg/kg respectively) prior to paracetamol treatment. Group F and G were pretreated with ginger extract (200mg/kg and 400mg/kg respectively) prior to the administration of paracetamol simultaneously for 7 days. Paracetamol, normal saline and the extracts were administered orally, once daily. Paracetamol at 400mg/kg induced liver damage in rats, manifested by statistically increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and thiobarbituric acid reactive substances, also reduced total serum protein. Pre-treatment of rats with ethanolic extracts of *Curcuma longa* and *Zingiber officinale* prior to paracetamol dosing lowered serum liver enzyme activities, bilirubin, thiobarbituric acid reactive substances and increased total serum protein. It was concluded from the result that the ethanolic extracts of *Curcuma longa* and *Zingiber officinale* possess hepatoprotective activity against paracetamol induced liver damage, with *Curcuma longa* (turmeric) having a higher potential when compared with *Zingiber officinale* (ginger).

KEYWORDS: paracetamol, *Zingiber officinale*, thiobarbituric, hepatoprotective.

INTRODUCTION

Hepatotoxicity is a common cause of severe metabolic disorders and even death (Patel et al, 2008). Some of the liver injuries are caused by the use and abuse of drugs. Conventional and/or synthetic drugs such as steroids, vaccines, antivirals and other medications can cause serious side effects, even toxic effects on the liver, especially when used for prolonged periods of time (Sehrawat et al., 2006). Xenobiotics are metabolized to inert metabolites that are excreted by the kidney, but some are metabolized to more reactive compounds that are more toxic than the parent compound. Examples of these drugs are: paracetamol, isoniazid and methotrexate. Paracetamol, a widely used over-the-counter (OTC) analgesic and antipyretic, is one of the best known experimental models of hepatotoxicity (Tunon et al., 2009). It is safe at therapeutic doses but causes a fatal hepatic necrosis and hepatic failure in overdose (Mitchell et al., 1973). Paracetamol at therapeutic doses is rapidly metabolized in the liver principally through glucuronidation and sulfation and only a small portion is

oxidized by cytochrome P-450 2E1 to generate a highly reactive and cytotoxic intermediate, N-acetyl -P-benzoquinoneimine (NAPQI), which is quickly conjugated by hepatic glutathione to yield a harmless water soluble product, mercapturic acid (Lee et al., 1996). When paracetamol is given at higher dose levels in animals or humans, its metabolism through glucuronidation and sulfation is saturated and NAPQI is synthesized in enough amounts to cause acute hepatotoxicity (Sun et al., 2009). Many research efforts are directed to the discovery and development of agents, which might protect cells from oxidative reactions with potential antioxidant and hepatoprotective effects (Knight et al., 2003).

There is a global trend towards the use of traditional herbal preparations for the treatment of liver diseases. Although many such modalities are available, herbal therapies are the most popular, and of these remedies, silymarin extracted from milk thistle plant (*silybum marianum*) is most widely subscribed to as a remedy for

liver disease (Bass, 2002). Botanical medicines have been used traditionally by herbalists and indigenous healers worldwide for the prevention and treatment of liver disease (Takeoka and Dao, 2003). They include: *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Glycyrrhiza glabra* (licorice), *Silybum marianum* (milk thistle), *Zingiber officinale* (ginger) and *Picrorhiza kurroa* (kutkin).

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

The fresh rhizomes of the plant (*Zingiber officinale*) were bought from the Mararaba market, Nasarawa, Nigeria. They were washed to remove sand and other impurities before cutting into small pieces and subsequently air dried for 14 days. Milling of the dried ginger was done using a sterilized manual hand grinder. Fresh rhizomes of *curcuma longa* were also purchased from the market, cleaned, dried and powdered.

The coarse powder of the plants was extracted with 90% ethanol in Soxhlet apparatus at 80°C for 72 h separately. Concentration of the extract was carried out in a water bath. The concentrated extract was then weighed and stored in a refrigerator for further use.

Phytochemical Analysis

The individual extracts were subjected to the qualitative phytochemical screening for the presence of some chemical constituents.

Test for alkaloids

This was carried out according to the method described by Ciulci, (1994). To 1.0ml of each extract in two separate test tubes, 3 drops of Dragendoff's reagent was added. An orange red precipitate/turbidity with Dragendoff's reagent indicates the presence of alkaloids.

Test for Flavonoid

Alkaline reagent test: Extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.

Test for saponins

This was carried out according to the method of Brain and Turner (1975). 0.5g of each extract was placed in a test tube and then 0.5ml of distilled water was added. The tube was then shaken vigorously. A persistent froth

that lasted for at least 15mins indicated the presence of saponins.

Test for reducing sugars

This was carried out according to the method of Brain and Turner (1975). 1ml of stock solution of each extract was diluted with 2ml of distilled water, followed by the addition of Fehling's solution (A+B) and the mixtures warmed. Brick red precipitate at the bottom of the test tubes indicated the presence of reducing sugars.

Test for Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

Test for Steroid

1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H_2SO_4 acid was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroid.

Experimental Design

Healthy weanling female albino rats weighing between 100–155 g were used throughout the study. The rats were obtained from the Animal House Facility, ABU Zaria. They were housed at room temperature and fed ad libitum with food pellet and water. The animals were acclimatized to laboratory conditions for 2 weeks before the commencement of experiments. The animals were housed in polycarbonate cages with steel wire tops and wood-cube bedding (five rats per cage).

The rats were grouped into seven groups (A, B, C, D, E, F and G). Group A served as the normal (negative) control, Group B served as the positive control (paracetamol treated group) in which the rats were treated orally with paracetamol at dose of 400mg/kg body weight for 7 days according to Madhu Kiran et al. (2012) for induction of hepatotoxicity. Group C was pretreated with a standard drug, silymarin -200mg/kg plus paracetamol for 7 days. Group D and E are turmeric (200mg/kg and 400mg/kg respectively) and paracetamol groups. Group F and G are ginger (200mg/kg and 400mg/kg respectively) and paracetamol groups in which the rats are pretreated with ginger prior to the administration of paracetamol simultaneously for 7 days.

Table 1: Experimental Design.

Group	Treatment
A	Normal saline
B	Paracetamol (400mg/kg)
C	Silymarin (200mg/kg) + Paracetamol (400mg/kg)
D	Turmeric extract (200mg/kg) + Paracetamol (400mg/kg)
E	Turmeric extract (400mg/kg) + Paracetamol (400mg/kg)
F	Ginger extract (200mg/kg) + Paracetamol (400mg/kg)
G	Ginger extract (400mg/kg) + Paracetamol (400mg/kg)

After 24 hours induction, the animals were lightly anesthetized using diethyl ether and blood collected by cardiac puncture in sterilized centrifuge tubes which was then centrifuged to get serum for biochemical analysis. The animals were then sacrificed by cervical dislocation and the liver was removed for histopathological studies.

BIOCHEMICAL ANALYSIS

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum levels of (BIL), were measured using assay kits.

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) present in the test sample which is produced during lipid

peroxidation according to the method of Varshney and Kale, (1990).

Statistical analysis

Results are reported as mean $\pm$ S.E.M. ANOVA was used to evaluate differences between groups. If significance was observed between groups, the Student's t-test was used to compare the means of specific groups, with $P < 0.05$ considered as significant.

RESULTS

Phytochemical Screening

The phytochemical screening and the biochemical analysis are presented in tables 2 and 3 respectively.

Table 2. Result for phytochemical screening of turmeric and ginger extracts.

TEST	TURMERIC	GINGER
Alkaloids	-	+
Reducing sugar	-	+
Tannin	-	-
Steroids	-	+
Flavonoids	+	+
Saponins	+	+

Alanine aminotransferase levels were significantly higher in paracetamol control groups throughout the duration of the study compared to all other treatment groups, while it was significantly higher in all groups compared to the normal control group (1ml/kg of normal saline). The ALT levels in ginger treated groups were higher compared to the turmeric treated groups. The increasing dosages (200 and 400mg/kg) of turmeric ethanolic extract produced a significant ($p < 0.05$) reduction in the alanine aminotransferase level when compared with those of the paracetamol and ginger treated groups.

The increasing dosages (200 and 400mg/kg) of turmeric ethanolic extract produced a significant ($p < 0.05$) reduction in the aspartate aminotransferase level when compared with those of the paracetamol and ginger treated groups. Aspartate aminotransferase level were significantly higher in paracetamol control groups during the study as compared to all other treatment groups, while it was significantly higher in all groups compared to the normal control group. The extracts reduced aspartate aminotransferase level in a dose dependent manner.

The increasing dosages (200 and 400mg/kg) of turmeric ethanolic extract produced a significant ($p < 0.05$) reduction in the aspartate aminotransferase level when compared with those of the paracetamol, silymarin and ginger treated rats. Total serum bilirubin level were

significantly higher in paracetamol control groups during the study as compared to all other treatment groups, while it was significantly higher in all groups compared to the normal control group. The extracts reduced total serum bilirubin level in a dose dependent manner.

The data represented in Table 2 showed that administration of paracetamol significantly decreased serum total protein. The increasing dosages (200 and 400mg/kg) of turmeric ethanolic extract produced a significant ($p < 0.05$) increase in the serum total protein when compared with those of the paracetamol, silymarin, ginger treated rats and normal control groups. Serum total protein were significantly lower in paracetamol control groups during the study as compared to all other treatment groups, while it was significantly higher in all groups, except the ginger treated rats, compared to the normal control group. The extracts increased serum total protein level in a dose dependent manner.

The increasing dosages (200 and 400mg/kg) of turmeric ethanolic extract produced a significant ($p < 0.05$) reduction in the lipid peroxidation level when compared with those of the paracetamol and ginger treated groups. Lipid peroxidation level were significantly higher in paracetamol control groups during the study as compared to all other treatment groups, while it was significantly higher in all groups compared to the normal control group. The extracts reduced lipid peroxidation level in a dose dependent manner.

The levels of serum marker enzymes and liver thiobarbituric acid reactive substances are given in Table 3.

Table 3. Effect of ethanolic turmeric and ginger extract on Levels of ALT, AST, Total bilirubin, Total protein and Lipid peroxidation.

PARAMETERS TREATMENT	ALT (U/L)	AST (U/L)	TOTAL BILIRUBIN (mg/dL)	TOTAL PROTEIN (g/dL)	LIPID PEROXIDATION (nmol/mg protein)
CONTROL	20.24± 2.16	65.42± 2.12	0.42± 0.03	6.82 ± 0.28	163.07 ± 5.63
CONTROL+ PARACETAMOL (400mg/kg)	48.22± 4.79	96.78± 4.02	3.16± 0.14	4.60 ± 0.92	285.7 ± 10.3
SILYMARIN (200mg/kg) +PARACETAMOL (400mg/kg)	22.40± 2.32	73.48± 1.52	0.76± 0.07	6.96 ± 0.16	184.63 ± 6.06
TURMERIC (200mg/kg) +PARACETAMOL (400mg/kg)	26.41± 2.44	73.74± 1.07	0.54± 0.12	7.32 ± 0.12	219.99 ± 10.19
TURMERIC (400mg/kg) +PARACETAMOL (400mg/kg)	24.18± 2.06	74.83± 1.33	0.50± 0.04	7.67 ± 0.07	206.58 ± 13.77
GINGER (200mg/kg) +PARACETAMOL (400mg/kg)	32.14± 2.03	75.48± 2.05	0.64± 0.05	5.09 ± 1.34	252.48 ± 14.37
GINGER (400mg/kg) +PARACETAMOL (400mg/kg)	28.78± 2.14	76.63± 2.57	0.61± 0.06	5.17 ± 1.44	226.62 ± 12.05

DISCUSSION

Liver is a very important and vital part of the animal body. The liver is a major target organ for toxicity of xenobiotics and drugs, because most orally ingested xenobiotics and drugs pass through the liver and some chemicals are metabolized into toxic intermediates in the liver (Jaeschke et al., 2002). Paracetamol, when used at high doses, could cause acute liver injury most probably via formation of N-acetyl-p-benzoquinoneimine, a toxic metabolite, by cytochrome P4502E1 (CYP2E1). N-acetyl-p-benzoquinoneimine is usually inactivated by hepatic glutathione, but when produced excessively, covalently binds to centriobular hepatic proteins, contributing to hepatic toxicity (Gardner et al., 1998; 2002). In the assessment of liver damage by paracetamol, the determination of enzyme activities such as ALT and AST is largely used. In the present study, the increase in serum activities of ALT, AST in paracetamol treated rats had been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm, mitochondria or microsomes and are released into the circulation after cellular damage (Sallie et al., 1991) or due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases (Nuduka, 1999). These results were in accordance with those of Kuvandik et al., (2008) who found that the serum levels of both ALT and AST were elevated almost fourfold in paracetamol treated group in comparison with the control group. Also, Kanchana and Sadiq (2011) showed that oral administration of 400 mg/kg paracetamol in rats increased serum activities of ALT, AST, LDH, ALP and GGT. It has also been reported in some other studies that paracetamol intoxication can result in severe hepatic damage characterized by hemorrhagic centrilobular necrosis in both humans and animals (Thomas, 1993; Valentovic et al., 2004).

The present results agree with Abdel-Azeem et al., (2013) who showed that acute paracetamol toxicity induced remarkable increase in plasma ALT, AST, ALP activities and significant decrease in plasma level of total protein and albumin of rats. Most significant is the observation that treatment of rats with turmeric and ginger significantly reduced serum level of the transaminases. Ginger products exert their antioxidant effect by quenching free radicals due to the effect of polyphenol compounds (6-gingerols and its derivatives (Wilkinson, 2000). The basic mechanism of paracetamol toxicity in the liver is related to the covalent binding of its reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) to sulfhydryl groups of GSH and various thiol containing proteins and their subsequent oxidation (Bessems and Vermeulen, 2001). In the present study, paracetamol administration was accompanied by increased lipid peroxidation, From the results, it can be speculated that (i) increasing level of ALT, AST, total bilirubin and increasing level of liver thiobarbituric acid reactive substance produced in rat treated with paracetamol were due to hepatocellular damage and (ii) turmeric and ginger extracts afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection against paracetamol induced liver damage by the following extracts is by self-action as free radical scavengers intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Its ability is to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1,1- diphenyl-2-picrylhydrazyl radical (DPPH). Thus, by trapping oxygen related free radicals the extracts could hinder their interaction with polyester fatty acids and would abolish the enhancement of lipids peroxidative processes leading to MDA formation (Gupta et al, 2006).

The abnormal high level of serum ALT, AST and bilirubin observed in the study (Table 3) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with turmeric and ginger reduced the enhanced level of serum ALT, AST and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells, with turmeric showing more reduction as compared to ginger. A reduction in total serum protein (TSP) (Table 3) observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result in decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight (Table 3). However, when the turmeric and ginger extracts were given along with paracetamol, the significant increase in TSP was observed indicating the hepatoprotective activity of the extracts and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration.

CONCLUSION

From the results, it can be concluded that the increased levels of serum marker enzymes and increased liver thiobarbituric acid reactive substance level in albino rats treated- paracetamol was due to hepatocellular damage. Extracts of turmeric and ginger afforded protection from such paracetamol induced liver damage, with turmeric affording more protection when compared to ginger. Possible mechanism that may be responsible for the protection of paracetamol induced liver damage by the extracts may be because the extracts could act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Therefore, turmeric and ginger extracts are promising hepatoprotective agents. The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular damage (Gupta *et al*, 2006).

REFERENCE

1. Abdel-Azeem, A. S., Hegazy, A.M., Ibrahim, K. S., Farrag, A.R. and El-Sayed, E.M. 2013. Hepatoprotective, antioxidant and ameliorative effects of ginger (*Zingiber officinale* Roscoe) and vitamin E in acetaminophen treated rats. *J. Diet. Suppl.* 10(3): 195-209.
2. Abdul Rahman and Derus, M. D, 1996. Pengenalan dan Penggunaan Herba Ubatan. Orient Press Sdn. Bhd. Kuala Lumpur, Malaysia.
3. Ali, A.S. and Fahmy, G.E. 2009. Effects of water extracts of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale* Roscoe) on alcohol abuse. *Food Chem. Toxicol.*, 47: 1945-1949.
4. Ammon, H.P. and Wahl, M.A. 1991. Pharmacology of *Curcuma longa*. *Planta Med.*, Feb; 57(1): 1- 7.
5. Apisariyakul, A., Vanittanakom, N. and Buddhasukh, D. 1995. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). *J Ethnopharmacol*, 49: 163-169.
6. Arana, A., Morton, N.S. and Hansen, T.G. 2001. Treatment with paracetamol in infants. *Acta Anaesthesiol Scand*, 45: 20-29.
7. Araujo, C, C and Leon L, L., 2001. Biological activities of *Curcuma longa* L. *Memorias Do Instituto Oswaldo Cruz*, 96: 723-728.
8. Bailey, D.N. and Briggs, J.R. 2004. The binding of acetaminophen, lidocaine and valproic acid to human milk. *Am J Clin Pathol*, 121: 754-757.
9. Bannwarth, B. and Pehourcq, F. 2003. Pharmacologic basis for using paracetamol: Pharmacokinetic and pharmacodynamic issues (abstract). *Drugs*, 63: 5-13.
10. Bass, N.M., 2002. Is there any use for nontraditional or alternative therapies in patients with chronic liver disease? *J.curr. Gastroenterol. Rep.*, 1: 50-56.
11. Bessems, J.G.M., Vermeulen, N.P.E. 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Crit. Rev. Toxicol.*, 31(1): 55-138.
12. Boyd, E.H. and Bereczk, G.M. 1966. Liver necrosis from paracetamol. *Br. J. Pharmacol.*, 26: 606-614.
13. Budavari, S, 1996. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biological. 12th Edn., Whitehouse Station, New York, Merck and Co. Inc., 1674.
14. Clements, J.A., Critchley, J.A. and Prescott, L.F. 1984. The role of sulfate conjugation in the metabolism and disposition of oral and intravenous paracetamol in man. *Br J Clin Pharmacol*, 18: 481-485.
15. Connell, D. and Sutherland, M., 1969. A re-examination of gingerol, shogaol and zingerone, the pungent principles of Ginger (*Zingiber officinale* Roscoe). *Aust J Chem*, 22: 1033-43.
16. Davidson, D.G and Eastham, W. N.1996. "Acute liver necrosis following overdose of paracetamol," *British Medical Journal*, 5512: 497-499.
17. DeFeuids, F.V., Papadopoulos, V. and Drieu K. 2003. Ginko biloba extracts and cancer a research area in its infancy. *Fundam Clin Pharmacol*, 17: 405-17.
18. de Jager, Prashanti. Turmeric: The Ayurvedic Spice of Life, 2nd ed. (Pioneer Imprints, 2010), 11-12.
19. Drug Evaluation Monographs. 1996. Paracetamol, Vol. 88. Greenwood Village, CO: Micromedex, Inc.
20. Duke, J.A. and Ayensu, E.S. 1985. Medicinal Plants of China. Medicinal Plants of the World. Vol. 1. Algonac, MI: Reference Publications, Inc, 362.
21. El-Sharaky, A.S., Newairy, A.A., Kamel, M.A. and Eweda, S.M. 2009. Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. *Food. Chem. Toxicol.*, 47(7): 1584-1590.
22. Farag, A.G.A., Elhalwagy, M.E.A. and Farid, H.E.A. 2010. Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats. *Pest. Biochem. Physiol.*, 97: 267-274.

23. Forrest, J.A., Clements, J.A. and Prescott, L.F. 1982. Clinical pharmacokinetics of paracetamol (abstract). *Clin Pharmacokinet*, 7: 93–107.
24. Gardner, C.R., Heck, D.E., Yang, C.S., Thomas, P.E., Zhang, X.J. and de George, G.L. 1998. Role of nitric oxide in acetaminophen induced hepatotoxicity in the rat. *Hepatol.*, 26: 748-754.
25. Gardner, C.R., Laskin, J.D., Dambach, D.M., Sacco, M., Durham, S.K., Bruno, M.K., Cohen, S.D., Gordon, M.K., Gerecke, D.R., Zhou, P. and Laskin, D.L. 2002. Reduced hepatotoxicity of acetaminophen in mice lacking inducible nitric oxide synthase: potential role of tumor necrosis factor- α , interleukin-10. *Toxicol. Appl. Pharmacol.*, 184: 27–36.
26. Gupta, A.K., Chitme, H., Dass, S.K. and Misra, N. 2006. Antioxidant activity of Chamomile recutita capitula methanolic extracts against CCl₄-induced liver injury in rats. *J. Pharmacol. Toxicol.*, 1: 101-107.
27. Gupta, A.K., Chitme, H., Dass, S.K. and Misra, N. 2006. Hepatoprotective activity of Rauwolfia serpentina rhizome in paracetamol intoxicated rats. *J. Pharmacol. Toxicol.*, 1: 82-88.
28. Gupta, A.K. and Misra, N. 2006. Hepatoprotective activity of aqueous ethanolic extract of Chamomile capitula in paracetamol hepatotoxic albino rats. *Am. J. pharm. toxicol.*, 1(1): 17-20.
29. Habib, S.H., Makpol, S., Abdul Hamid, N.A., Das, S., Ngah, W.Z. and Yusof, Y.A. 2008. Ginger extract (*Zingiber officinale*) has anti-cancer and anti-inflammatory effects on ethionine induced hepatoma rats. *Clinics (Sao Paulo)*, 63(6): 807–813.
30. Iyengar, M.A., 1995. Study of Crude Drugs. 8th ed., Manipal Power Press, Manipal, India., 2.
31. Jaeschke, H., Gores, G.J., Cederbaum, A.I., Hinson, J.A., Pessayre, D. and Lemasters, J.J. 2002. Mechanisms of hepatotoxicity. *Toxicol. Sci.*, 65: 166–176.
32. James, L.P., Mayeux, P.R. and Hinson, J.A. 2003 "Acetaminophen-induced hepatotoxicity," *Drug Metabolism and Disposition*, 31(12): 1499–1506.
33. Jurenka, J.S. 2009. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern Med Rev*. Jun; 14(2): 141-53.
34. Kanchana, N., Sadiq, A.M. 2011. Hepatoprotective effect of plumbago zeylanica on paracetamol induced liver toxicity in rats. *Int. J. Pharm. Sci.*, 3(1): 151-154.
35. Kapoor, L. D., 1990. CRC Handbook of Ayurvedic Medicinal Plants. Boca Raton: CRC Press, 149-150.
36. Knight, T.R., Fariss, M.W., Farhood, A., Jaeschke, H. 2003. Role of lipid peroxidation as a mechanism of liver injury after acetaminophen overdose in mice. *Toxicol. Sci.*, 76(1): 229–236.
37. Kota, N., Kirshna, P., Polasa, K. 2008. Alterations in antioxidant status of rats following intake of ginger through diet. *Food Chem.*, 106: 991–996.
38. Kuvandik, G., Duru, M., Nacar, A., Yonden, Z., Helvacı, R., Koc, A., Kozlu, T., Kaya, H. and Sogüt, S. 2008. Effects of erdosteine on acetaminophen induced hepatotoxicity in rats. *Toxicol. Pathol.*, 36: 714-719.
39. Lal, B., Kapoor, A.K., Asthana, O.P., Agrawal, P.K., Prasad, R., Kumar, P. and Srimal, R.C. 1999. Efficacy of curcumin in the management of chronic anterior uveitis. *Phytother Res*. Jun; 13(4): 318-22.
40. Lee, W.M. 2004. Acetaminophen and the U.S. Acute Liver Failure Study Group: lowering the risks of hepatic failure. *Hepatol.*, 40: 6-9.
41. Leung, A. Y and Foster, S, 1996. Encyclopedia of Common Natural Ingredients Used in Food and Cosmetics. 2nd Edn., New York, John Wiley and Sons Inc., 499-501.
42. Lubbad, A., Oriowo, M.A., Khan, I. 2009. Curcumin attenuates inflammation through inhibition of TLR-4 receptor in experimental colitis. *Mol Cell Biochem*. Feb; 322(1-2): 127-35. Epub 2008 Nov 11.
43. Luper, S., 1999. A review of plants used in the treatment of liver disease. Part two, Alternative medicine review. *J. Clinical Therapeutic*, 4: 178-188.
44. Madhu Kiran, P., Vijaya Raju, A. and Ganga Rao, B. 2012. Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rats. *Asi. Pac. J. Trop. Biomed.*, 2(5): 352-356.
45. Mascolo, N., Jain, R., Jain, S.C. and Capasso F. 1989. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *J Ethnopharmacol*, 27: 129-40.
46. Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillette, J.R. and Brodi, B.B. 1973. Acetaminophen induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.*, 187: 211-7.
47. Morris, M.E. and Levy, G. 1984. Renal clearance and serum protein binding of acetaminophen and its major conjugates in humans (abstract). *J Pharm Sci.*, 73: 1038–1041.
48. Nagabhushan, M. and Bhide, S.V. 1986. Nonmutagenicity of curcumin and its antimutagenic action versus chili and capsaicin. *Nutr Cancer*, 8(3): 201-10.
49. Nelson, S.D. 1990. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. *Semin Liver Dis.*, 10: 267–278.
50. Newall, C.A., Anderson, L.A. and Phillipson, J.D. 1996. Herbal medicines: a guide for health care professionals. London: Pharmaceutical Press, 1996; ix: 296.
51. Nichols, G. and Frampton, C.S. 1998. Physicochemical characterization of the orthorhombic polymorph of paracetamol crystallized from solution. *J Pharm Sci.*, 87: 684–693.
52. Nuduka, N. 1999. Clinical biochemistry for students of pathology, Longman Nigerian Plc., 1-236.

53. Ostapowicz, G., Fontana, R.J., Schiodt, F.V., Larson, A., Davron, J.T., Steven, H.B., Timothy, M. and Reish J. 2002. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med.*, 137: 947–954.
54. Patel, R.K, Patel, M.M, Patel, M.P., Kanzaria, N.R., Vaghela, K.R. and Patel, N.J. 2008: Hepatoprotective activity of *Moringa oleifera* Lam. Fruit on isolated rat hepatocytes. *Pharmacogn Mag*, 4: 118–123.
55. Platel, K. and Srinivasan, K. 1996. Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. *Int J Food Sci Nutr.*, Jan; 47(1): 55-9.
56. Prescott, L.F. 1996. The metabolism of paracetamol. In: Prescott LF, editor. *Paracetamol (Acetaminophen). A critical bibliographic review.* London: Taylor and Francis., 67-102.
57. Qureshi, S., Shah, A.H., Tariq, M. and Ageel, A.M. 1989. Studies on herbal aphrodisiacs used in Arab system of medicine. *Am J Chin Med.*, 17: 57-63.
58. Rossi, A., Savioli, A., Bini, M., Capsoni, D., Massarotti, V., Bettini, R., Gazzaniga, A., Sangalli, M.E. and Giordano, F. 2003. Solid state characterization of paracetamol metastable polymorphs formed in binary mixtures with hydroxypropylmethylcellulose. *Therm Acta*, 406: 55–67.
59. Sakr, S.A. 2007. Ameliorative effect of ginger (*Zingiber officinale*) on mancozeb fungicide induced liver injury in albino rats. *Liv.*, 1: 650–656.
60. Sallie, R., Tredger, J. 1991. Drugs and the liver. *Biopharm. Dru. Dispos.*, 12: 251-259.
61. Sehrawat, A., Khan, T.H., Prasad, L. and Sultana, S. 2006. Buteamonosperma and chemomodulation: protective role against thioacetamide mediated hepatic alterations in Wistar rats. *Phytomed.*, 13(3): 157–163.
62. Sohn, Y.T. 1990. Study on the polymorphism of acetaminophen (abstract). *J Kor Pharm Sci.*, 20: 97– 104.
63. Soni, K.B. and Kuttan, R. 1992. Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. *Indian J Physiol Pharmacol.*, 1992 Oct; 36(4): 273-5.
64. Soni, K.B., Rajan, A. and Kuttan, R. 1992. Reversal of aflatoxin induced liver damage by turmeric and curcumin. *Cancer Lett.*, Sep 30; 66(2): 115-21.
65. Srinivasan, K. and Sambaiah, K. 1991. *Int J The effect of spices on cholesterol 7 alpha hydroxylase activity and on serum and hepatic cholesterol levels in the rat.* *Vitam Nutr Res.*, 1991; 61(4): 364-9.
66. Srivastava, K.C., Bordia, A. and Verma, S.K. 1995. Curcumin, a major component of food spice turmeric (*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. *Prostaglandins Leukot Essent Fatty Acids.*, 1995 Apr; 52(4): 223- 7.
67. Srivastava, R., Dikshit, M., Srimal, R.C. and Dhawan, B.N. 1985. Anti-thrombotic effect of curcumin. *Thromb Res.*, 1985 Nov 1; 40(3): 413-7.
68. Sun, J., Schnackenberg, L.K. and Beger, R.D. 2009. Studies of acetaminophen and metabolites in urine and their correlations with toxicity using metabolomics. *Drug Metab. Lett.*, 3: 130-136.
69. Takeoka, G.R. and Dao, L.T. 2003. Antioxidant constituent of almond [*Prunus dulcis* (Mill.) D.A. Webb. hulls. *J. Agric. Food Chem.*, 51: 496-501.
70. Tapsell, L.C., Hemphill, I., Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A. and Inge, K.E., 2006. Health benefits of herbs and spices: the past, the present, the future. *Med. J. Aust.*, 185: S4–S24.
71. Thomas, S.H. 1993. Paracetamol (acetaminophen) poisoning. *Pharmacol. Ther.*, 60: 91–120.
72. Tirmenstein, M.A. and Nelson, S.D. 1989. Subcellular binding and effects on calcium homeostasis produced by acetaminophen and a nonhepatotoxic regioisomer, 3_ hydroxyacetanilide, in mouse liver. *J Biol Chem*, 264: 9814–9819.
73. Tsokos-Kuhn, J.O., Hughes, H., Smith, C.V. and Mitchell, J.R (1988) Alkylation of the liver plasma membrane and inhibition of the Ca2_ ATPase by acetaminophen. *Biochem Pharmacol*, 37: 2125–2131.
74. Tunon, M.J., Alvarez, M., Culebras, J.M. and Gonzalez-Gallego, J. 2009. An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure. *W. J. Gastr.*, 15(25): 3086–3098.
75. Valentovic, M., Terneus, M., Harmon, R.C. and Carpenter, A.B. 2004. S-adenosylmethionine (SAME) attenuates acetaminophen hepatotoxicity in C57BL/6 mice. *Toxicol. Lett.*, 154: 165-174.
76. WHO. 2003. WHO Model List of Essential Medicines, 13th edition. URL: www.who.int/medicines/organization/par/edl/expcom13/eml13_en.doc. Wilkinson JM (2000). Effect of ginger tea on the fetal development of Sprague Dawley rats. *Reprod. Toxicol.*, 14: 507–512.
77. Zapater, P., Lasso De La Vega, M.C., Horga, J.F., Such, J., Frances, R., Esteban, A., Palazon, J.M., Carnicer, F., Pascual, S. and Perez-Mateo, M. 2004. Pharmacokinetic variations of acetaminophen according to liver dysfunction and portal hypertension status. *Aliment Pharmacol Ther*, 20: 29–36.