Biokemistri
Vol. 33, No. 2, June 30, 2021
Printed in Nigeria

0795-8080/2021 $10.00 + 0.00

http://journals.niseb.org
Also available online at http://www.bioline.org.br/bk

BKR 2021005/33202

Hepatoprotective effect of ethyl acetate extract of *Curcuma longa* on alcohol-induced liver damage in female Wistar rats

Moses Ceaser ANTIYA[1]*, Ofem Effiom ETENG [1], Musa Adio ALIMI [1], David Oluwagbemi ADEYI [1], Esther Olubisi ADEYI [1], Ijeoma OKOLO [2] and Oluseyi Adeboye AKINLOYE

[1]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria; [2]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

***Corresponding author:** email address: antiyaceaser@gmail.com Tel: +2347065565685

(Received February 13, 2021; Accepted June 16, 2021)

ABSTRACT: Chronic alcohol intake predisposes an individual to liver damage. This study investigated the effect of ethyl acetate extract of *Curcuma longa* (EEC) against alcohol-induced liver damage in female Wistar rats. Thirty rats (120–220g) were randomly selected into six groups (n=5); Control, 20% ethanol (5g/kg), various dosage of EEC (100, 200 and 350mg/kg) + 20% ethanol and EEC only (350 mg/kg) groups. Animals in the treatment groups were administered EEC 2 hours before intoxicated with ethanol daily for 14 days. At the end of the treatment period, glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities, malondialdehyde (MDA) levels, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities were determined. Histopathology of the liver was also performed. Ethanol caused a significant ($p<0.05$) decrease in hepatic GSH level, SOD, GPx and CAT activities while significantly ($p<0.05$) increasing MDA level, plasma AST, ALT and ALP activities. Pre-treatment with EEC prevented these effects. Histopathology result shows that group that received the highest dose of EEC showed mild necrosis of the hepatocyte. This study shows that *Curcuma longa* ethyl acetate extract exerts a protective effect against ethanol-induced liver damage in rats via antioxidant activity.

Keywords: Ethanol, ethyl acetate, hepatic, necrosis, pre-treatment.

Introduction

Alcohol is one of the natural products consumed by humans for thousands of years. It is also one of the most common cause of reactive oxygen species (ROS) generation in the body (1). Excessive intake of alcohol over a long period is detrimental to all the body organs, especially the liver. The liver is an essential organ in the body which has detoxification of toxicant as one of its principal function. It is also the primary site of alcohol metabolism in the body (2). Globally alcohol attributed mortality was at 38.8 per 100,000 with 8.3 per 100,000 deaths due to alcohol liver damage (ALD) (3).

Metabolism of ethanol involves multiple pathways and various molecular mechanisms which contributes to the pathogenesis of ALD. Alcohol dehydrogenase and aldehyde dehydrogenase enzyme systems are involved in the metabolism of ethanol. Ethanol is first oxidized to acetaldehyde by alcohol

dehydrogenase before converted to acetic acid by acetaldehyde dehydrogenase (4-8). The acetate is metabolized in the Kreb cycle to generate energy or other intermediates. Although the reduction of acetaldehyde to acetate by alcohol dehydrogenase is beneficial to the cell, it also creates free radicals (8). Increased in free radical generation due to high intake of alcohol damages mitochondria, endoplasmic reticulum, and other cellular structures (10).

Natural compounds isolated from plants have been shown to possess significant pharmacological potential, they have been used in treatment of various ailments which include alcohol-related diseases such as the alcoholic fatty liver. *Curcuma longa* (Tumeric) is one of the most commonly used medicinal plant popularly used as a remedy against a wide range of ailments, both in Nigeria and several other countries in the world (11,12). *Curcuma longa* is a herbaceous evergreen plant that belongs to family Zingiberaceae. It is a tropical plant which is extensively used as a dietary pigment and as a spice, which is derived from the dried, ground rhizome. *Curcuma longa* possesses antioxidant, antitumor, antimicrobial, anti-inflammatory, wound healing, lipid-reducing, chemo-preventive, immunomodulatory, and gastroprotective activities and all these are well documented (13-15). In this study, we investigated the protective effect of ethyl acetate extract of *Curcuma longa* against alcohol-induced liver damage.

Materials and Methods

Chemicals

Ethanol (99.8%) and 5,5'–dithiobisnitrobenzoic acid (DTNB) were purchased from Sigma-Aldrich Chemical Co. (USA). AST, ALP and ALT assay kits were purchased from Randox Ltd. (United Kingdom). All other chemicals used were of analytical grade and were purchased from a reputable manufacturer.

Preparation of Extract

Fresh *Curcuma longa* rhizomes were harvested from their natural habitat at Ajasa village in Ile-Ise Awo in Abeokuta, Ogun State, Nigeria. The rhizomes were authenticated by a taxonomist at the Department of Botany, Federal University of Agriculture, Abeokuta, Ogun State. A voucher specimen (reference number FUNAAB H-0065) was deposited at the herbarium for reference purpose.

The rhizomes were descaled, air-dried and pulverized using a mechanical blender. The pulverized rhizome was weighed (1000g) and dissolved with 2000ml of ethyl acetate. The mixture was left to stay for 3 days, it was subsequently filtered using Whitman No.10 filter paper and evaporated. The extracts were stored for further use.

Experimental Animals

Adult female Wistar rats (weighing 150 – 220g) were purchased from Tayo farms, Ajibode, University of Ibadan, Ibadan. The animals were kept in well ventilated plastic cages at the animal house at a controlled temperature (28±2^{o}C) with 12 hours light-dark cycle. The animals were provided with food and water *ad*-libitum. They were acclimatized for two weeks before the commencement of the experiment.

Experimental Design

The experiments in this project was approved by the Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta Ethical Committee (FUNAAB-BCH-MC-020).

After acclimatization the animals were selected into six (6) groups consisting of five (5) animals each.

- Group 1: Control administered normal saline (0.5ml).
- Group 2: 20% ethanol (EtOH) (5g/kg b.wt) was administered.
- Group 3: 100 mg/kg b.wt of EEC+ 20% EtOH (5g/kg b.wt)
- Group 4: 200 mg/kg b.wt of EEC + 20% EtOH (5g/kg b.wt)
- Group 5: 350 mg/kg b.wt of EEC + 20% EtOH (5g/kg b.wt)
- Group 6: 350 mg/kg b.wt of EEC.

Groups 3, 4 and 5 were pre-treated with various dose of ethylacetate extract of *Curcuma longa* (100, 200 and 350 mg/kg b.wt) 2 hours before administering 20% ethanol.

Blood and tissue sampling

After the last day of administration, the animals were fasted overnight for 12 hours, and weighed. Blood was collected via ocular into a 10ml heparinized tube. They were then sacrificed using cervical dislocation. The liver was harvested, washed with ice-cold normal saline and weighed.

Blood collected was centrifuged at 4000 rpm for 5 minutes (Beckman GS-6R, Germany) to collect the supernatant as plasma. Liver homogenate (10%) was prepared using 0.25M sucrose solution.

Biochemical analysis

Antioxidant assays

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were determined according to method described by Li (16), Rotruck *et al.* (17) and Claiborne (18) respectively. Oxidative stress markers; reduced glutathione (GSH) and malondialdehyde (MDA) were determine according to the method of Tipple and Rodger (19) and Draper *et al.* (20) respectively.

Liver function test

Liver function markers such as Alkaline phosphatase (ALP), Aspartate transaminase (AST) and Alanine transaminase (ALT) activities were determined using standard kits purchased from Randox Ltd. (United Kingdom). All the enzymatic estimation was assessed using spectrophotometer as described by the manufacturer.

Histopathology

Small piece of liver sections from each group was fixed in 10% formalin for histopathology, and the remaining were kept in an Eppendorf tube for further analysis. Fixed liver tissues were dehydrated and embedded in paraffin wax. Section 5µ thickness were made, stained with Hematoxylin and Eosin and examined under microscope.

Statistical analysis

All the data were expressed as mean ± standard error of the mean (S.E.M). Differences between the experimental groups were assessed by one-way ANOVA followed by Duncan's test. Values were considered statistically significant when $p < 0.05$.

Results

Effect of EEC on GPx, SOD, CAT activities and GSH levels in the liver

Administration of ethanol resulted in a significant ($p<0.05$) decrease of liver GPx, SOD, CAT activities (enzymatic antioxidant) and GSH level (non-enzymatic antioxidant) in the ethanol-only group when compared with the control group (Table 1). Pretreatment with EEC at the dose of 100, 200 and 350mgkg significantly ($p<0.05$) increase the liver GPx, SOD, CAT activities and GSH level. The groups pretreated with the highest dose (350mg/kg) of EEC recorded significant ($p<0.05$) increase in CAT activity and GSH level when compared to other pretreatment groups. On the other hand, increase in dose of EEC showed no effect on the GPx and SOD activities.

Table 1. Effects of ethyl acetate extract of *Curcuma longa* (EEC) on hepatic GSH level and SOD, GPx, and CAT activities in ethanol treated rat

Group (n=5)	GSH (µmol/mg protein)	Activity (U/mg protein)		
		GPx	SOD	CAT
Control	1.87 ± 0.14^{d}	0.83 ± 0.02^{b}	2.44 ± 0.19^{d}	0.70 ± 0.06^{d}
20% EtOH only	1.09 ± 0.03^{a}	0.62 ± 0.02^{a}	1.56 ± 0.06^{a}	0.51 ± 0.04^{a}
EEC: 100mg/kg + 20% EtOH	1.25 ± 0.10^{b}	0.76 ± 0.04^{b}	1.96 ± 0.06^{b}	0.53 ± 0.01^{a}
EEC: 200mg/kg + 20% EtOH	1.40 ± 0.06^{c}	0.78 ± 0.02^{b}	1.96 ± 0.06^{b}	0.58 ± 0.02^{b}
EEC: 350mg/kg + 20% EtOH	1.49 ± 0.03^{c}	0.82 ± 0.02^{b}	1.95 ± 0.04^{b}	0.62 ± 0.02^{c}
350 mg/kg EEC only	1.95 ± 0.09^{d}	0.84 ± 0.02^{b}	2.25 ± 0.08^{c}	0.68 ± 0.03^{d}

*Values are expressed as mean ± SEM. Values with different superscript along the same column are statistically different ($p < 0.05$).

Effect of EEC on lipid peroxidation in the liver

The effects of EEC on malondialdehyde (MDA) in the liver is shown in Figure 1. Ethanol administration significantly ($p<0.05$) increased the MDA level in ethanol-only when compared to the control group. According to the result, pre-treatment with EEC at various doses (100, 200 and 350 mg/kg) significantly ($p<0.05$) reduced the MDA level when compared to the ethanol-only group with the group that received 350 mg/kg of *Curcuma longa* recording the highest decrease.

Effect of EEC on plasma ALP, AST and ALT.

The effects of EEC on liver marker enzymes are shown in Figure 2. Intoxication with ethanol caused a significant ($p<0.05$) increase the activities of hepato-specific enzymes; ALP, ALT and AST in the plasma of the ethanol-only group when compared with the control group. Pre-treatment with EEC, at the dose of 100, 200 and 350mg/kg significantly ($p<0.05$) reduced the plasma activities of ALP, ALT and AST. At the dose of 350mg/kg, a significantly ($p<0.05$) higher reduction in AST was recorded when compared with groups that received dose of 100 and 200mg/kg but no significant ($p>0.05$) difference in the activities of ALP and ALT within the treatment groups.

Histopathological evaluation

Histopathological assessment of the liver was done for all the groups (Figure 3). In the control group, no pathological abnormality was observed in the liver tissue with no visible lesion (Figure 3a). In EtOH group (Figure 3b), there is mild dilatation of sinusoid, necrosis of hepatocytes and infiltration by inflammatory cells in the periportal area. However, liver tissues of all the rats in the groups that were pre-treated with *Curcuma longa* before administering EtOH (Figure 3c, 3d, 3e) showed mild and moderate dilation of the sinusoid and hepatocyte necrosis.

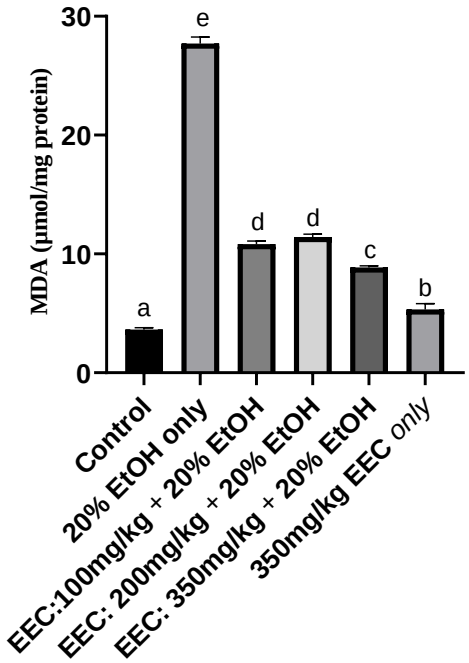

Figure 1. Effects of ethyl acetate extract of *Curcuma longa* (EEC) on level of malondialdehyde (MDA) induced with ethanol in rat liver. *Values are expressed as mean ± SEM. Values with different superscript along the same are statistically different ($p < 0.05$).

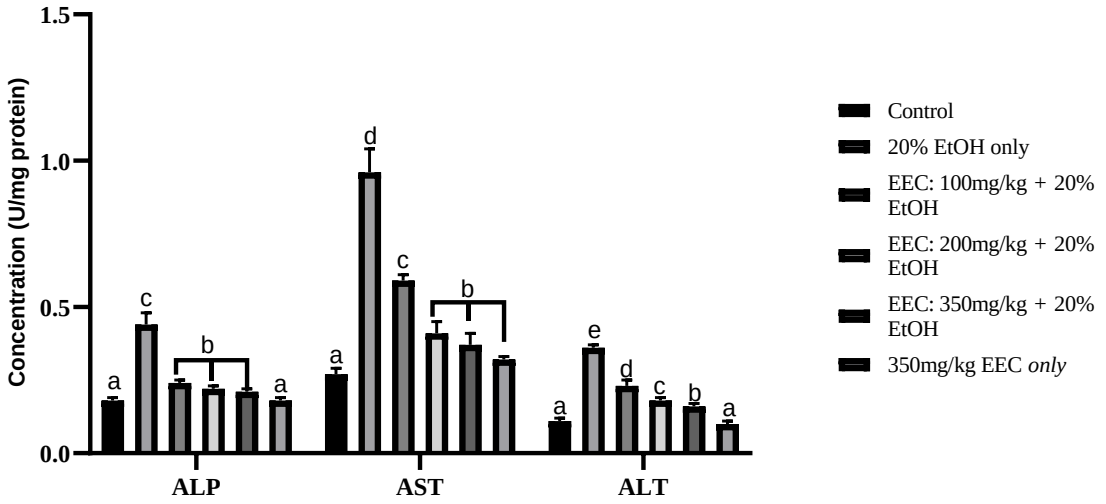

Figure 2. Effects of ethyl acetate extract of *Curcuma longa* (EEC) on activities of plasma ALP, AST and ALT activities in ethanol treated rat. *Values are expressed as mean ± SEM. Values with different superscript along the same are statistically different ($p < 0.05$).

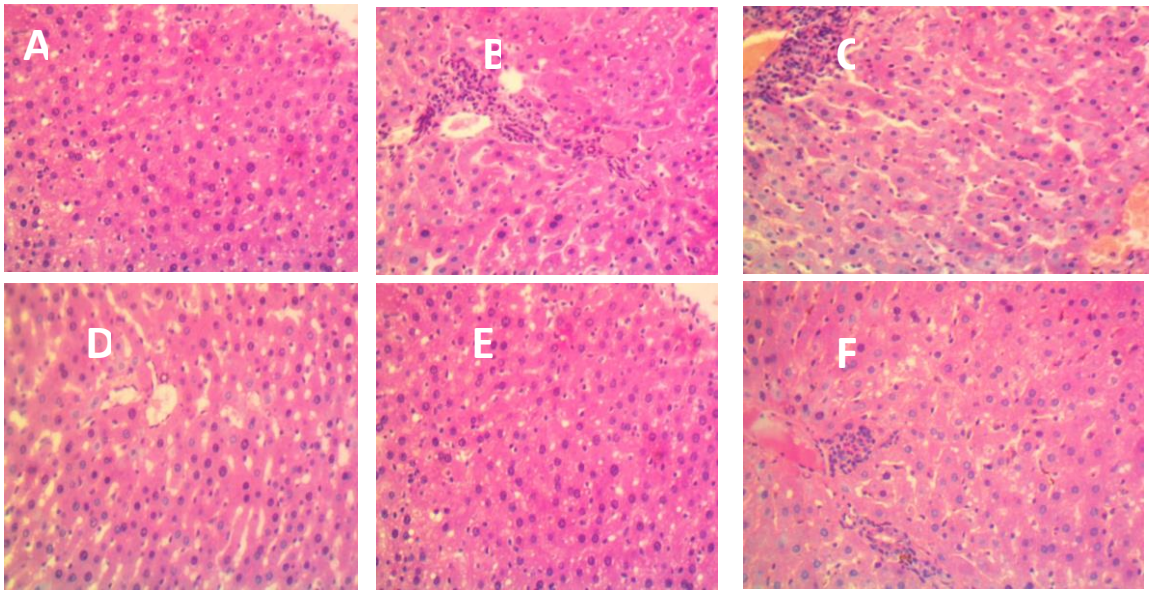

Figure 3. **The histological representation of the rat's tissues**
Representative histological section of liver in acute toxicity (Light microscopy, ×400; haematoxylin and eosin staining). A: Control group, no visible lesions seen. B: EtOH group, there is mild dilatation of sinusoid, necrosis of hepatocytes and infiltration by inflammatory cells in the periportal area. C: Group III, there is mild necrosis of hepatocytes and dilatation of the sinusoid and infiltration of inflammatory cells in the periportal area. D: Group IV, mild dilatation of the sinusoid and moderate necrosis of hepatocyte. E: Group V, moderate dilatation of the sinusoid and necrosis of hepatocyte. F: Group VI, there is mild dilatation of the sinusoid.

Discussion

The result of this study confirmed the hepatoprotective effect of ethyl acetate extract of *Curcuma longa* in ethanol induced liver toxicity. Ethanol has been widely used to induce hepatic damage in rodent and mice model of liver disease (21).

Increase in oxidative stress due to chronic consumption of alcohol can damage the antioxidant defence system and increase the production of free radicals such as reactive oxygen species (ROS); hydroxyethyl radical, superoxide radical, hydroxyl radical, peroxyl radicals and hydrogen peroxide (22). In this study, alcohol intoxication considerably decreased the level of GSH and activities of antioxidant enzymes (GPx, SOD and CAT) in the liver. Pre-treatment with EEC significantly prevented oxidative stress by alleviating the activities of the antioxidant enzymes and GSH level toward normal. *Curcuma longa* extract has apparent antioxidative effect and potential radical scavenging potential/activity. This result is consistent with the findings of Salama *et al.* (11) who showed that ethanolic extract of *Curcuma longa* increase the activities of antioxidant enzymes in thioacetamide induced liver cirrhosis in rats.

It has been reported that lipid peroxidation, reduction in antioxidant enzymes activity and increase in ROS are primary cause of hepatic injury in ethanol induced liver damage (23). MDA is a secondary product of poly-unsaturated fatty acid peroxidation (24) and serves as a major marker to estimate the level of lipid peroxidation (25). Furthermore, the level of MDA is an indicator of cell membrane damage (26). In this study, administration of ethanol increased the level of MDA in the liver due to increase in lipid peroxidation caused by ethanol dependent overproduction of ROS (27). Pre-treatment with EEC was able to prevent hepatic cell membrane oxidation by free radicals leading to the decrease in the MDA level. Therefore, *Curcuma longa* extract showed antioxidative effects *in vivo* by increasing the total antioxidant capacity of the liver tissues preventing increase in the MDA levels (9, 14, 28).

ALT, AST and ALP are important metabolic enzymes in the hepatocytes and are at a low concentration in the plasma. Therefore, one of the ways for estimating the extent of hepatic damage is through the determination of the plasma activities of AST, ALT, and ALP which are a direct reflection

of alterations in the hepatic structural integrity, possibly, necrosis of hepatocytes that results in the leakages of transaminases and ALP in to the blood (27, 29, 30, 31). Plasma ALP, AST and ALT activities were elevated in the ethanol only group, indicating a severe liver damage (30, 31). The significant decreased in transaminases (AST and ALT) and ALP activities in the groups pre-treated with EEC before ethanol administration demonstrated their hepatoprotective ability (27). The hepatoprotective potential of *Curcuma longa* extract can be attributed to the antioxidant capacity, preventing lipid peroxidation and thus maintaining the structural integrity of hepatocytes (14).

Alcoholic liver damage is normally characterized with changes in the hepatocellular morphology (32). From our results, morphological abnormalities in the liver histology of the rat that received ethanol only support the biochemical test results obtained. This is in accordance to the findings of Saravanan *et al.* (33), who reported changes in liver histology partially attributed to cytochrome P_{450} dependent enzyme activities in liver that tends to be present in higher concentration near the central vein and lower near the peripheral site. Pre-treatment with EEC prevented liver injury through preservation of the liver membrane integrity and prevention of oxidative stress.

Conclusion

Based on the results observed it is evident that exposure to ethanol causes damages to the structural integrity of the liver via oxidative stress and pre-treatment of rats for 14 days with *Curcuma longa* exhibited antioxidant and hepatoprotective property in ethanol-induced liver damage.

Acknowledgement

The authors are extremely grateful to Head of Department and all members of Biochemistry Department, Federal University of Agriculture, Abeokuta for providing an enabling environment to carry out this work

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of interest

The authors report no conflict of interest.

References

1. Das SK, Vasudevan DM: Alcohol-induced oxidative stress. Life sciences 2007;81(3):177-187. doi: 10.1016/j.lfs.2007.05.005
2. Lieber CS: Alcoholic liver disease: new insights in pathogenesis lead to new treatments. Journal of hepatology 2000. 32:113-128. doi: 10.1016/s0168-8278(00)80420-1
3. World Health Organization. Global status report on alcohol and health 2018. Poznyak V, Rekve D, eds. Geneva: WHO; 2018. 450 p.
4. Thurman, RG, Bradford BU, Iimuro Y, Frankenberg MV, Knecht KT, Connor HD, Adachi Y, Wall C et al.: Mechanisms of alcoholinduced hepatotoxicity: studies in rats. Journal of Frontier Bioscience 1999; 4:42–6.
5. Lieber CS: Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Journal of Alcohol metabolism 2004; 34(1): 9–19. DOI: 10.1016/j.alcohol.2004.07.008
6. Zakhari S: Overview: how is alcohol metabolized by the body?. Alcohol Research and Health 2006; 29(4): 245.
7. Liu J: Ethanol and liver: recent insights into the mechanisms of ethanol-induced fatty liver. World journal of gastroenterology: WJG 2014; *20*(40), 14672. doi: 10.3748/wjg.v20.i40.14672
8. Mello T, Ceni E, Surrenti C, Galli, A: Alcohol induced hepatic fibrosis: role of acetaldehyde. Molecular Aspects of Medicine 2008; 29(1-2):17-21. doi: 10.1016/j.mam.2007.10.001
9. Wang J, Du H, Ma X, Pittman B, Castracane L, Li T, Behar KL, Mason, GF: Metabolic products of [2-13C] ethanol in the rat brain after chronic ethanol exposure. Journal of Neurochemistry 2013; 127(3): 353-364. DOI: 10.1111/jnc.12405
10. Reddy JK, Rao MS: Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. American Journal on Physiology and Gastrointestinal 2006; 290(5): G852-G858. DOI: 10.1152/ajpgi.00521.2005

11. Salama SM, Abdulla MA, AlRashdi AS, Ismail S, Alkiyumi SS, Golbabapour S: Hepatoprotective effect of ethanolic extract of Curcuma longa on thioacetamide induced liver cirrhosis in rats. BMC Complementary and Alternative Medicine 2013; 13(1): 56. doi: 10.1186/1472-6882-13-56
12. Ramana KV, Singhal SS, Reddy AB: Therapeutic potential of natural pharmacological agents in the treatment of human diseases. Biomed Research International 2014 https://doi.org/10.1155/2014/573452
13. Maizura M, Aminah A, Wan Aida WM: Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric *Curcuma longa*) extract. International Food Research Journal 2011; 18: 526-531.
14. Miquel J, Bernd A, Sempere JM, Dıaz-Alperi J, Ramırez A: The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Archives of Gerontology and Geriatrics 2002; 34(1): 37-46. DOI: 10.1016/s0167-4943(01)00194-7
15. Jo B, Vijaykumar M, Lokesh BR: Biological properties of curcumin — cellular and molecular mechanisms of action. Critical Reviews in Food Science and Nutrition 2004; 44(2): 97-111. doi: 10.1080/10408690490424702.
16. Li X: Improved pyrogallol autoxidation method: a reliable and cheap superoxide-scavenging assay suitable for all antioxidants. Journal Of Agricultural And Food Chemistry 2012; 60(25): 6418-6424. https://doi.org/10.1021/jf204970r
17. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman, DG, Hoekstra WG: Selenium:biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588–590. doi: 10.1126/science.179.4073.588.
18. Claibome A: Catalase activity. In: Greenwald, R.A., ed. CRC handbook of methods for oxygen radical research; Boca Raton, FL: CRC Press. 283-284, 1986
19. Tipple TE, Rogers LK: Methods of the determination of plasma or tissue glutathione levels. Developmental Toxicology pp.315-324. Human Press, Totowa, N.J, 2012.
20. Draper HH, Squires EJ, Mahmoodi H, Wu J, Agarwal S, Hadley M: A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radical Biology and Medicine 1993;15(4): 353-363. doi: 10.1016/0891-5849(93)90035-s
21. Arteel GE: Animal models of alcoholic liver disease. Digestive Diseases 2010; 28(6), 729-736.
22. Girotti AW: Lipid hydroperoxide generation, turnover, and effector action in biological systems. Journal of Lipid Research 1998; 39(8):1529-1542.
23. Mo Q, Zhou G, Xie B, Ma B, Zang X, Chen Y, Wang Y: Evaluation of the hepatoprotective effect of Yigan mingmu oral liquid against acute alcohol-induced liver injury in rats. BMC Complementary Medicine and Therapies 2020; 20(1), 32.
24. Amat N, Upur H, Blažeković B: In vivo hepatoprotective activity of the aqueous extract of Artemisia absinthium L. against chemically and immunologically induced liver injuries in mice. Journal of ethnopharmacology 2010; 131(2), 478-484.
25. Cho BO, Ryu HW, So Y, Jin CH, Baek JY, Park KH, Jeong IY: Hepatoprotective effect of 2, 3-dehydrosilybin on carbon tetrachloride-induced liver injury in rats. Food chemistry 2013; 138(1), 107-115.
26. Kepekçi RA, Polat S, Çelik A, Bayat N, Saygideger SD: Protective effect of Spirulina platensis enriched in phenolic compounds against hepatotoxicity induced by CCl4. Food chemistry 2013; 141(3), 1972-1979.
27. Lodhi P, Tandan N, Singh N, Kumar D, Kumar M: Camellia sinensis (L.) Kuntze extract ameliorates chronic ethanol-induced hepatotoxicity in albino rats. Evidence-Based Complementary and Alternative Medicine 2014; https://doi.org/10.1155/2014/787153
28. Sarhan R, El-Azim S A, Motawi TM, M. Hamdy MA: Protective effect of turmeric, Ginkgo biloba, Silymarin separately or in combination, on iron-induced oxidative stress and lipid peroxidation in rats. International Journal of Pharmacology 2007;3: 375-384. **DOI:** 10.3923/ijp.2007.375.384
29. Baldi E, Burra P, Plebani M, Salvagnini M: Serum malondialdehyde and mitochondrial aspartate aminotransferase activity as markers of chronic alcohol intake and alcoholic liver disease. The Italian journal of gastroenterology 1993; 25(8): 429.
30. Goldberg DM, Watts C: Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterology 1965; 49:256-261.
31. Yanardag RO, Ozsoy-Sacan O, Bolkent S, Orak H, Karabulut-Bulan O: Protective effects of metformin treatment on the liver injury of streptozotocin-diabetic rats. Human and Experimental Toxicology 2005; 24(3): 129-135. doi: 10.1191/0960327104ht507oa.
32. Itagaki H, Shimizu K, Morikawa S, Ogawa K, Ezaki T: Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. International journal of clinical and experimental pathology (2013): 6(12), 2683.
33. Saravanan R, Viswanathan P, Pugalendi KV: Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. Life Sciences 2013. DOI: 10.1016/j.lfs.2005.05.060