



Protective Effect of Terminalia Chebula Fruit Extract on Ethanol-Induced Hepatotoxicity in Albino Rat

Vuyyala Balakrishna*, Takhalapally Lakshmi, Deepak Pawar, Chedurupally Lavanya

Asst. Professor, Department of pharmacology, GNITC-School of pharmacy, Ibrahimpatnam, Hyderabad, India

ABSTRACT

The purpose of this study was to gauge the impact of Terminalia chebula fruit extract on liver inhibitor enzymes in ethanol-induced hepatotoxicity in rats. Rats were divided into six completely different teams every having six. Group one served as a control, cluster two received forty percent of ethyl alcohol (2 ml/100 g, oral), in sterile water, Groups 3, 4 and 5 served as extract treatment teams and received 50, 100 and 200 mg/kg, orally, ethanolic fruit extract of T. chebula (TCE) and cluster three served as standard cluster and received silymarin twenty five mg/kg orally. All the treatment protocols followed twenty-one days, and when those rats were sacrificed, the liver was taken for inhibitor and microscopic anatomy studies, severally. The ethanol-treated cluster rats (G2) showed variable decrease in inhibitor parameter (catalase, glutathione, and glutathione reductase) levels. In the present study treatment with T. Chebula fruit extract significantly decreased the elevated levels of SGOT, SGPT, ALP, Total bilirubin and increased the levels of Total proteins. Administration of ethanolic trichloroethane considerably prevented ethanol-induced elevation within the levels of malondialdehydelipide peroxidation and attenuated inhibitor parameters in experimental teams of rats. The impact of extract was compared with a standard drug, silymarin. The changes in antioxidant parameters were supported by histological profile. It is concluded that the ethanolic fruit TCE protects against ethanol-induced oxidative liver injury in rats.

Key Words: Terminalia chebula, Ethanol, Hepatotoxicity, Silymarin, Extract of Terminalia chebula.

eIJPPR 2019; 9(1):44-50

HOW TO CITE THIS ARTICLE: Vuyyala Balakrishna, Takhalapally Lakshmi, Deepak Pawar, Chedurupally Lavanya (2019). "Protective Effect of Terminalia Chebula Fruit Extract on Ethanol-Induced Hepatotoxicity in Albino Rat", International Journal of Pharmaceutical and Phytopharmacological Research, 9 (1), pp.44-50.

INTRODUCTION

Conventional drugs derived from medicinal flowers are utilized by approximately 60% of over-the-counter population, though over the counter strategies to lessen over-the-counter unwell outcomes of various sicknesses and its secondary headaches, herbal formulations are desired due to lesser facet effects and low cost [1]. A number of medicinal flowers, historically used for over 1000 years, are found in a set of herbal preparations of over the counter Indian traditional fitness care gadget (Ayurveda) named Rasayana proposed for their exciting antioxidant activities [2].

Alcoholic liver disease (ALD) develops in patients consuming excessive amounts of alcohol. Alcohol

dependency is not always a prerequisite for ALD development. In fact, some patients develop ALD and, in particular, cirrhosis without a history of dependence. Moreover, the severity of sickness doesn't perpetually correlate with the quantity of alcohol intake, and environmental and genetic factors likely play a crucial role in ALD development. Although a dose-effect relationship between alcohol intake and alcohol-induced hepatic damage has been reported, there is no set amount of alcohol consumption that could surely predict the development of ALD [3]. In fact, the bulk of long-term serious drinkers develop liver disease, but only 10-35% develops hepatitis and only 8-20% will progress to cirrhosis, and daily ethanol consumption exceeding 40-80 g/day for males and 20-40 g/day for females for 10-12 years can virtually definitely cause ALD. In a large survey conducted in Northern Europe, the relative risk of

Corresponding author: Vuyyala Balakrishna

Address: Vuyyala Balakrishna, Department of pharmacology, GNITC-School of pharmacy, Ibrahimpatnam, Hyderabad.

E-mail: balakrishnavuyyala@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 November 2018; **Revised:** 23 January 2019; **Accepted:** 02 February 2019



ALD significantly increased above a threshold of 7-13 drinks/week for women and 14-27 drinks/week in men [4]. Alcohol leads to increased liver oxidative stress through the generation of highly reactive oxygen species (ROS) and adducts. Alcohol dehydrogenase (ADH) generates acetaldehyde, which is subsequently oxidized to acetate by aldehyde dehydrogenase (ALDH). Acetaldehyde can form hybrid-adducts with reactive residues (e.g. malondialdehyde [MDL] adduct) acting on proteins or small molecules (e.g. cysteines), mediating lipid peroxidation, and nucleic acid oxidation. Further oxidations in alcohol metabolism are accompanied by an excessive reduction of nicotinamide adenine dinucleotide (NAD), with a shift in the NADH/NAD ratio. Under traditional circumstances, reduction of NAD (NAD/NADH) is finely regulated by the cell Krebs cycle. The shift caused by excessive alcohol consumption is assumed to impair saccharide and lipid metabolism, finally causing impairment of gluconeogenesis and diversion of metabolism to ketogenesis and fatty acid synthesis. The magnified quantity of reducing equivalents, such as NADH, leads to their shattering into mitochondria, which induces the electron transport chain components to assume a reduced state. This facilitates the transfer of associate degree negatron to molecular chemical element to get reactive species as superoxide. Mitochondrial ROS generation can even derive from the alterations made in mitochondrial complexes I and III, which have been discussed above. In fact, such alteration can also promote superoxide anion generation within the mitochondria [5]. Thus, mitochondria represent a main site where huge amount of ROS are generated, leading, in turn, to cell damage and necrosis. Finally, the NADH-induced inhibition of mitochondrial β -oxidation leads to accumulation of intracellular lipids, thus promoting steatosis. Excessive alcohol consumption is additionally related to the accelerator induction of CYP2E1 pathway of alcohol metabolism. The recruitment of this pathway may indirectly contribute to ALD development by excess production of superoxide radicals through the interaction of CYP2E1 with cytochrome reductase, which leads to electron leaks in the respiratory chain and ROS production. The species produced in this cascade can interact with iron (Fenton reaction) generating even more potent hydroxyl, ferryl, and perferryl radicals which perpetuate liver damage [6, 7]. Terminalia chebula fruits contain wide range of tannins, triterpenoids, glycosides, anthraquinones, and flavonoids. Due to its antioxidant activity it protects the body's cells and DNA from free radical damage [8]. The present investigation was undertaken to study the effect of T. chebula fruit extract on liver inhibitor enzymes in grain alcohol evoked hepatotoxicity in rats.

MATERIALS AND METHODS

Plant:

Terminalia chebula fruits were freshly collected from the medicinal plants farm, Mulugu, Warangal district surroundings Telangana state. The material was taxonomically known and echt by adult male.P.V.Prasanna, person 'E'/Officer In-charge, BSI, Govt.of India and the voucher specimen No.BSI/DRC/2014-2015/Tech/746.

Animal selection:

Thirty six male Wistar Albino rats weighing 150-250g were obtained from National Institute of Nutrition, Hyderabad. The rats were housed in polypropene cages and maintained below commonplace conditions (12 h light-weight and dark cycles, at $25\pm 3^\circ$ C and 35-60% humidity). Standard pelletized feed and water were provided spontaneously. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2013-14/MPCOL/11).

Extraction:

The fruits were carefully washed under running tap water followed by distilled water. These were air dried and then ground to coarse powder. This dried coarse powder (300g) was used for soxhlet extraction. Extraction was done by using the soxhlet apparatus at a temperature of 60° C for 48 hours. Powder was extracted with 95% ethanol. The solvent thus obtained was evaporated under vaccum to get a semi-solid form of the extract [9]. Percentage yield was 12.1% with respect to dried powder. Oral emulsion containing 50mg, 100mg and 200mg of extract were prepared by using distilled water. This was used for evaluation of the activity.

Experimental animals design:

Induction of experimental hepatotoxicity Rats were treated with four-hundredth plant product (2 ml/100 g, orally) for 21 days to study the effect of ethanolic which was used as a standard drug in this study. Treatment protocol Thirty-six Wistar Albino male rats of weight 150-250 g were selected for this study. Animals were divided into six teams of six animals every.

Group 1: Control group (distilled water) for 21 days.

Group 2: Inducer (Ethanol 2ml/100g body weight, p.o) for 21 days.

Group 3: T.chebula fruit extract 50 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Group 4: T.chebula fruit extract 100 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Group 5: T.chebula fruit extract two hundred mg/kg weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Group 6: Silymarin 25 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Biochemical estimation: At the tip of the study, animals were sacrificed by cervical dislocation with ether anesthesia; blood was obtained from carotid artery. The blood samples were allowed to clot for 45min at temperature. Serum was separated by centrifugation using Remi cool centrifuge at 4000 rpm for 15 min and utilized for the estimation of various biochemical parameters like SGPT, SGOT, ALP, Total proteins and Total bilirubin.

A part of the liver homogenate was taken and mixed with equal volume of 10% trichloroacetic acid (TCA) for the estimation of MDL. Homogenate was centrifuged using Remi cool centrifuge at 8000 rpm for 30 minutes. The supernatant was separated and used for estimation of MDL [10] and antioxidant levels of different enzymes, i.e., CAT [11], GR, and reduced GSH [12] in the liver tissue.

Statistical Analysis:

The results square measure expressed as mean±standard error of the mean. The analysis of the info was done victimization unidirectional multivariate analysis followed by Dunnett’s multiple comparisons tests. P values<0.001 were considered statistically significant.

RESULTS:

The phytochemical screening of the phytochemical screening of *T.chebula* fruits alcoholic extract revealed that it contained large amounts of flavonoids and glycosides; moderate amounts of tanins, and proteins and a few triterpinoids as depicted in Table (1).

Table 1: Preliminary phytochemical screening of ethanolic extract of *T.chebula* fruits.

S.No.	TEST	INFERENCE
1	Test for tannins	
	1.Ferric chloride test	+
	2.Lead acetate test	+
2	Test for proteins	
	1. Biuret test	+
	2. Millon’s test	+
3	Test for alkaloids	
	1. Dragendorff’s test	-
	2. Mayer’s test	-
	3. Hager’s test	-
4	Test for flavonoids	
	1. Shinoda test	+
	2. Ferric chloride test	+
	3. Alkaline reagent test	+
5	Test for anthraquinone glycosides	
	1.Hydroxyanthraquinone Test	+
6	Test for carbohydrates	
7	Test for Triterpenoids	
	1. Salkowski test	+
8	Test for glycosides	
	1. Baljet test	+
	2. Legals test	+
9	Test for saponins	
	1.Foam test	-
10	Test for reducing sugars	
	1.Fehlings test	+
	2.Benedicts test	+

+ = found - = not found.

Table 2: Effect of *T.chebula* fruit extract on serum parameters (SGOT, SGPT, ALP, Total proteins, Total bilirubin) in ethanol induced hepatotoxicity in rats

Groups	Treatment	ALT(IU/L)	ALP(IU/L)	AST(IU/L)	Total proteins (mg/dL)	Totalbilirubn (mg/dL)
I	Normal control	159.1±5.774	185.1±5.76	26.49±0.09	69.74±0.27	1.3±0.32
II	Inducer(ethanol)	560.5±5.77***	674±5.77***	68.8±0.052***	26.77±0.14***	2.12±0.49***
III	TCE (50mg/kg)+ethanol (40%)	410.2±5.77*	556±5.77**	60.80±0.03**	32.91±0.10*	1.92±0.37*
IV	TCE (100mg/kg) ethanol (40%)	290.1±5.768**	431.3±5.768*	47.06±0.239**	41.22±0.13**	1.74±0.35*
V	TCE (200mg/kg)+ethanol(40%)	183.9±5.77**	223.3±5.74**	37.7±0.096**	56.8±0.19**	1.41±0.29**
VI	Silymarin(25mg/kg)+ethanol 40%	213.8±5.80**	198.8±5.74**	30.29±0.033**	59.28±0.25**	1.37±0.38**

Values are expressed as mean ± SEM,n=6. The values were finding out by using ONEWAY ANOVA followed by DUNNETS test.

***p<0.001 as compared with normal control

*p<0.05, **p<0.01 as compared with inducer ethanol



Table 3: Effect of *T.chebula* fruit extract on Antioxidant parameters (MDA, GSH, Catalase and GR) in ethanol induced Hepatotoxicity in rats.

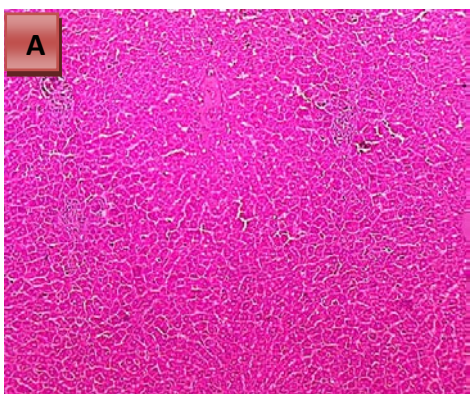
GROUPS	TREATMENT	MDA (nmol/g)	GSH (μmol/min/mg)	CATALASE (μmol/min/mg)	GR(U/ml)
I	Normal control	53.33±2.108	21.58±0.510	20.1±0.03	34.6±0.22
II	Inducer (ethanol40%)	107.24±8.83***	13.88±0.38***	10.3±0.045***	20.5±0.4***
III	TCE (50mg/kg) +ethanol (40%)	92.33±2.2*	16.14±0.17*	12.2±0.010**	25.6±0.5*
IV	TCE(100mg/kg) +ethanol (40%)	73.67±1.89**	19.24±0.220**	15.4±0.044*	29.6±0.4**
V	TCE(200mg/kg) +ethanol (40%)	56.67±2.61**	21.42±0.344**	18.5±0.021**	31.6±0.3**
VI	Silymarin(25mg/kg)+ ethanol40%	49.0±2.27**	26.12±0.155**	17.8±0.028**	33.5±0.3**

Values are expressed as mean ± SEM, n=6. The values were finding out by using ONEWAY ANOVA followed by DUNNETS test.

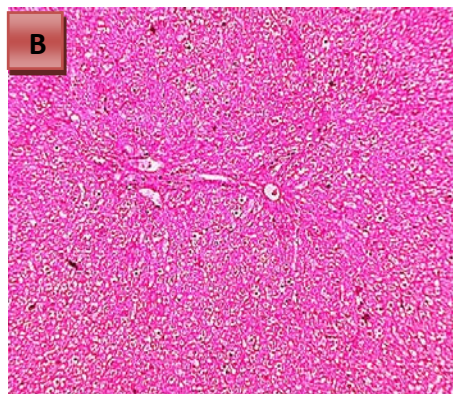
***p<0.001 as compared with normal control

*P<0.05, **p<0.01 as compared with inducer ethanol.

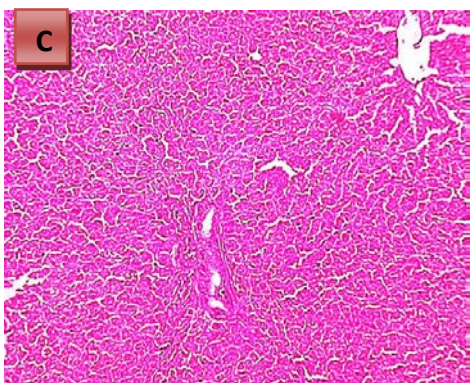
Histopathology: Effect of ethanolic extract of *T.chebula* fruiton Liver histopathology.



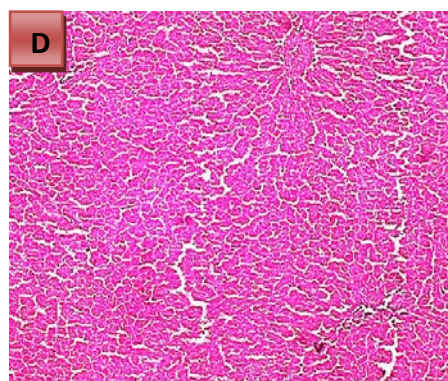
A. Normal control



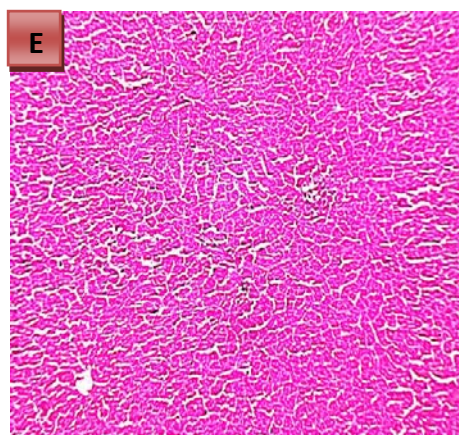
B. Ethanol (40%) treated



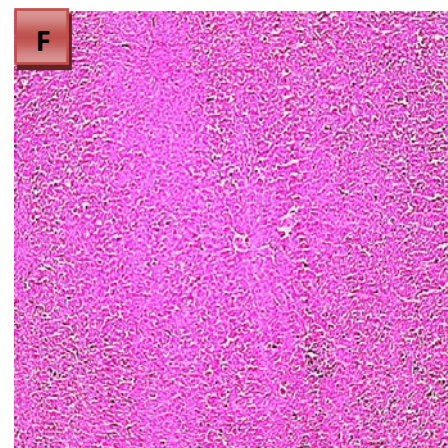
C. TCE (50mg/kg) +Ethanol (40%)



D. TCE (100mg/kg) +Ethanol (40%)



E. TCE (200mg/kg) +Ethanol (40%)



F. Silymarin(25mg/kg)+Ethanol(40%)

- A. Hepatocytes are normal in cell morphology with moderate eosinophilic cytoplasm contains fine chromatin. hepatic vein, central vein and portal tracts are in normal position.
- B. There are ballooning degeneration of hepatocytes with patchy and perivascular inflammatory component comprising of lymphomononuclear cell aggregates. There is mild central vein dilation and focal fibrosis seen in toxicant ethanolic group.
- C. The liver section show still persisting of degenerated hepatocytes with sparse inflammatory component. Bile duct, central vein, portal tract are normal in TCE (50mg/kg).
- D. The liver section show disappearance of trabeculae and hepatocytes appeared to be normal. Very less mononuclear inflammatory infiltration is found in TCE (100mg/kg).
- E. Liver section show still persisting of less degenerated hepatocytes with sparse inflammatory component. Bile duct, central vein, portal triad are normal in TCE (200mg/kg).
- F. Liver section show hepatocytes are normal in cell pattern and arrangement. No inflammatory component, no thrombosis, portal triad, central vein and bile duct are more or less normal in standard silymarin (25mg/kg).

DISCUSSION

Alcohol is one in all the foremost necessary and frequently used toxic agents within the experimental study of liver connected disorders. The toxic effects of alcohol are mostly thanks to its active substance trichloromethyl radical. This activated radical bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (PUFA). This process leads to excessive formation and accumulation of lipids in tissues such as liver. Lipids from peripheral adipose tissue are translocated to liver for accumulation [13, 14].

Ethanol exposed rats exhibited decrease in catalase level when compared to normal group. Treatment with various doses of T.chebula fruit extract significantly increased catalase levels when compared to ethanolic group [15].

Glutathione reductase, also known as GSR or GR, is an enzyme, reduces glutathionedisulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. The monitored by NADPH consumption [16, 17]

The protective action of antioxidants is usually due to the inhibition of free radical chain reaction and the resultant prevention of peroxidative deterioration of structural lipids in membranous organelles [18].

The protein and non-enzymatic like enzyme (SOD), catalase (CAT) and reduced glutathione (GSH) play important role in alleviating tissue damage due to the formation of free radicals [19, 20].

Serum AST, ALT, mount are the foremost sensitive markers utilized within the diagnosing of internal organ injury as a result of these are protoplasm in location and discharged into the circulation once cellular injury. The multiplied activities of AST, ALT, ALP and Total bilirubin level in serum manifested the ethanol induced hepatocellular damage. Treatment with TCE significantly decreased the activities of AST, ALT, ALP and Total bilirubin in serum suggesting that they offer protection by preserving the structural integrity of hepatocellular membrane against Ethanol [21].

In this study, we tend to determine the hepatoprotective result of Terminalia chebula in ethyl alcohol induced hepatotoxicity in rats. A significant elevation was determined within the levels of humour AST, ALT, ALP, Total bilirubin and significant decrease level Total protein in ethanolic group which received ethanol as compared to control group rats who received distilled water [22].

Elevated levels of those parameters in liquid body substance are presumptive markers of toxic lesions within the liver. Co-administration of ordinary Silymarin and varied doses of (50mg/kg, 100mg/kg & 200mg/kg) T.chebula fruit ethanolic extract with ethanol in various extract groups, maintained the levels of AST, ALT, ALP, and serum total macromolecule and Total hematoidin towards traditional as compared to ethyl alcohol elicited rats [23]. This was presumably thanks to the anti-oxidizing agent result of Terminalia chebula constituents on morphological examination in low dose Terminalia chebula showed partial inflammation in internal organ cells.

While in high dose (200mg/kg) extract of Terminalia chebula fruit showed an extremely recovery compared to traditional.

CONCLUSION

In this study, the Wistar albino rats pretreated with ethanolic extract of Terminalia chebula fruit ahead ethanol and then determined the effect of this extract on various biochemical parameters and antioxidants.

Tissue parameters:

In ethanol - induced Hepatotoxicity with liver damages were inhibited by oral treatment of T.chebula fruit extracts 50mg/kg, 100mg/kg, and 200mg/kg. In addition, they additionally increased the liver inhibitor defense systems —they dose-dependently repressed Ethanol-induced will increase of LPO and changes on the GSH contents, GR and enzyme activities as direct evidences that T.chebula fruit extracts have favorable ameliorating

effect on the oxidative damage and related organ damages induced by Ethanol through antioxidant effects.

Serum parameters:

In case of Hepatotoxicity induced groups, there were increased in levels of SGOT, SGPT, ALP, and Total bilirubin levels and decreased in levels of Total proteins. In the present study treatment with T.Chebula fruit extract significantly decreased the elevated levels of SGOT, SGPT, ALP, Total bilirubin and increased the levels of Total proteins.

The hepatoprotective role of Ethanolic extract of Terminalia chebula fruit could be thanks to its inhibitor potential mechanism suggesting that the extract of plant could also be helpful to forestall the ethanol induced Hepatotoxicity. More analysis is needed during this View-point to develop an honest hepatoprotective drug from fruits of Terminalia chebula. Purification of extract and identification of the active principle might yield active Hepatoprotective ingredients.

REFERENCES

1. ManishaModak, Priyanjali Dixit, JayantLondhe, SarojGhaskadbi, Thomas Paul A, Devasagayam. Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes. *J ClinBiochemNutr.*2007; 40(3): 163–167.
2. Scartezzini P., Sproni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.* 2000; 71: 23–43.
3. Mandayam S, Jamal MM, Morgan TR. Epidemiology of alcoholic liver disease. *Semin Liver Dis* 2004; 24(3):217-32.
4. Roizen R, Kerr WC, Fillmore KM. Cirrhosis mortality and per capita consumption of distilled spirits, United States, 1949-1994: trend analysis. *West J Med* 1999; 171(2):83-7.
5. Suguna L, Singh S, Sivakumar P, Sampath P, Chandrakasan G. Influence of Terminalia chebula on dermal wound healing in rats. *Phytother Res* 2002; 16(3):227-31.
6. Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. *Alcohol Res Health* 2003; 27(4):277-84.
7. Thurman RG. Mechanism of hepatic toxicity: II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *Am J Physiol* 1998; 275:G605-11.
8. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of Terminalia chebula, Terminalia bellirica and Emblicaofficinalis. *BMC Complement AlternMed*2010; 10:20.
9. Sabu MC, Ramadasan K. Anti-Diabetic Activity of Medicinal Plants and Its Relationship with Their Antioxidant Property. *J. Ethnopharmacol* 2002. 81:155-160.
10. Bergmeyer HU, Hørder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). *J ClinChemClinBiochem* 1986; 24(7):481-95.
11. Georg LE. Tissue sulfhydryl group. *Arch BiochemBiophys* 1959; 82(1):70-7.
12. Saravanan N, Nalini N. Antioxidant effect of Hemidesmusindicus on ethanol-induced hepatotoxicity in rats. *J Med Food* 2007; 10(4):675-82.
13. Bowers GN and McCommb RB. *ClinChem* 1972; 18:97.
14. Rajalingam D, Varadharajan R, Palani S. Evaluation of hepatoprotective and antioxidant effect of Combretumalbidum g. Don against ccl4 induced hepatotoxicity in rats. *Int J Pharm PharmSci* 2016; 8(9): 218-23.
15. Balakrishna V, Lakshmi T. Hepatoprotective activity of ethanolic extract of Terminalia chebula fruit against ethanol induced hepatotoxicity in rats. *Asian j pharm clin res* 2017; 10 (11): 55-58
16. Venditti P, Balestrieri M, Di Meo S. Effect of thyroid state on lipid peroxidation, antioxidant defences and susceptibility to oxidative stress in rat tissues. *Journal of Endocrinology.* 1997; 151–157.
17. Valko M, Leibfritz D, Moncol J. Free radical and antioxidants in normal physiological functions and human disease. *J Biocell* 2006; 7(1):45-78.
18. TorenFinkel and Nikki J H olbrook. Oxidants, oxidative stress and biology of ageing. *Nature.* 2000; 408: 239-247.
19. Priyanka S, Anupam DT, Manabendra DC. Antioxidant potential profile of Pajanelialongifolia (Willd.) k. Schuman; Potential new sources of natural antioxidant. *Asian J Pharm Clin Res* 2017; 10(6):184-8.
20. Chance B, Green Stein DS and Roughton RJW. The mechanism of catalase action- steady state analysis. *Archives of Biochemistry and Biophysics.* 1952; 37:301-39.
21. Navarro CM, Montilla MP, Martin A, Jimenez J, Utrilla MP. Free Radical Scavenging and Antihepatotoxic Activity of



- Rosamarinustomentosus. *PlantaMedica*. 1993; 59:312-316.
22. Aquaisua NA, Bassey BR, Ikpeme MB, Bassey IE. Effect of Crude Extracts of *Blighiaunijugata* on histology of the Liver and Kidney of Adult Wistar Rats. *Toxicology International*. 2011; 19(1):63-67.
23. Oboso E. Etim, Jessie I.Ndem, Utibe Evans B. Effect of ethanol leaves extract of *Justiciaschimperi* on liver enzymes, serum proteins and bilirubin. *International Journal of Research in Medical Sciences* 2016; 4(9); 2593-2597.