Int J Pharm Bio Sci 2014 Jan; 5(1): (P) 166 - 175

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PREVENTIVE AND CURATIVE EFFECT OF ETHYL ACETATE FRACTION OF *GEODORUM LAXIFLORUM* EXTRACT ON PARACTEMMOL (PCM)-INDUCED HEPATOTOXICITY IN RATS.

RAM CHANDRA BESRA*, LAKHAN MAJHEE AND JANARDAN SHARMA

Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

ABSTRACT

Polyphenolic of medicinal plants and nutraceuticals are well known for the antioxidant property. In pathogenic conditions, antioxidants are being used to curtail the deleterious effect of free radicals globally. *Geodorum laxiflorum* has been used as folklore medicine in India for liver diseases. Therefore, the aim of this study was to investigate the preventive and curative activity of ethyl acetate fraction; compare the effect of fraction given by oral and intraperitoneal route of *Geodorum laxiflorum* methanolic extract in Paracetamol- induced liver damage rats. Study was conducted in Paracetamol-induced hepatoxicity model. Results of ethyl acetate fraction indicate that intraperitoneal route of administration was more effective than the oral route in both preventive and curative study. The study not only supports the traditional use but also provides the basis to identify, isolate molecule and develop a formulation from ethyl acetate fraction of the plant *Geodorum laxiflorum.*

KEYWORDS: Preventive and curative, Hepatoprotective, Paracetamol, Hepatotoxicity, Ethyl acetate fraction

RAM CHANDRA BESRA
Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

***Corresponding author**

INTRODUCTION

Liver is the vital organ and gland present in the body of vertebrate. Liver is responsible for metabolism of food materials, drugs and chemicals. It synthesizes many potent biochemicals' such as carbohydrate, proteins and fats which is necessary for normal body functions. Hepatic injury is mainly associated with disturbance of metabolic functions. Variety of liver ailments may arise due to long and continuous exposer of infections, toxicants, xenobiotics, drugs, alcohols etc. Thus liver diseases are remaining serious problem to health[1]. Globally, about 796,000 deaths per year reported is because of liver cirrhosis[2,3,4]. Many folklore herbal remedies are being used for liver diseases due to absence of reliable liver protective agent in modern medicine. Therefore, various plants have been evaluated for their hepatoprotective activity against different chemical induced liver damage in experimental animals[5,6,7,8]. Liver damage was induced by Paracetamol, which involves in biotransformation of free radicals. Increased free radicals level responsible for lipid per-oxidation and thus lead into cell death in liver tissue. Therefore, model of Paracetamol-induced liver injury are been used in new drug development widely for liver diseases.

Geodorum laxiflorum is a terrestrial orchid plant, abundantly found in Jharkhand[9]. Traditionally, pseudo-bulbs of this plant are being used for the treatment of jaundice, malignant tumor and joint pain by the tribes of Jharkhand in India. In our previous preliminary study, crude methanolic extract of pseudo-bulbs of *Geodorum laxiflorum* have shown positive results regarding the hepatoprotective and anti-oxidant activity[10]. It is necessary to conduct more systematic and comprehensive study in another model of hepatotoxicity for validation of hepatoprotective activity of fraction of extract of *Geodorum laxiflorum* in Paracetamol-induced liver injury. Therefore, aim of present study is to examine preventive and curative effect of ethyl acetate fraction given by oral and parenteral route of *Geodorum laxiflorum* extract on Paracetamol induced liver injury.

MATERIALS AND METHODS

(1) Chemicals

Paractemmol was purchased of Sigma Aldrich. Silymarin was procured from Sigma Aldrich India. Commercially available diagnostic kits (SGOT, SGPT, Alkaline phosphatase and total bilirubin) were procured from the central store of the institute. The diagnostic kits used were made of Biocon Ltd, Crest Biosystem and Accrex India.

(2) Plant Materials

The whole plant of *Geodorum laxiflorum* including pseudo-bulbs were collected from the local forest at Kalamati, Ranchi, Jharkhand, India. The collected plant was authenticated by Dr. A. K. Bariar, Post-Graduate Department of Botany, Ranchi College, Ranchi University, Ranchi, India. Pseudo-bulbs of plant were crushed, dried in the shade and powdered.

(3) Preparation of Extract and its Fractions

250 g powdered pseudo-bulbs of the plant was extracted with 500 mL volume of methanol for 12hrs in a Soxhlet apparatus. The extract was evaporated in Rotatory vaccum evaporator and then dried in laboratory vacuum pump. A sticky residue obtained (Yield was 25 g, 10%). Fractions were obtained from 50 g of residue of total methanolic extract when fractionated with 10X250 mL each using solvent e.g. toluene, *n*-butanol, chloroform and ethyl acetate. Water was not used because powder of pseudo-bulbs getting swell and make highly mucilaginous and viscous mass, which render from extraction and fractionation. The isolated yield obtained for toluene fraction, n-butanol fraction, chloroform fraction and ethyl acetate fraction (EAF) were 5% (2.5g), 8.72% (4.36g), 18.5% (9.25g) and 55% (27.5 g) respectively.

(4) Animals

All adult Wistar albino rats were procured from the central animal house, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. The animals were acclimatized in-house conditions.

All the animals were allowed to feed a commercial pellet diet made of Hindustan Liver Limited and water *ad libitum.* All the experimental protocol was undertaken in accordance with animal ethical guidelines. Studies were approved by Institutional Animal Ethics Committee (IAEC) of the Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

(5) Preliminary phytochemical tests of Ethyl acetate fraction

It involves testing of Ethyl acetate fraction (EAF) of methanolic extract of *Geodorum laxiflorum* for different classes of phytochemicals. The common qualitative tests were performed to get the general idea regarding nature of compounds present in fraction [11].

(6) Acute oral toxicity study

Acute oral toxicity study of the ethyl acetate fraction (EAF) of methanolic extract of pseudo-bulbs of *Geodorum laxiflorum* was performed according to OECD 423 guidelines. Adult Wistar female rats were used in this study[12].

(7)Evaluation of Preventive and Curative effect of EAF on PCM-induced Hepatotoxicity rats:

Experimental design

The animals weighing 150-250 g were divided into eight groups of six rats each and subjected to the following treatments[13].

Group I: received the vehicle, normal saline, per oral (*p.o.)* only and served as normal control.

Group II: served as toxicant control and treated with Paracetamol.

Preventive study group (Pre-treatment with EAF)

Group III: received Silymarin (standard) at the dose of 100 mg kg^{-1} body weight.

Group IV: received EAF at the dose of 200 mg kg^{-1} body weight, *p.o.*

Group V: received EAF at the dose of 200 mg kg^{-1} body weight, intraperitoneal (*i.p.)*

Curative study group (Post-treatment with EAF)

Group VI: received Silymarin (standard) at the dose of 100 mg kg^{-1} body weight.

Group VII: received EAF at the dose of 200 mg kg^{-1} body weight, *p.o.*

Group VIII: received EAF at the dose of 200 mg kg^{-1} body weight, *i.p.*

Group I was kept as normal control group and untreated and received only single daily dose of normal saline (5 mL/kg) as vehicle. Group II was considered as toxicant control and PCM (750 mg/kg) injected intraperitoneally for 7^{th} day after 7^{th} day of vehicle treatment.

Preventive study (Pre-treatment with EAF)

Group III was considered as standard control (Silymarin 100 mg/kg body wt., p.o.) and animals were pre-treated once daily for 7 days. Animals of group IV and V were pre-treated with EAF 200 mg/kg body wt. once daily by oral (p.o.) and intraperitoneal (i.p.) route of administration for 7 consecutive days. Then, group III, IV and V were treated with toxicant PCM (750 mg/kg) injected intraperitoneally once daily for another 7 days after 7^{th} day pre-treatment with standard and EAF.

Curative study (Post-treatment with EAF)

Group VI was considered as standard control (Silymarin) and animals were intoxicated with PCM (750 mg/kg) injected once daily intraperitoneally for 7 days followed by treatment with Silymarin 100 mg/kg body wt., p.o once daily for another 7 days. Animals of group VII and VIII were treated with toxicant PCM (750 mg/kg) injected once daily intraperitoneally for 7 days followed by treatment with EAF 200 mg/kg body wt. once daily by oral (p.o.) and intraperitoneal (i.p.) route of administration for another 7 consecutive days. All the animals were maintained under normal diet and water during the whole treatment period. On 15^{th} day, i.e. twenty four hour after the last treatment all the animals were anesthetized under light ether anesthesia and blood was collected by puncturing retro-orbital plexus for estimation of liver marker enzymes.

Preparation of Paracetamol suspension

Desired quantity (750 mg/kg body weight of animal) of paracetamol was taken into mortar and pestle. Measured volume i.e. 40% w/v sucrose solution was added into it. Mixed the above content by triturating and adjusted the desired volume with remaining sucrose solution. Alteration in serum levels of marker enzymes among the study groups of rats were also expressed in percent change. Percent change was calculated using formula- % change = (initial reading - final reading/initial reading) x 100

(8) Statistical Analysis

The results were expressed as Mean±SEM. Statistical significant was determined by One-way Analysis of Variance (ANOVA) and subjected to Tukey's multiple comparison tests. Significant difference between the mean was accepted when $p<0.05$. SPSS 17 software was used for statistical analysis of data.

RESULTS

(1) Preliminary qualitative phytochemical tests of EAF

Phytochemical screening of ethyl acetate fraction of *Geodorum laxiflorum* extract confirmed the presence of bilogicaly potent compounds particulary glycosides and polyphenols and flavonoids (table 1).

(2) Oral toxicity study

All the doses 5, 50, 300 and 2000 mg/kg bw of ethyl acetate fraction (EAF) used for acute oral toxicity study were found non-toxic and safe. No mortality and behavioral changes were observed even in highest dose (2000 mg/kg bw) employed to animals. The highest screening dose (2000 mg/kg bw) was fixed as LD_{50} cut-off value. Therefore, 200 mg/kg bw ($1/10^{th}$ of 2000 mg/kg bw) was selected for evaluation of preventive and curative activity.

(3)Preventive and Curative Effect of Ethyl acetate fraction (EAF) on Biochemical parameter in PCM - damaged rat liver:

Preventive and curative effect of oral and intraperitoneally administered EAF at a dose of 200 mg/kg body weight was compared with standard. So, protective and curative potential of EAF was evaluated by the estimation of altered serum levels of SGPT, SGOT, alkaline phosphatase, total protein and total bilirubin (table 2).

Serum levels of liver marker enzymes

Results of hepatoprotective effects of EAF can be summarized as follows-

In this study, EAF have altered elevated serum levels significantly ($p<0.001$) of SGPT, SGOT, Alkaline phosphatase, Total protein and total bilirubin at a dose of 200 mg/kg body weight. Hepatoprotective effect of EAF (p.o.) and EAF (i.p.) was assessed by altering ability of the elevated serum levels of liver marker enzymes. Preventive and curative effect of EAF (p.o.) and EAF (i.p.) was compared and found highly significant ($p<0.001$) when compared to Paracetamol- intoxicated group (toxicant group). Percent of change (%) of Paracetamol-intoxicated groups (toxicant control) in serum levels of SGPT, SGOT, alkaline phosphatase, total protein and total bilirubin were 110.36, 157.20, 108.04, 22.46 and 203.70 respectively in relation to normal control group.

Maximum percent of change obtained in serum SGPT level with EAF (200 mg/kg, i.p.) for preventive and curative effect was 31.77% and 28.81% respectively in relation to Paracetamol - intoxicated groups. While, 18.84% and 15.51% change in serum SGPT level for preventive and curative effect of EAF (200 mg/kg, p.o.) was observed in relation to Paracetamol- intoxicated rats (toxicant group).

31.01% and 28.81% change of serum SGOT level in curative and preventive effect was obtained with EAF (200 mg/kg, i.p.) in relation to Paracetamol- intoxicated rats. Similarly, 17.75% and 15.27% change of serum SGOT level in curative and preventive effect was obtained with EAF (200 mg/kg, p.o.) in relation to Paracetamol-intoxicated rats (toxicant group). 11.20% and 8.96% change of serum alkaline phosphatase level in preventive and curative effect was obtained with EAF (200 mg/kg, i.p.) in relation to Paracetamol-

intoxicated rats. Similarly, 5.70% and 4.58% change of serum alkaline phosphatase level in preventive and curative effect was obtained with EAF (200 mg/kg, p.o.) in relation to Paracetamol- intoxicated rats (toxicant group). 15.78% and 21.84% change of serum total protein level in preventive and curative effect was obtained with EAF (200 mg/kg, i.p.) in relation to Paracetamol- intoxicated rats. Similarly, 12.89% and 14.21% change of serum total protein level in preventive and curative effect was obtained with EAF (200 mg/kg, p.o.) in relation to Paracetamol- intoxicated rats (toxicant group). .

47.56% and 46.95% change of serum total bilirubin level in preventive and curative effect was obtained with EAF (200 mg/kg, i.p.) in relation to Paracetamol- intoxicated rats. Similarly, 40.85% and 31.70% change of serum alkaline phosphatase level in preventive and curative effect was obtained with EAF (200 mg/kg, p.o.) in relation to Paracetamol-intoxicated rats (toxicant group). Silymarin (100 mg/kg, p.o.) given to standard groups of animals have shown percent change (%) of preventive effect- 36.94, 34.94, 17.00, 27.63, 53.04 in serum SGPT, SGOT, alkaline phosphatase, total protein and total bilirubin respectively in relation to Paracetamol-intoxicated rats (toxicant group). Silymarin (100 mg/kg, p.o.) given to standard groups of animals have shown percent change (%) of curative effect- 37.06, 40.78, 15.89, 25.78, 52.43 in serum SGPT, SGOT, alkaline phosphatase, total protein and total bilirubin respectively in relation to Paracetamol-intoxicated rats (toxicant group). EAF (200 mg/kg) given to different treatment group through intraperitoneal route (i.p) of administration was found more effective than the treatment given by oral route (p.o.) of administration in PCM- induced hepatotoxicity in rats. Although, preventive and curative effects of either EAF (200 mg/kg, p.o) or EAF (200 mg/kg, i.p.) were found less than the silymarin (100 mg/kg, p.o.) in PCM-induced hepatotoxicity in rats. Thus, EAF (200 mg/kg,) given by intraperitoneal route (i.p.) of administration to the different treatment groups have shown more preventive effect than curative effect in Paracetamol -induced hepatotoxicity in rats.

Table 1

Results of Phytochemical screening of Ethyl acetate fraction (EAF) of Geodorum laxiflorum extract

Phytochemicals	Qualitative tests	Ethyl acetate fraction
Glycosides	Raymond's test	+++
Alkaloids	Wagner's test	-
	Dragendorff's test	-
	Mayer's test	-
	Hager's test	-
	Tannic acid test	
Polyphenols	Ferric chloride test	+++
& **Tanins**	Mitchell's test	++
	Alkaline reagent test	++
Flavonoids	Shinoda test	+++
	Sulphuric acid test	+++
	Alkaline reagent test	+++
Zinc Hydrochloride reduction test		+++
Sterols	Salkowski reaction	++
	Libermann- Buchard test	++
Saponins	Production of foam	+
Proteins &	Millons test	-
Amino Acids	Ninhydrin test	-

+ = Present; - = absent

Table 2

Preventive and Curative effect of EAF treatments given by oral (p.o) and intraperitoneal (i.p.) route of Geodorum laxiflorum on the biochemical parameters of Paracetamol (PCM)-intoxicated rats

Treatment Group	*Dose*	*SGPT IU/L*	*SGOT IU/L*	*ALP IU/L*	*Total protein g/dL*	*Total bilirubin mg/dL*
Normal	Normal saline	64.33±0.882	57.67±1.706	78.67±1.520	5.42±0.114	0.54±0.007
PCM	750 mg/kg, b.w., *i.p.*	135.33±1.174[a] *+110.36**	148.33±1.308 [a] *+157.20**	163.67±1.820 [a] *+108.04**	3.80±0.516 [a] *-29.88**	1.64±0.060 [a] *+203.70**
Preventive Effect						
Silymarin (Standard)	100 mg/kg, b.w., *p.o.*	85.33±1.202[b] *+32.64** *-36.94***	96.50±1.544[b] *+67.33** *-34.94***	135.83±1.138[b] *+72.64** *- 17.00***	4.85±0.034[b] *-10.51** *+27.63***	0.77±0.013[b] *+42.59** *- 53.04***
EAF	200 mg/kg, b.w., *p.o.*	109.83±2.455 [b] *+70.72** *- 18.84***	125.67±.882 [b] *+117.91** *-15.27***	154.33±1.256 [b] *+96.17** *-5.70***	4.29±0.007 [b] *-20.84** *+12.89***	0.97±0.006 [b] *+79.62** *- 40.85***
EAF	200 mg/kg, b.w., *i. p.*	92.33±1.202 [b] *+43.55** *-31.77***	108.00±1.414 [b] *+87.27** *-27.18***	145.33±1.282 [b] *+96.17** *-11.20***	4.40±0.070 [b] *-18.81** *+15.78***	0.86±0.008 [b] *+59.25** *- 47.56***
Curative Effect						
Silymarin (Standard)	100 mg/kg, b.w., *p.o.*	85.17±1.108 [b] *+32.39** *-37.06***	87.83±1.778 [b] *+52.29** *-40.78***	137.67±1.308 [b] *+74.99** *- 15.89***	4.78±0.040 [b] *-11.80** *+25.78***	0.78±0.009 [b] *+44.44** *-52.43***
EAF	200 mg/kg, b.w., *p.o.*	114.33±1.282 [b] *+77.72** *- 15.51***	122.00±.966 [b] *+111.54** *-17.75***	156.17±1.046 [b] *+98.51** *- 4.58***	4.34±0.006 [b] *-19.92** *+14.21***	1.12±0.047 [b] *+107.40** *-31.70***
EAF	200 mg/kg, b.w., *i. p.*	96.33±1.022 [b] *+49.74** *-28.81***	102.33±1.542 [b] *+77.44** *-31.01***	149.00±0.775 [b] *+89.37** *-8.96***	4.63±0.049 [b] *-14.57** *+21.84***	0.87±0.010 [b] *+61.11** *- 46.95***

*Numbers of animals (n = 6). a = P<0.001, when compared to the normal group. b = P<0.001, when compared to the PCM group. * = % of change in relation to normal control, ** = % of change in relation to toxicant control (PCM), b.w. = body weight, p.o. = oral, i.p. = intraperitoneal EAF = Ethyl acetate fraction. PCM = paracetamol*

DISCUSSION

The aim of the present study was to investigate the preventive and curative effects of ethyl acetate fraction (EAF) of *Geodorum laxiflorum* extract given by oral and intraperitoneal route to Paracetamol induced liver damage in rats.

Liver damage induced by Paracetamol is widely accepted experimental model for the screening and in search of novel hepatoprotective agent[14,15]. The basic mechanism behind paracetamol induced liver toxicity is related to the covalent binding of its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) to sulfhydryl groups of GSH as well as with various thiol-containing proteins and their subsequent oxidation[16]. The depletion of GSH causes the endogenous reactive oxygen species (ROS) to bind to cellular macromolecules leading to lipid peroxidation, membrane breakdown, and cell death[17]. The significant reduction ($p<0.001$) in Paracetamol-induced elevated serum level of SGPT, SGOT, Alkaline phosphatase, total bilirubin and increased serum total protein were found with oral and intraperitoneally administered ethyl acetate fraction(EAF) of *Geodorum laxiflorum* extract. The significant reduction ($p<0.001$) in Paracetamol induced elevated serum level of marker liver enzymes were observed in both preventive and curative mode of treatment with oral and intraperitoneally administered ethyl acetate fraction (EAF) of *Geodorum laxiflorum* extract. This activity demonstrates that the ethyl acetate fraction (EAF) of *Geodorum laxiflorum* extract has ability to restore the normal functional status of injured liver. Ethyl acetate fraction (EAF) has shown to prevent and cure liver against Paracetamol induced toxicity.

Antioxidants are the molecule of synthetic and natural origin having ability to scavenge, deactivate and stabilize the highly reactive free radicals. It is well documented that reactive free radicals are responsible for cellular injury including liver damage[18]. Polyphenols and Flavonoids are major phytoconstitutents having antioxidant activity[19,20,21]. Phytoconstitutents have the ability to accelerate the excretion of hepatotoxins or inhibit the lipid peroxidation[22]. Investigators have already reported the hepatoprotective potentials of glycosides, polyphenols and flavonoids[23,24]. In disease conditions, the defense against free radicals is weakened or damaged and thus the oxidant load increases. In such conditions, external supplement of antioxidants is necessary to countervail the deleterious consequences of oxidative stress[25,26]. It is proposed that polyphenols as antioxidants can act by a number of mechanisms, such as free radical scavenging, in which the polyphenols can either break the free radical chain reaction or suppress the free radical formation by regulation of enzyme activity and chelating metal ions responsible for free radical production. It is also been proposed that the interaction between polyphenolic compounds with other physiological antioxidants can be possible antioxidant pathway for these compounds[27,28]. In preliminary, phytochemical tests of ethyl acetate fraction of methanolic extract of pseudo-bulbs of *Geodorum laxiflorum* indicate the presence of potent phytoconstitutents mainly glycosides, polyphenols and flavonoids. One of the main mechanisms of hepatoprotective activity is due to the presence of antioxidants in medicinal plants as all antioxidants of synthetic and natural origin inhibit the covalent binding of free radicals to vital macromolecules[29]. Thus, antioxidants protect liver damage from free radicals. Therefore, there is possibility that biologically active antioxidant like phytoconstitutents present in ethyl acetate fraction of methanolic extract in alone or combination responsible for preventive and curative effect against Paracetamol- induced liver injury.

CONCLUSION

In conclusion, the present study revealed the preventive and curative effect of ethyl acetate fraction given by oral and intraperitoneal route

to Paracetamol -induced liver damaged rats. The protective and curative effect of EAF may be because of its free radical scavenging ability. Further comprehensive investigation of this fraction is required to isolate the active compounds and validate the hepatoprotective activity

Conflict of interest statement

We declare that we have no conflict of interest

ACKNOWLEDGEMENT

We would also like to acknowledge to Dr. AK Bariar, Post-Graduate Department of Botany, Ranchi College, Ranchi University for identifying the plant.

REFERENCES

1. Wolf PL. Biochemical diagnosis of liver diseases. Ind J Clin Biochem; 14: 59-90, (1999).
2. Matthers CD, Stein C, Ma Fat D, Rao C, Global burden of disease-2000. Version 2, methods and results. GPE discussion paper no. 50. Geneva World Health Organization; 1-2, (2002).
3. Salomon JA, Murray CJThe epidemiological transition revisited: Composition models for causes of desths by age and sex. Popul Dev Rev; 28: 205-28, (2002).
4. WHO, World Health Report. Reducing risks promoting healthy life. Geneva WHO; 1-13, (2002).
5. Asima C, Satyesh CP, The treatise of Indian Medicinal Plants. Vol.IV, Publications and Information Directorate, CSIR, New Delhi; 85-87, (2001).
6. Dahanukar SA, Kulkarni R.A, Rege NN, Pharmacology of medicinal plants and natural products. Indian J Pharmacol; 32: 81-118, (2002).
7. Ram VJ, Herbal preparations as a source of hepatoprotective agents. Drug News Perspect; 14(6): 353, (2001).
8. Valiathan MS, Healing plants. Curr sci; 75: 1122-1127, (1998).
9. Kumar P, Jalal JS, Rawat GS, Orchidaceae, Chotanagpur, State of Jharkhand, India. Check List; 3: 297-304, (2007).
10. Besra RC, Majhee L, Sharma J, Evaluation of Phytochemical, Anti-oxidant and Hepatoprotective activity of tuber of *Geodorum laxiflorum* Griff. J.Pharmacol. Toxicol; 6(7): 610-623, (2011).
11. Silva GL, Lee IS and Kinghorn AD, Special Problem with the Extraction of Plants. In: Natural Products Isolation, Cannell, R.J.P. (Ed.). Humana Press, Totowa, New Jersey; ISBN: 9780896033627, 343-363, (1998).
12. Ecobion DJ. The basis of Toxicology Testing 1st Edn., CRC Press, New York; 43-86, (2007).
13. Nandeep SS, Chopra D, Kaur S, Amleioration of Paracetamol-induced hepatotoxicity by a protein isolated from the leaves of the herb *Cajanus acutifolius* Linn. International Journal of Pharmacy and Pharmaceutical Sciences; 2(3): 75-80, (2010).
14. Rumyana S, Vessela V, Magdalena K, Ilina K, Vassil M. Hepatoprotective and Antioxidant Effects of Saponarin, Isolated from*Gypsophila trichotoma* Wend. on Paracetamol-Induced Liver Damage in Rats. Biomed Res Int; 2013:1-10,(2013).
15. Knight TR, Fariss MW, Farhood A, Jaeschke H, Role of lipid peroxidation as a mechanism of liver injury after acetaminophen overdose in mice. Toxicological Sciences*;*76(1):229–236, (2003).
16. Bessems JGM, Vermeulen NPE, Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Critical Reviews in Toxicology*;* 31(1):55–138, (2001).

17. Udem SC, Madubunyy II, Okoye JOA, Anika SM. Anti-hepatotoxic effects of the ethanolic extracts of Combretum dolichopetalum root bark and Morinda lucida leaf. Fitoterapia;68(1):21–26, (1997).
18. Halliwel B, Free radicals, antioxidants and human disease: curiosity, cause or consequences. Lancet; 344:721-724, (1994).
19. Zhou K., Wang H, Li X, Luo Y and Dai H, Antioxidant activity of papaya seed extracts. Molecules; 25: 6178-6192, (2011).
20. Till B, Christian L, Gaby K, Stephanie G, Ulrich HE. Bioavailability and antioxidant potential of rooibos flavonoids in humans following the consumption of different rooibos formulations. Food Chem;128: 338-347, (2011).
21. Jaggadeo J, Brody N, Complementary antioxidant function of caffeine and green tea polyphenols in normal human skin fibroblasts. J. Drugs Dermatol; 10:753-761, (2011).
22. Mehta RS, Shankar M, Saliyu AK, Hepatoprotective activity of *Trianthema portulacastrum* Indian Drugs; 36: 241-244, (1999).
23. Ma T, Huang C, Zong G, Zha D, Meng Li X J and Tang W, Hepatoprotective effects of geniposide in a rat model of nonalcoholic steatohepatitis. J Pharm Pharmacol; 63:587-593, (2011).
24. Yue-Tao L, Bi-Nan L and Jin-Yong P, Hepatoprotective activity of total flavonoids from *Rosa laevigata* Michx fruit in mice treated by paracetamol. Food Chemistry; 125: 719-725, (2011).
25. Ratnam, DV, Ankola DD, Bhardwaj V, Sahana DK and Kumar MN, Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. Journal of Controlled Release; 113(3): 189-207, (2006).
26. Reuter S, Gupta SC, Chaturvedi MM and Aggarwal BB, Oxidative stress, inflammation, and cancer: how are they linked? Free Radical Biology & Medicine; 49(11): , 1603-1616, (2010).
27. Fraga CG, Galleano M, Verstraeten SV and Oteiza PI, Basic biochemical mechanisms behind the health benefits of polyphenols. Molecular Aspects of Medicine; 31(6): 435-445, (2010).
28. Perron NR and Brumaghim JL, A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochemistry and Biophysics; 53(2): 75-100, (2009).
29. Lake BG, Harris RA, Philip JC, Gangolli SD, Studies on the effect of L-ascorbic acid on Acetaminophen-induced hepatotoxicity-1 inhibition of the covalent binding of acetaminophen metabolite to hepatic microsomes in vitro. Toxicol Appl Pharmacol; 60:229-40, (1981).