

Evidences of hepatoprotective and antioxidant effect of *Citrullus colocynthis* fruits in paracetamol induced hepatotoxicity

Syed Wakiloddin^{1*}, Neeraj Fuloria², Shivkanya Fuloria², Sokkalingam Arumugam Dhanaraj², Kaveti Balaji² and Sundram Karupiah²

¹Anuradha College of Pharmacy (University of Amravati), Anuradha Nagar, Chikhli, Dist. Buldana (M.S.), India

²Faculty of Pharmacy, AIMST University, Semeling Campus, Jalan Bedong-Semeling, Bedong, Kedah Darul Aman, Malaysia

Abstract: The objective of present study was to explore the hepatoprotective and antioxidant profile of *Citrullus colocynthis* fruits. Hepatoprotective profile of methanolic extract of *Citrullus colocynthis* fruits (MECCF) was investigated on rats, which were made hepatotoxic using paracetamol. The antioxidant profile of MECCF was evaluated by conducting Catalase, Super oxide Dismutase, Lipid Peroxidation and Diphenyl Picryl Hydrazyl tests. During hepatoprotective investigation, the Paracetamol treated group II showed significant increase in total bilirubin (TB), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) level. The results so obtained showed that pretreatment of rats with MECCF 300mg/kg p.o. decreases the elevated TB, SGOT, SGPT and ALP serum levels. Also, MECCF inhibitory profile was found comparable with toxicant group (Paracetamol 2g/kg, p.o.). The present study concludes that MECCF fruit possess significant hepatoprotective and antioxidant activity.

Keywords: Hepatoprotective, antioxidant, MECCF, Paracetamol.

INTRODUCTION

In human body liver is an imperative organ (Ahmad and Sharafatullah, 2008). The liver is considered imperative because the nutritional level of a person does not depends only on what eatables consumed, but also on what liver processed. Regrettably it is not possible to diagnose early symptoms of imbalance in liver metabolism. A person could be a chronic patient of some liver disorder without known to it. Apart from treatment of liver disorders, daily care of liver can be a keystone for good health of a person (Kiran *et al.*, 2011).

Liver is associated with vital functions such as it maintains and regulates the homeostasis in body. It plays an astonishing role in human body as it mediates various biochemical pathways like pathways for body defense against diseases, for body growth, for energy production and for supply of nutrition. Liver acts as a centre for metabolism of proteins, lipids, carbohydrates, and it also removes unwanted metabolites. Liver secretes a biochemical called bile, which plays an important role in digestion (Naskar *et al.*, 2011).

Universal health problem is nothing but the liver disease. It is fact that for treatment for liver disorders the conventional or synthetic drugs are inadequate and are generally associated with various side effects. Although, plant sources provides many folk remedies with potential antioxidant and hepatoprotective activities. In India there exist three different systems for traditional medicines

namely Ayurveda, Siddha and Unani that makes use of plant materials. The three features of herbal drugs such as safety, cost effectiveness and efficacy, made herbal drugs to gain high popularity and importance (Das *et al.*, 2012).

From a long time in India herbal-based therapeutics for liver disorders has been in use and has been famous world over by leading pharmaceuticals. As per literature, numerous plants and formulations are reported to possess hepatoprotective profile. It is an estimate that from more than 100 plants about 160 phytoconstituents are known to have hepatoprotective action. Moreover, in India, about 87 plants are reported for their use in different commercial herbal multi component preparations (Saleem *et al.*, 2010).

Citrullus colocynthis (L.) Schrad belonging to the melon family of cucurbitaceae (Ramanathan *et al.*, 2011). Plant of *Citrullus colocynthis* is most commonly distributed in East Asian continent. Bitter apple or bitter cucumber is another name of *Citrullus colocynthis*. Generally, the fruit of *Citrullus colocynthis* plant is traditionally used for treatment of urinary disorders, jaundice and diabetes as well. The pulp of *Citrullus colocynthis* fruit contains a strong laxative phytoconstituent called colocynth (Shasthree *et al.*, 2010).

MATERIALS AND METHODS

Plant material

The *Citrullus colocynthis* fruit used for the present studies was collected from the province of Akola district of Maharashtra, India. Plant was further authenticated by

*Corresponding author: e-mail: syedvakil@gmail.com

Taxonomist Dr. S. P. Rothe, Shri Shivaji college of Arts, Commerce and Science, Akola, University of Amravati.

Preparation of the extract

The powdered drug was dried and packed well in Soxhlet apparatus and then was successively extracted with benzene, chloroform and methanol (Agarwal *et al.*, 2012).

Qualitative phytochemical screening

The three obtained herbal extracts (benzene, chloroform and methanolic) of *Citrullus colocynthis* were further subjected to different standardized tests to detect presence of different phytoconstituents such as carbohydrates, alkaloids, triterpenoids, glycosides, phenolics, tannins, saponins, flavones, flavonoids and phytosterols (Agarwal *et al.*, 2012, Ramachandra *et al.*, 2011).

Animals

In present study Male albino Wistar rats (weight between 150 to 200g) and albino mice (weight between 20 to 25g) of either sex were used. The animals were procured from Anuradha College of pharmacy, Chikhli, Dist-Buldhana (MS) India and were kept in animal house at a room temperature of $25\pm1^{\circ}\text{C}$. The animals were exposed to 12 h light and 12 h dark cycle; and were fed with standard pellets and tap water. The relative humidity of animal house was maintained up to 60%. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) after careful evaluation of research project during 2013 meeting (Proposal No. 751/03/abc/CPCSEA and Ref. No. IAEC/ 2012-2013).

Acute oral toxicity studies

The acute oral toxicity studies were performed on mice, using guidelines of OECD-423. The delivery dose was chosen from one of the four levels such as 5, 50, 500 and 2000 mg/kg. To determine mortality and behavioral response, the mice were kept under strict observation for 48 h, and thereafter once daily up to fourteen days.

Experimental design

A total of 24 rats were procured and equally divided into 4 groups. Each group contained six rats. The Normal control administered group was named as Group I, and was a recipient of daily oral dose of 2 ml/kg of saline up to seven days. Toxicant control administered group was named as Group II, and was a recipient of daily oral dose of 2 ml/kg of saline up to seven days. The methanolic extract of *Citrullus colocynthis* fruit (MECCF) administered group was named as Group III, and was a recipient of daily oral dose of 300 mg/kg up to seven days. The reference control (Standard) administered group was named as Group IV and was a recipient of daily oral dose of 1ml/kg of marketed preparation of Liv 52 up to seven days.

Moreover, on day seven, the rats of group II, III, and IV were orally administered with 2 g/kg of Paracetamol, that too after 30 min of the last dose (Rajesh *et al.*, 2009). Next, after 36h of oral administration of 2g/kg of Paracetamol all groups of rats were anesthetized using ether and blood sample from respective groups of rats were withdrawn and collected in sterile tube for clotting. Later, the sterile tubes were subjected to centrifugation for 15 min at 2500 rpm to separate the serum. The serum's so obtained were subjected to several biochemical assays. On the other hand, after last blood sample withdrawn all groups of rats were sacrificed and liver were isolated for histopathological studies from all groups of rats respectively.

Physical parameters (Qureshi *et al.*, 2010)

Determination of wet liver weight

The respective livers of sacrificed animal group were isolated and were subjected to saline washing. Next, the weights of livers were determined using an electronic balance. The liver weights were expressed with respect to its body weight i.e. g/100g.

Determination of wet liver volume

After recording the weight, all the livers were dropped individually in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

Biochemical parameters

The various groups of animals were assayed (according to standard methods) for different bio components such as total bilirubin (TB) (Ghosh *et al.*, 2007), serum glutamate oxaloacetate transaminase (SGOT) (Khan *et al.*, 2008), serum glutamate pyruvate transaminase (SGPT) (Balaraman and Bafna, 2013), and alkaline phosphatase (ALP) (Rathi *et al.*, 2010).

Antioxidant parameters

All the four groups were estimated for In-vivo antioxidant parameters Catalase (CAT), Super oxide Dismutase (SOD) and Lipid Peroxidation (LPO) (Pietrzycka *et al.*, 2006, Naik and Panda, 2008, Ruana *et al.*, 2010). In-vitro diphenyl picryl hydrazyl (DPPH) radical scavenging activity (Qureshi *et al.*, 2010, Padmanabhan and Jangle, 2012, Patil *et al.*, 2011).

Histopathological studies

Animals from all groups after treatment were sacrificed and small portions of each liver were fixed for 48 h in 10% formalin solution. Further, processing of livers was done by using paraffin embedding method. Next, the liver sectioning (cutting up to 5µm thickness) was performed. Later, the liver sections were subjected to staining with eosin and hematoxylin. Lastly, the stained liver sections were kept under microscope for histopathological studies.

STATISTICAL ANALYSIS

After following the experimental methodology the resultant data so obtained were subjected to statistical investigation. Resultant values were expressed in Mean $\pm$ SEM. The statistical significance of resultant data was assessed by ANOVA test. The resultant values with $P < 0.05$ were considered significant.

RESULTS

Phytochemical constituents present in Citrullus colocynthis fruits

After extraction of *Citrullus colocynthis* fruits, the extracts were subjected to different standard qualitative tests for phytochemical identification. The data so obtained is represented in table 1.

According to table 1, the identified phytochemical constituents in *Citrullus colocynthis* fruits in three different extracts were found to be carbohydrates, alkaloids, glycosides, triterpenoids, phenolics and tannins, saponins, flavones & flavonoids, and phytosterols

In, preliminary phytochemical studies of extracts of *Citrullus colocynthis* confirmed the strong presence of desired phytochemicals in methanolic extracts when compared to benzene and chloroform extracts. Hence, for the further investigational studies, the MECCF was selected.

Physical parameters

Wet liver weight and volume

Treatment of rats with Paracetamol caused liver enlargement which was evident by increased in the wet liver weight and volume. The groups treated with Paracetamol displayed a significant increase in the wet liver weight and wet liver volume, in comparison to group I. Administration of MECCF (300mg/kg) and Liv 52 syrup (1ml /kg) in rats caused significant reduction in the wet liver weight and wet liver volume in comparison to group II. The results and data on Wet liver weight and Wet liver volume are shown in table 2.

Biochemical parameters

Effect of MECCF on biochemical parameters in Paracetamol induced hepatotoxic rats.

Total bilirubin level

Elevation of total bilirubin levels after administration of Paracetamol indicates its hepatotoxicity. Pretreatment with Liv 52, MECCF significantly reduced level of total bilirubin when compared to toxic control group, which indicates Hepatoprotective effect of MECCF. Resultant data of bilirubin levels obtained during this study are shown table 3.

SGOT, SGPT and ALP level

The group II, Paracetamol treated rats suffered from significant hepatotoxicity. This was confirmed by observing high serum levels of several bio chemicals such as SGOT, SGPT and ALP in comparison to normal control. Pretreatment with Liv 52, MECCF showed significant reduction in SGOT, SGPT and ALP serum level against paracetamol induced toxicity in liver. Resultant data of SGOT, SGPT and ALP serum level are shown in table 4.

In- vivo antioxidant parameters

From the results it was found that rats treated with Paracetamol showed a marked decrease in activities of catalase and super oxide dismutase in comparison to group I. The rats pretreated with syrup of Liv 52 and MECCF caused a marked increase in activities of catalase and super oxide dismutase when compared to toxicant group. During *In vivo* lipid peroxidation study Paracetamol treated group displayed a marked elavation in Malondialdehyde (MDA) level in comparison to group I. From present study it was revealed that MECCF and Liv 52 were able to significantly prevent the rise in MDA level, which is evident from table 5.

In-vitro antioxidant activity of MECCF

DPPH radical scavenging activity

After commencement of DPPH radical scavenging experiment, it was found that scavenging of free radicals by MECCF was concentration dependent. The MECCF extract at concentration of 800 μ g/mL showed 76% inhibition of DPPH radicals (table 6). Whereas standard ascorbic acid drug at a concentration of 200 μ g/mL displayed 90% inhibition of the DPPH radicals.

Histopathological studies of liver in paracetamol induced hepatotoxicity

The histopathological evaluation of Paracetamol toxicity in all the groups was examined and showed in fig. 1. According to fig. 1, Section 1 of rat liver treated with vehicle control group showed liver parenchyma with intact architecture, which is the normal appearance. Section 2 of liver in toxicant control group showed partially effaced architecture. Where Section 2 showed extensive vacuolar changes with congestion indicating severe degree of hepatotoxicity. It also shows round cell infiltration. Center lobular vein also shows hyperplasia of bile duct. Section 3 of rat liver in test drug treated groups (300mg/kg) showed intact architecture, at few places granular changes. Hemorrhages at few places seen. Whereas Section 4 of liver treated with standard (Liv 52 syrup) group showed mild vacuolar tubules in focal areas indicating incomplete restoration of parenchyma with intact architecture. No hyper plastic changes of the periphery of central tubular vein suggesting changes of restoration as compare to MECCF.

Table 1: List of tests for determination of various phyto constituents in *Citrullus colocynthis* fruits extracts

S. No	Phytochemical constituents	Test	Benzene Extract	Chloroform Extract	Methanolic Extract
1	Carbohydrates	Molish's test	+	+	+
2	Alkaloids	Dragandroff 's test	-	-	+
3	Glycosides	Borntrager's test	-	-	-
4	Triterpenoids	Tin + thionyl chloride	-	-	+
5	Phenolics and tannins	Ferric chloride test	-	-	+
6	Saponins	Foam test	+	+	+
7	Flavones and Flavonoids	Shinoda test	+	+	+
8	Phytosterols	Libermann-Burchards test	-	-	-

+, Presence of component -, Absence of component

Table 2: Effect of MECCF on Wet liver weight and Wet liver volume in Paracetamol induced hepatotoxic rats

Group	Treatment	Dose	Wet Liver weight (gm/100gm)	Liver volumes (ml/100gm)
I	Normal Control (saline solution)	2 ml/kg p. o.	2.50±0.03	2.57±0.01
II	Toxicant Control (Paracetamol)	2 g/kg, p. o.	4.81±0.09	4.55±0.024
III	MECCF + Paracetamol	300 mg/Kg p.o.+ 2 g/kg, p. o.	2.75±0.02**	2.798±0.01**
IV	Standard (Liv 52) + Paracetamol	1ml /kg p.o. + 2 g/kg, p. o.	2.64±0.02***	2.62±0.02***

Table 3: Effect of MECCF on bilirubin levels in Paracetamol induced hepatotoxic rats

Group	Treatment	Dose	Total bilirubin levels (mg/dl)
I	Normal Control (saline solution)	2 ml/kg, p. o.	1.158±0.02
II	Toxicant Control (Paracetamol)	2 g/kg, p. o.	2.96±0.02
III	MECCF + Paracetamol	300 mg/Kg p. o.+ 2 g/kg, p. o.	1.343±0.02*
IV	Standard (Liv 52) + Paracetamol	1ml /kg p. o. + 2 g/kg, p. o.	1.475±0.02***

Table 4: Effect of MECCF on SGOT, SGPT & ALP levels in Paracetamol induced hepatotoxic rats

Group	Treatment	Dose	SGOT levels (U/L)	SGPT levels (U/L)	ALP levels (U/L)
I	Normal Control (saline solution)	2 ml/kg p. o.	97.07±1.31	114.6±1.62	163.8±1.99
II	Toxicant Control (Paracetamol)	2 g/kg, p. o.	275.8±2.04	294.9±4.38	322.4±1.78
III	MECCF + Paracetamol	300 mg/Kg p. o.+ 2 g/kg, p. o.	209.8±3.47*	219.3±6.46**	251.3±1.09*
IV	Standard (Liv 52) + Paracetamol	1ml /kg p. o.+ 2 g/kg, p. o.	171.6±2.09***	156.4±4.15**	201.0±5.23**

Values are mean ± SEM for six rats each. Values are statically significant *at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 Toxicant rats were compared with normal control rats. MECCF treated rats were compared with Toxicant rats, Liv 52 treated rats were compared with Toxicant.

DISCUSSION

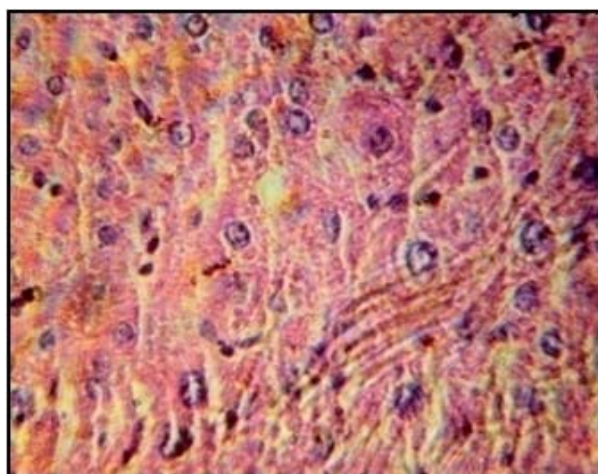
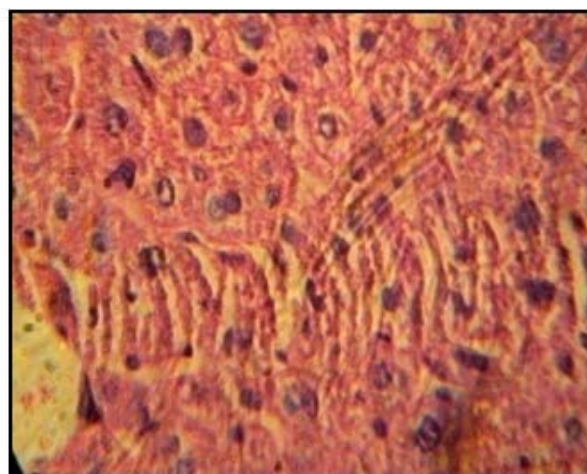
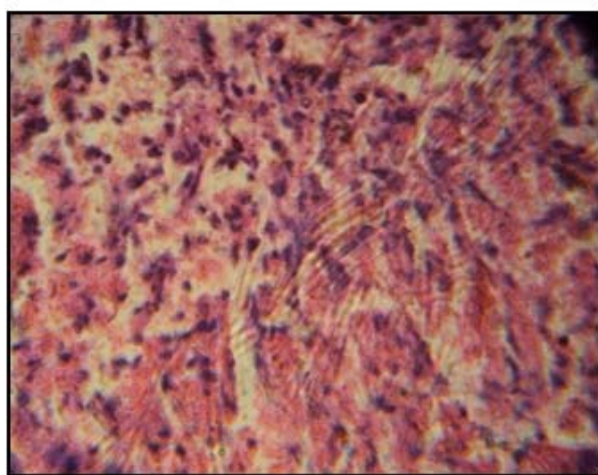
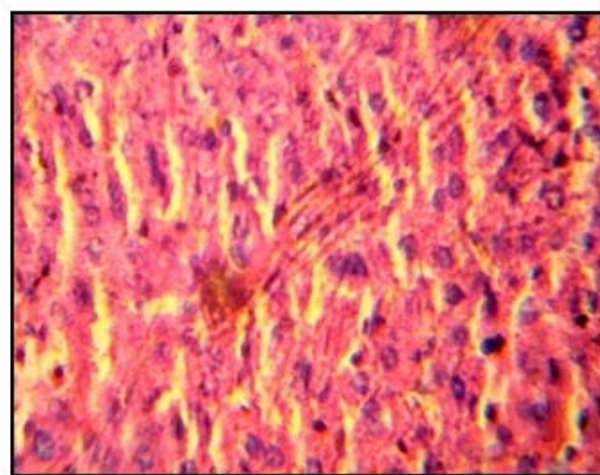
The preliminary phytochemical investigations showed presence of various secondary metabolites. The qualitative phytochemical investigation on different extracts of *Citrullus colocynthis* showed positive test for carbohydrate, triterpenes, steroids, tannins and flavonoids. The methanolic extract showed significant hepatoprotective activity. This might be due to higher content of the triterpenes, tannins and flavonoids.

In toxic liver both wet liver weight and volumes were increased. In this case water was trapped in the cytoplasm of hepatic cells. Which caused enlargement of hepatic cells, thereby caused enhancement of total liver volume and mass. The treatment of rats with MECCF showed a remarkable reduction in wet liver weight and volumes of rats. Application of statistical tests over the obtained results again declared that MECCF possesses statistically significant hepatoprotective activity.

Table 5: Effect of MECCF on Catalase, SOD and Lipid peroxidation in Paracetamol induced hepatotoxic rats

Group	Treatment	Dose	SOD (units/mg protein)	CAT (units/mg protein)	LPO (nM of MDA/mg of protein)
I	Normal Control (saline solution)	2 ml/kg, p. o.	12.48±0.20	95.22±1.59	4.360± 0.16
II	Toxicant Control (Paracetamol)	2 g/kg, p. o.	3.95±0.32	25.22±0.99	10.04± 0.89
III	MECCF + Paracetamol	300 mg/Kg p. o.+ 2 g/kg, p. o.	5.588±0.16*	35.54±1.46*	7.28± 0.48*
IV	Standard (Liv 52) + Paracetamol	1ml /kg p. o. + 2 g/kg, p. o.	8.53±0.14***	82.76±1.01***	6.65±0.28***

Values are mean ± SEM for six rats each. Values are statically significant *at $p < 0.05$, ** represents highly significant at $p < 0.01$, and *** represents very significant at $p < 0.001$ Toxicant rats were compared with normal control rats. MECCF treated rats were compared with Toxicant rats, Liv 52 treated rats were compared with Toxicant.

**Section 1. Normal Control****Section 2. Toxicant (Paracetamol)****Section 3. MECCF + Paracetamol****Section 4. Standard + Paracetamol****Fig. 1:** Histopathology of the liver in Paracetamol induced hepatotoxicity studies

In toxic liver, level of bilirubin gets elevated to cause hyperbilirubinemia, which can be attributed to impaired hepatic uptake of unconjugated bilirubin. This condition commonly occurs in general injury of hepatic cell. In present investigation MECCF treatment to rats significantly decreased bilirubin serum level. Such observation further supports to hepatoprotective profile of MECCF.

Table 6: DPPH scavenging activity of MECCF

MECCF (µg/ml)	% Inhibition
25	8.52±0.09
50	39.44±0.05
100	48.92±0.04
200	56.80±0.03
400	63.37±0.04
800	76.35±0.23
Ascorbic acid (200)	90.32±0.24

Values represents number of readings in each group = 3mean ± SEM

Liver toxicity elevates the SGOT and SGPT levels. Alcoholic liver damage and cirrhosis can also be associated with mild to moderate elevation of transaminases. In the present investigation the MECCF treatment to rats caused a remarkable reduction in SGOT and SGPT serum levels. This indicates and confirms the hepatoprotective profile of MECCF.

Furthermore, in hepatotoxicity, the level of alkaline phosphatase was very high. This must be due to defect in hepatic excretion or must be due to higher production of ALP by duct cells or hepatic parenchymal cells. The present study revealed that MECCF treatment to rats reduced ALP serum level, which supports to hepatoprotective profile of MECCF.

The present investigation further supports that chronic exposure of paracetamol reduced the reactive oxygen species (ROS) scavenging activities of CAT and SOD. The present study reveals that MECCF have the ability to restore the activity of both CAT and SOD. So, MECCF may reduce hepato cellular damage and free radicals generation.

Paracetamol induced hepatotoxicity is also associated with high lipid peroxidation. The present study supports that MECCF administration reduced lipid peroxidation. This indicates that MECCF possesses antioxidant activity and reduces paracetamol induced membrane lipid peroxidation.

The plant extract MECCF showed maximum percentage inhibition of DPPH radicals. Free radicals of DPPH are comparatively stable. These DPPH radicals when comes in contact with antioxidants undergoes quenching and

their absorbance is reduced. The result suggested that in comparison with ascorbic acid DPPH radicals showed scavenging activity of the MECCF.

Histopathological liver sections also revealed that liver's microscopic structure was disrupted in Paracetamol treated group. On the other hand the hepatic section of the rats treated with the MECCF and toxicants, retained normal cellular architecture as compared with the toxicant group. Hence study confirms the significant hepatoprotective effect of 300mg/kg of orally administered MECCF.

CONCLUSION

Based on the results of serum marker enzyme levels, physical, antioxidant, and histopathological studies the present study concludes that *Citrullus colocynthis* fruits possesses hepatoprotective activity and thus supports the traditional use of same for treatment of liver disorder.

REFERENCES

- Agarwal V, Sharma AK, Upadhyay A, Singh G and Gupta R (2012). Hypoglycemic effects of *Citrullus colocynthis* roots. *Acta Poloniae Pharmac. Drug Res.*, **69**: 75-79.
- Ahmad U and Sharafatullah T (2008). Hepato-protective natural compounds. *Pak. J. Pharmacol.*, **25**: 59-68.
- Balaraman R and Bafna PA (2013). Protective effect of DHC-1, a polyherbal formulation, against CCl₄ induced liver damage. *Hygeia. J. Drugs. Med.*, **5**: 10-18.
- Das C, Mishra A, Mishra MR, Pradhan DK, Velraj M and Sahoo D (2012). Hepatoprotective herbal medicine: A review. *Int. Res. J. Pharm. Sci.*, **3**: 1-12.
- Ghosh T, Kumar TM, Das M, Bose A and Das DK (2007). *In vitro* antioxidant and Hepatoprotective activity of ethanolic extract of *Bacopa monnieri* Linn. aerial parts. *Ira. J. Pharmacol. & Therapeutics*, **6**: 77-85.
- Khan S, Pradeep HA, Kumar ABS, Kumar AM, Manimaran S, Chandrasekhar R and Vijayan P (2008). Chemoprotective effect of *Anogeissus latifolia* bark extract on paracetamol-induced hepatotoxicity. *Pharmacology online*, **2**: 303-327.
- Kiran RY, Manjunath C, Kumar RM, Brahmaiah Y, Kumar AU and Tamizhmani T (2011). A review on traditional plants with hepatoprotective activity. *Pharmacology online*, **3**: 653-658.
- Naik SR and Panda VS (2008). Hepatoprotective effect of *Ginkgoselect Phytosome* in rifampicin induced liver injury in rats: Evidence of antioxidant activity. *Fitoterapia.*, **79**: 439-445.
- Naskar S, Mazumder UK, Halder PK and Amitava Ghosh (2011). Evaluation and review of hepatoprotective drugs from natural resources. *Pharmacology online*, **3**: 993-1006.

- Padmanabhan P and Jangle SN (2012). Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. *Int. J. Pharm. Sci. & Drug Res.*, **4**: 143-146.
- Patil R, Gadakh R, Gound H and Kasture S (2011). Anti-oxidant and anti-cholinergic activity of *Rubia Cordifolia*. *Pharmacology online*, **2**: 272-278.
- Pietrzycka A, Pniewski MST, Waszkielewicz AM, Marona H, Krzyzanowska A, Klosowska K and Solarz O (2006). Effect of vita glucan on some anti-oxidant parameters of the human blood. *In vitro* study. *Acta Poloniae. Pharmac. Drug Res.*, **63**: 547-551.
- Qureshi MN, Kuchekar BS, Logade NA and Haleem MA (2010). *In vitro* Antioxidant and *In vivo* Hepatoprotective activity of *Leucas ciliata* leaves. *Rec. Nat. Prod.*, **4**: 124-130.
- Rajesh SV, Rajkapoor B, Kumar RS and Raju K (2009). Effect of *Clausena dentata* (willd.) M. Roem. against paracetamol induced hepatotoxicity in rats. *Pak. J. Pharm. Sci.*, **22**: 90-93.
- Ramachandra YL, Kavitha BT, Shruthi SD and Rai SP (2011). Phytochemical analysis and hepatoprotective properties of *Tinospora cordifolia* against carbon tetrachloride-induced hepatic damage in rats. *J. Basic & Clin. Pharmacy*, **2**: 139-142.
- Ramanathan T, Gurudeeban S, Satyavani K and Dhinesh T (2011). Anti-microbial effect of coastal medicinal plant *Citrullus colocynthis* against pathogenic Microorganisms. *Afr. J. Pure. Applied. Chem.*, **5**: 119-122.
- Rathi MA, Rajalakshmy BM, Thirumoorthi L and Gopalakrishnan VK (2010). Potential effect of *Bacopa monnieri* on nitrobenzene induced liver damage in rats. *Ind. J. Clin. Biochem.*, **25**: 401-404.
- Ruana J, Zhoua D, Caia Y, Xionga Z, Fua W and Wei A (2010). Antioxidant and hepatoprotective activity of ethanol extract of *Arachniodes exilis* (Hance) Ching. *J. Ethnopharmacol.*, **129**: 232-237.
- Saleem MTS, Chetty CM, Ramkanth S, Rajan VST, Kumar KM and Gauthaman K (2010). Hepatoprotective herbs a review. *Int. J. Res. Pharm. Sci.*, **1**: 1-5.
- Shasthree T, Savitha R, Sudhakar and Mallaiah B (2010). High frequency of plantlet regeneration and multiple shoot induction from leaf and stem explant of *Citrullus colosynthis* (L.) Schrad, an essndangered medicinal cucurbit. *Int. J. Pharma. Bio. Sci.*, **1**: 1-8.