IJPRAS International Journal of Pharmaceutical Research & Allied Sciences

Research Article
Available online at
www.ijpras.com

Volume 4, Issue 4 (2015):33-38

ISSN 2277-3657

International Journal of Pharmaceutical Research & Allied Sciences

Hepatoprotective Agent Present In Pods of *Clitoria Ternatea* with Evidence of Histopathological Analysis

Bairam Balaji[1*], Kishan Bookya[1], G.Vijaya Bai[2] ,Teelavath Mangilal[3].

[1*]*Department of General Medicine,KMC(MGMH),Warangal,Telangana,India.*
[1]*Department of Pathology, ACSR Govt medical College, Nellore, Andhra Pradesh, India.*
2Department of Biochemistry, St Ann's Hospital,Warangal,Telangana,India.
[3]*Department of Pharmacy,UCT,Osmania University,Hyderabad,Telangana,India.*
Email:teelavath@gmail.com

Subject: Medical Sciences

Abstract

The aim of the present research work was to extract the potential biomolecules present in the pods of *Clitoria ternatea* and to evaluate the *in vitro* and *in vivo* Hepatoprotective activity. The in *vitro* Hepatoprotective activity was carried out by DPPH assay**.** The IC_{50} value (50% inhibition) of the EEFSCT was found to be 212.96 µg/ml. The *in-vivo* Hepatoprotective activity was carried out by using albino rats. The results displayed that the elevated levels of SGOT, SGPT, ALP and Serum bilirubin were mainly due to CCl_4 intoxication, reduced significantly in rats, after treatment with ethanolic extract of pods of *Clitoria ternatea* (EEFSCT). Treatment with EEFSCT at a dose of 250 mg/kg decreased the SGOT, SGPT, ALP, Serum Bilirubin levels by 6.23%,28.96,8.81and11.11% respectively, while a higher dose of 500 mg/kg was more effective, causing a reduction of 25.02, 47.65, 24.09, and 27.35%. Silymarin used as standard showed a reduction of 55.09, 68.98, 57.46 and 35.04% receiving CCl_4 alone and depending upon the experimental data it were confirmed that the biochemical parameters of the group treated with ethanolic extract was significantly lower than the CCl_4 treated group. Moreover the treatment with the extract significantly reduced the previously raised levels of AST, ALT, ALP and bilirubin in hepatotoxic rats. Histopathological investigation displayed that at both doses, the EEFSCT was possessed moderate to good hepatoprotective activity, but at 500 mg/kg showed excellent hepatoprotective activity against CCl_4 induced damaged hepatocytes.

Keywords: *Biomolecules, DPPH assay, Intoxication, Hepatocyte, SGOT, SGPT, SALP.*

Introduction

Clitoria ternatea, common names including butterfly pea, blue pea, Cordofan pea and Asian pigeonwings, is a plant species belonging to the Fabaceae family. The flowers of this vine have the shape of human female genitals, hence the Latin name of the genus *"Clitoria"*, from "clitoris".[1]

In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent.[2] In traditional Chinese medicine, owing to its similarity to the female reproductive organ, this plant has been ascribed properties affecting the same (a phenomenon also found in connection with the mandrake, among other plants). It was used traditionally in an attempt to treat sexual ailments, like infertility and gonorrhea, to control, menstrual discharge, and also as an aphrodisiac. This practice aligns with an ancient belief recorded in the Doctrine of Signatures.[3] In animal tests the methanolic extract of *Clitoria ternatea* roots demonstrated nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity.[4]

The active constituents include tannins, resins, starch,taraxerol, and taraxerone. Recently, several biologically active peptides called cliotides have been isolated from the heat-stable fraction of *Clitoria ternatea* extract. Cliotides belong to the cyclotides family [5] and activities studies show that cliotides display potent antimicrobial activity against *E. coli, K. pneumonia, P. aeruginosa* and cytotoxicity against Hela cells.

These peptides may have potential to be developed an antimicrobial and anti-cancer agents.[6] The enzyme responsible for the biosynthesis and the backbone cyclization of cliotides has recently been isolated. It was named butelase1 in accordance with its local name in Singapore. Butelase 1 is the fastest peptide ligase known capable of catalyzing peptide cyclization at an extraordinary efficiency.[7] The present study the *in-vivo* hepatoprotective activity of the extract, the potential biomolecules present in the pods of

Clitoria ternatea was evaluated by CCl_4 induced hepatotoxicity model in rats.

Materials and Methods

Materials: The all chemicals used for the extraction and phytochemical screening were of LR and AR grade. The standard drug Silymarin was purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from the Institutional Store of AR grade.

Methods

Experimental animals: White albino rats weighing about 200-250g were used. They were obtained from the animal house of National Institute Nutrition, Hyderabad. They were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection, had free access to normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle under hygienic conditions.

Method of extraction (Soxhlet Extraction): When an organic substance is to be reserved from a solid, it is extracted by means of an organic solvent in which impurities are insoluble. In actual practice the extraction of solids is often tedious and requires through contact and heating with the solvent. This is done in a special apparatus, the Soxhlet Extractor. It consists of a glass cylinder having a side tube and siphon. The cylinder carries a water condenser at the top and is fitted below into the neck of a boiling round bottom flask.[4]

Methodology: First the dried fruits and seeds are triturate to make a fine powder and the powdered material is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol is heated on a water bath or on a heating mantle. As the solvent boil, its vapors rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here arises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind.[4] Afterwards the ethanolic extract, transfer in a clean and dried beaker and is concentrated by placing on a water bath and then cool, keep it in a freeze. From this concentrated extract that is Ethanolic Extract of pods of *Clitoria ternatea* (EEFSCT) the preliminary phytochemical screening has to be carried out.

Preliminary Phytochemical screening: Preliminary Phytochemical screening of EEFSCT had shown the presence of various biologically active molecules such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, alkaloidsds (higher concentration), terpenoids especially diterpenoids, Tri and tetra terpinoids die etc.. [5-8]

Evaluation of acute toxicity: In the present study the acute oral toxicity of the EEFSCT was performed by the acute toxic class method. In this method the toxicity of the extract was planned to test using stepwise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food, but not water should be withheld) for three to four hours. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg. Animals were observed individually after dosing at least once during the first 30 min; period the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. [9]

Evaluation of In vitro Antioxidant activity by DPPH assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay evaluates free radical scavenging activity by measuring the color change that occurs when a DPPH radical is quenched by a free radical scavenger that donates a hydrogen atom. [10]

Method: The ethanolic plant extract was tested for the DPPH free radical scavenging activity, according to the method of Pan et al. with minor modification. 0.2 ml of the extract solution in ethanol (95 %) at different concentrations was added to 8 ml of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–Visible spectrophotometer. As a positive control, synthetic antioxidant gallic acid was used. All determinations were performed in triplicate. The DPPH radical scavenging activity (S%) was calculated using the following equation:

S% = [(A control – A sample) /A control] ×100

Where A control = absorbance of the blank control (containing all reagents except the extract solution) and A sample = absorbance of the test sample.[11]

Experimental protocol for the Evaluation of *In vivo* Hepatoprotetive activity

A total of 30 rats was taken and divided into 5 groups of 6 rats each

Group I: Normal Control Group (only the vehicle (1 ml/kg/day of 1% CMC;)

Group II: Negative Control CCl_4 1 ml/kg (1:1 of CCl_4 in olive oil)

Group III: Positive Control/Standard Group [CCl_4 1 ml/kg (1:1 of CCl_4 in olive oil) + Standard Silymarin 100 mg/kg orally for 7 days]

Treatment Groups

Group IV: High Dose Group [CCl_4 1 ml/kg (1:1 of CCl4 in olive oil) + EEFSCT (500 mg/ kg)]

Group V: Low Dose Group [CCl_4 1 ml/kg (1:1 of CCl4 in olive oil) + EEFSCT (250 mg/ kg)]

Treatment was given daily for seven days orally.

Collection of blood: On the 8th day, blood was collected by retro orbital puncture, under mild ether anesthesia after 8 hours fasting. Blood samples were centrifuged at 3000 rpm for 20 mins. Serum was separated and stored at – 200 C until biochemical estimations were carried out.

Biochemical Analysis: The Serum samples were analyzed for Alanine Aminotransferase (ALT) (SGPT), Aspartate Aminotransferase (AST) (SGOT), Alkaline Phosphatase (ALP), Serum Bilirubin.

Histopathological Analysis: The liver tissue was dissected out and fixed in 10% formalin solution. It was then dehydrated in ethanol (50%-100%), cleared in Xylene and embedded in paraffin wax. Afterwards thick sections (5-6 mm) were made and then stained with hematoxylin and eosin dye for photo microscopic observation. The whole biochemical and histopathological analysis was carried out at Achala Subbareddy Government Hospital and Medical College, Nellore, Andhrapradesh, India. [12]

Result and Discussion

Table 1: Results of DPPH Scavenging Activity

S.NO	Concentration (ng/ml)	Absorbance (A)	S% = $[(A_0\text{-}A) \div A_0]$ X100	IC_{50}(µg/ml)
CONTROL (DPPH Solution)				
1	0.1mm in ethanol	1.174 (A_0)	-	-
STANDARD (ASCORBIC ACID)				
1	4	0.984	16.25	-
2	6	0.953	18.74	-
3	8	0.916	21.89	-
4	10	0.870	25.88	39.88
5	25	0.564	51.86	-
6	50	0.038	96.84	-
ETHANOLIC EXTRACT OF FRUIT-SEEDS OF *CLITORIA TERNATEA* (EEFSCT)				
1	10	1.043	11.08	-
2	25	0.935	20.10	-
3	50	0.850	27.58	212.95
4	75	0.785	33.02	-
5	100	0.701	40.20	-
6	250	0.567	51.60	-

Table 2: Results of Hepatoprotective Activity

GROUP	TREATMENT	AST(SGOT) IU/L	ALT(SGPT) IU/L	ALP(SALP) IU/L	SERUM BILIRUBIN mg/dL
1	Normal Control Group (only the vehicle, 1% CMC;)	$52.00 \pm 8.672^{***}$	$46.70 \pm 11.95^{***}$	$138.2 \pm 6.914^{***}$	$0.59 \pm 0.08^{***}$
2	Negative Control (1:1 of CCl_4 in olive oil.)	201.2±30.45	203.4±47.74	398.2±16.18	1.18±0.16
3	Low dose [(1:1 of CCl_4 in olive oil)+ EEFSCT (250 mg/ kg.)]	188.6 ± 14.48^{ns}	$144.2 \pm 39.75^{*}$	$363.0 \pm 16.52^{*}$	1.03± 0.15
4	High dose [(1:1 of CCl_4 in olive oil) + EEFSCT (500 mg/ kg)]	$152.6 \pm 13.52^{***}$	$108.0 \pm 19.47^{***}$	$302.0 \pm 38.78^{***}$	$0.84 \pm 0.20^{**}$
5	Positive Control/Standard Group[(1:1 of CCl_4 in olive oil)+ Silymarin 100 mg/kg orally]	$90.70 \pm 17.61^{***}$	$63.41 \pm 15.73^{***}$	$168.8 \pm 8.55^{***}$	$0.75 \pm 0.14^{***}$

Data are expressed as mean±SD (n = 6). One-way ANOVA followed by Dunnett's Multiple Comparison Test (* P< 0.05) compared with group 2 ;(ns=non significant).

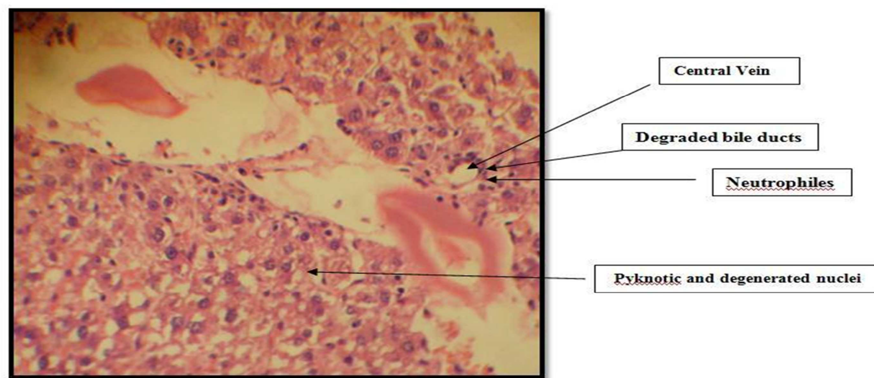

Fig.1: LIVER SECTION OF CCl_4 TREATED RATS.

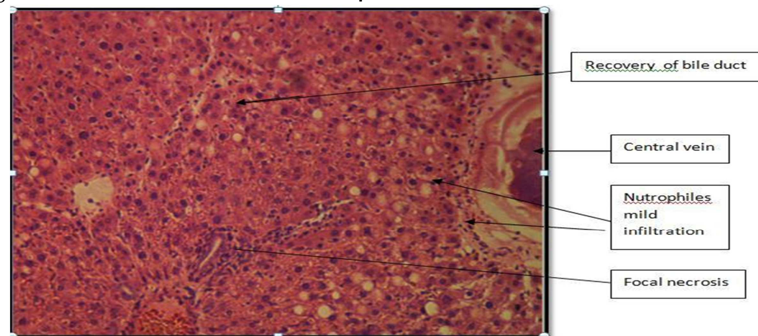

Fig.2: LIVER SECTION OF RATS TREATED CCl_4 AND 100 mg/kg OF SILYMARIN.

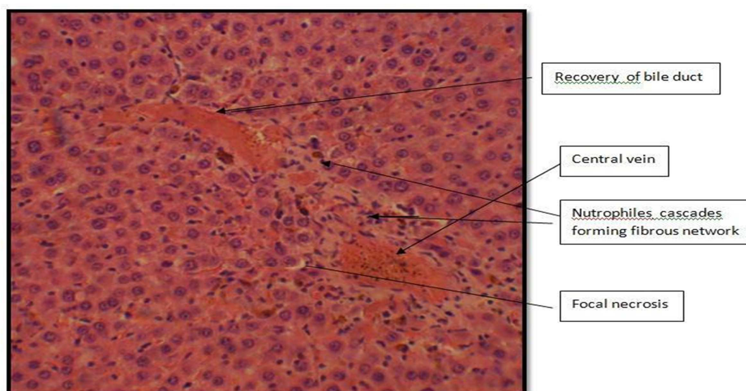

Fig.3: LIVER SECTION OF RATS TREATED CCl_4 AND 500 mg/kg OF EEFSCT.

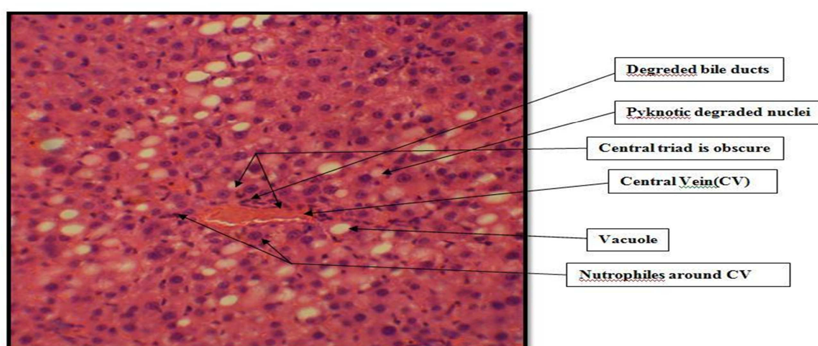

Fig.4: LIVER SECTION OF RATS TREATED CCl_4 and 250 mg/kg of EEFSCT.

1. Phytochemical screening: Preliminary Phytochemical screening of EEFSCT had shown the presence of various biologically active molecules such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, alkaloidsds (higher concentration), terpenoids especially diterpenoids, Tri and tetra terpinoids dye and aromatic acids and alcohols etc.

2. Acute Oral Toxicity Studies: In this study the acute oral toxicity was evaluated by "Acute toxic class methods (OECD guideline-423)". The extract was administered orally at a dose of 2000 mg/Kg During the surveillance period, no significant toxicity occurred along with minute non-considerable behavioral changes. After a statistical analysis of trial and error, the significant doses were chosen at 250 mg/kg (LD) and 500 mg/kg. (HD) considerably.

3. Hepatoprotective activity

(i) Statistical analysis: The data were expressed as mean ± SD. Statistical differences at $^{*}P < 0.05$ between the groups were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison Test using the Graph Pad Prism 5.04 Instate software package. The data's were compared with group 2 i.e. Negative Control group.

(ii) Analysis of DPPH free radical scavenging activity: The extract was planned to be evaluated for *in-vitro* antioxidant activity by DPPH free radical scavenging activity. The study was carried out taking ascorbic acid as the standard antioxidant, which is also a natural antioxidant. The results of antioxidant activity by DPPH free radical scavenging activity were expressed in terms of % inhibition of generating free radicals respectively with respect to various concentrations. Concentration dependent effects were observed, i.e.; higher concentrations were found to exhibit higher % inhibition. The IC_{50} value (50% inhibition) of the EEFSCT and the standard ascorbic acid were determined in all the studies.

The DPPH radical scavenging activity ethanolic extract of fruit-seeds of *Clitoria ternatea* (EEFSCT) was evaluated and compared with Ascorbic acid and the results are given in Table 1. The % inhibition at various concentrations (4-50 µg/ml) of an ethanolic extract of fruit-seeds of *Clitoria turn-out* as well as standard Ascorbic acid (10 -250 µg/ml) were calculated and plotted in Figure 6.3 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found to be 39.87µg/ml (Ascorbic acid) and 212.96 ((EEFSCT).

(iii) Biochemical analysis: The effects of EEFSCT on liver marker enzymes and serum bilirubin content are displayed in Table 2. The data exhibited that Normal Control Group demonstrated a normal range of AST, ALT, and bilirubin levels while the CCl_4-treated group showed elevated levels of AST, ALT, and bilirubin, thus confirming that CCl_4 causes hepatocellular degeneration at higher doses. The elevation of cytoplasmic AST and ALT is considered an indicator for the release of enzymes from disrupted liver cells. Bilirubin concentration has been used to evaluate chemically induced hepatic injury.

The results displayed in Table 2 indicate that the elevated levels of SGOT, SGPT, ALP and Serum bilirubin due to CCl_4 intoxication were reduced significantly ($^{*}P<0.05$) in rats, after treatment with EEFSCT. Treatment with ethanolic extract at a dose of 250 mg/kg decreased the SGOT, SGPT, ALP, Serum Bilirubin levels by 6.23%, 28.96, 8.81, and 11.11% respectively, while a higher dose of 500mg/kg was more effective, causing a reduction of 25.02, 47.65, 24.09, and 27.35%. Silymarin used as standard showed a reduction of 55.09, 68.98, 57.46 and 35.04% receiving CCl_4 alone. So depending upon the data of Table 2 it was confirmed that the biochemical parameters of the group treated with EEFSCT was significantly lower than the CCl_4-treated group. Moreover the treatment with the EEFSCT significantly reduced the previously raised levels of AST, ALT, ALP and bilirubin in hepatotoxic rats.

(iv) Histopathological Analysis:

1. Figure 1 Liver section of CCl_4 treated Rats showed massive fatty changes, necrosis, ballooning degeneration, and severe infiltration of the lymphocytes around central vein and the loss of cellular boundaries, pyknotic and degenerated nuclei.
2. Figure 2 Liver section of rats treated CCl_4 and 100 mg/kg of Silymarin displayed signs of the inflammatory cascade around the central vein indicating a mild degree of fatty change, and necrosis and focal necrosis (dilatation).
3. Figure 3 Liver section of rats treated CCl_4 and 500 mg/kg of EEFSCT demonstrated minimal inflammatory cellular infiltration around central vein, absence of necrosis, neutrophil cascades forming fibrous network, considerable protection and large septa of connective tissue flowing together and penetrating into the parenchyma, normal liver architecture.
4. Figure 4 Liver section of rats treated CCl_4 and 250 mg/kg of EEFSCT had shown the very less recovery, with the obscure central tread and infiltration of neutrophils around central vein, degraded fatty change, and necrosis and focal necrosis (dilatation), loss of cellular boundaries.

Conclusion

In conclusion, we report here that the IC_{50} value (50% inhibition) of the Ethanolic Extract of Fruit-

seeds of *Clitoria ternatea* (EEFSCT) were found to be 212.96 μg/ml and EEFSCT had ability to regenerate the hepatocytes *in vivo* and also possessed potential anti-inflammatory activity which was confirmed by liver biopsy. The hepatoprotective activity of the EEFSCT could be considered as excellent with regards to the standard drug silymarin.

“Cite this Article”

B Balaji, K Bookya, G. VijayaBai, T Mangilal “Hepatoprotective Agent Present In Pods of Clitoria Ternatea with Evidence of Histopathological Analysis” Int. J. of Pharm. Res. & All. Sci. 2015;4(4):32-38

References

1. Pharmacopia Indica Awl
2. Mukherjee PK, Kumar V, Kumar NS, Heinrich M"The Ayurvedic medicine Clitoria ternatea-From the traditional use to scientific assessment." *J Ethnopharmacol*. 2008 Sep 20;
3. Fantz, Paul R. (1991). "Ethnobotany of *Clitoria* (Leguminosae)". *Economic Botany* (New York Botanical Garden Press) 45 (4): 511–20. doi:10.1007/BF02930715. JSTOR 4255394.
4. Jain, N; Ohal, C.C; Shroff, S.K; Bhutada, R.H; Somani, R.S; Kasture, V.S; Kasture, S.B (2003). "Clitoria ternatea and the CNS". *Pharmacology Biochemistry and Behavior* **75** (3): 529. doi:10.1016/S0091-3057(03)00130-8.
5. Nguyen, GK; Zhang, S; Nguyen, NT; Nguyen, PQ; Chiu, MS; Hardjojo, A; Tam, JP. (Jul 2011). "Discovery and characterization of novel cyclotides originated from chimeric precursors consisting of albumin-1 chain a and cyclotide domains in the Fabaceae family". *J Biol Chem.* 286 (27): 24275–87. doi:10.1074/jbc.m111.229922.
6. Nguyen, Kien Truc Giang; Zhang, S; Nguyen, N. T.; Nguyen, P. Q.; Chiu, M. S.; Hardjojo, A.; Tam, J. P. (8 July 2011). "Discovery and Characterization of Novel Cyclotides Originated from Chimeric Precursors Consisting of Albumin-1 Chain a and Cyclotide Domains in the Fabaceae Family". *Journal of Biological Chemistry* 286 (27): 24275–24287. doi:10.1074/jbc.M111.229922. PMC 3129208. PMID 21596752. Retrieved July 8, 2011.
7.Nguyen, Giang. "Butelase 1 is an Asx-specific ligase enabling peptide macrocyclization and synthesis". *Butelase 1 is an Asx-specific ligase enabling peptide macrocyclization and synthesis*.
8. Arun bhal, B.S Bhal. Advanced Organic Chemistry, First Edition 2010, pp 13.
9. P.C Dandiya, P.K.Sharma , Bio-chemistry and clinical pathology, second edition, PP- 17-18, 24, 47-48.
10. Dr. G. Devala Rao, A Manual of Practical Biochemistry,pp 17.
11. Jaswant Kaur, PV Chemistry of Natural Products, 2010 edition, PP-113-114, 116, 344-346, 381.
12. C.K. Kokate, A.P. Purohit, S.B.Gokhale, Nov 29, 2012. Pharmacognosy, 42 Editions, pp- A.1.
13. “OECD guidelines – 423” for testing of chemicals, 2001, pp 1-14.
14. Nagendrappa CG. An appreciation of free radical chemistry-3, free radicals in diseases and health, Resonance. 2005; 10: 65-73.
15. Kujala TS, Loponen JM, Klika KD, Pihlaja K, Phenolics and betacyanins in red beetroot (Beta vulgaris) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds, J Agric Food Chem, 2000; 48:5338–5342.
16 . Garrat DC. The Quantitative Analysis of Drugs, Japan, Chapman and Hall, 1964, 456.