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HEPATOPROTECTIVE ACTIVITY OF CHLOROFORM EXTRACT OF LEAVES OF *BARLERIA GIBSONII* AGAINST CARBON TETRA CHLORIDE INDUCED HEPATOTOXICITY IN RATS

C.R.Akila[1*], Gazala Perveen[2] and G.Jeyabalan[3]

[1]Scient Institute of Pharmacy, Ibrahipatnam, Hyederabad, Telangana, India.
[2]SunRise University, Alwar, Rajasthan, India.
[3]Alwar College of Pharmacy, Alwar, Rajasthan, India.

ABSTRACT

The aim of the study is to investigate the hepatoprotective activity of Chloroform extract of leaves of *Barleria gibsonii* against Carbon tetrachloride induced hepatotoxicity. The phytochemical screening was carried on the leaves extracts of *Barleria gibsonii* revealed the presence of some active ingredients such as Carbohydrates, Tannins, Saponines, Phenols, steroids, terpenoids and flavonoids. Leaves of *Barleria gibsonii* was successively extracted with Chloroform and it is treated against Carbon tetra Chloride (0.8 ml / kg i.p) induced hepatotoxicity using Standard drug Silymarin (100mg/kg). There was a significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Serum alanine phosphatase (SALP), reduces serum bilirubin, Cholesterol and triglyceride levels and reduces the total proteins and albumin content) in carbon tetra chloride treated rats, which were restored towards normalization in *Barleria gibsonii* (250 mg/kg, 400 mg /kg and 500 mg/kg) treated animals. Thus the present study ascertains that the Chloroform leaf extract of *Barleria gibsonii* possesses significant hepatoprotective activity.

Keywords: *Barleria gibsonii*, hepatoprotective activity, Carbon tetra chloride, Chloroform and Silymarin.

INTRODUCTION

In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. Existing documents revealed that around two lakh fifty thousand varieties of traditionally used plants haven't been scientifically proven for their therapeutic efficacy. Numerous herbs have been researched as reliable medicine in assorted therapeutic areas and significantly more study is continual in such a way. Thus, drug development from vegetation own remarkable opportunity and desires cumulative influence of plant scientists, specialists of pharmacognosy, phytochemcial experts and additional researchers. In depth knowledge of chemical and geographical diversity, improvement of isolation techniques of bioactive molecules from correctly identified plants and high through put screening are the real challenges of drug discovery from plant source.

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw

Corresponding Author

C.R.Akila
Email id: appharma12@gmail.com

materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials.

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction [1-3]. The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [4-6].

Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders [7]. The disorders associated with the liver are also numerous and varied [8]. More than 900 drugs have been implicated in causing liver injury [9] and it is the most common reason for a drug to be withdrawn from the market. Drug- induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures [10-15].

Liver disease is the serious health problems. This is rising in all age groups worldwide. There is a numerable scope to study the hepato protective activity of plants in suitable animal models. In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions of herbs or to confirm the claims made about them in the official books of Ayurveda.

The present study was carried out with an objective to extract *Barleria gibsonii* (leaves) with Chloroform. This extract was used for testing photochemical and hepato protective activity in carbon tetrachloride induced hepato toxicity in animal models.

MATERIALS and METHODS

Plant materials

Fresh leaves of *Barleria gibsonii* were collected from Kadapa district of Andhra Pradesh, India, in the month of October and authentified by Prof. Dr. K. Madhava Chetty, Taxonomist, S.V. University, Tirupati. A.P. Specimen vouchers (Ref No: SCIENT/SVU/2011- 107) were deposited at Department of Pharmacognosy for further reference.

Preparation of Chloroform extract

The leaves were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder. The coarsely powder leaves of *Barleria gibsonii* were used for extraction. The shade dry coarsely leaves of *Barleria gibsonii* were used for extraction with Chloroform. *Barleria gibsonii* leaf powder (250 g) was loosely packed in the thimble of soxhlet apparatus and extracted with chloroform at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

Phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in Chloroform extracts [16-20]

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of SCIENT INSTITUTE OF PHARMACY, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment was carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

Acute toxicity study

In order to find out the fifty percent of lethal dose of CEBG, a toxicology study named acute oral toxicity study was performed following OECD test guidelines 425

Experimental design for hepatoprotective activity

Animals were divided into six different groups, each having 6 rats and treated accordingly. Group 1: rats fed with a normal standard diet for 21 days. Group II rats receives CCL_4 (0.8ml/kg. i.p) as toxicant control. Group III receives silymarin (100mg/kg p.o) with CCL_4 (0.8ml/kg. i.p). Finally Group IV, V and VI rats receive CCL4 (0.8ml/kg. i.p) along with *Barleria gibsonii* leaf extracts (250mg/kg, 400mg/kg and 500mg/kg.p.o respectively for 21days). On the final day of study, rats were sacrificed by cervical disruption and blood was collected and serum was separated by permitting the bloodstream to clot for thirty minutes at thirty-seven degree centigrade. The coagulated blood was centrifuged at 3000 rpm for fifteen minutes as well as the supernatant blood serum had been utilized towards calculating distinctive biochemical variables such as hepatic enzymes, total protein and total bilirubin amounts. Hepatic tissue was isolated; part of it was used for histopathological studies.

Histopathology

Histopathology of liver was carried out by a modified Luna [21]. In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h. Then the livers were paraffin embedded and 5 µ thickness microtone sections were made [22]. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light micro-scope for any histological damage/protection.

Statistical analysis

The data are represented as mean ± S.E.M. Students't-test is used for statistical analysis of blood serum parameters and for statistical analysis of liver enzymes

RESULTS

The phytochemical screening of *Barleria gibsonii* shows the presence of Alkaloids, Carbohydrates, Steroids, Tannins, Flavonoids and Glycosides (Table 1). Acute toxicity studies of CEBG did not show any sign and symptoms of toxicity or mortality up to 5000 mg/kg body weight on oral administration. Thus, the extracts could be considered as category 5 as per OECD guidelines 425. Body weight before and after administration were noted and any changes in skin, fur, eyes, mucous membranes, breathing, vascular, autonomic and central nervous system were observed, sign of salivation, diarrhoea, tremors, convulsions, lethargy, sleep and coma were comprehended. The onset of toxicity and signs of toxicity were not seen in the rats up to 72 h of observation period. This indicates the safety of extract. Hence, the 250, 400 and 500 mg/kg dose were selected for the further study.

The effect of Chloroform extract of *Barleria gibsonii* an serum transaminases, alkaline phosphates, bilirubin and total protein level in CCl_4 intoxicated rats are summarized in Table 2. Rats treated with CCl_4 developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal group. After treatment with silymarin, Chloroform extracts had showed good protection against CCl_4 induced toxicity to liver. Dunnet's 't' test indicates a significant reduction in elevated serum enzymes levels with extracts treated animals compared to toxic control animals. The hepatoprotective activity was expressed as percentage reduction in biochemical parameters comparing with toxic control as 100 % elevation.CCl_4 treatment has increased bilirubin levels after injection. Treatment with silymarin and Chloroform extracts (250mg/kg, 400mg/kg and 500mg/kg) significantly reduced levels of direct bilirubin levels when compared to with toxicant group. CCl_4 treatment has considerably reduced serum total protein and albumin levels. Pretreatment with silymarin and Chloroform extracts of leaves of *barleria gibsoni* had showed a significantly increased the total protein and albumin level as compared with toxicant group. From the results it was found that rats treated with CCl_4 have showed a marked increase in cholesterol and triglycerides levels when compared to normal control group. The rats pretreated with silymarin and Chloroform extracts of leaves of *barleria gibsoni*, the serum cholesterol and triglycerides levels had significantly decreased when compared to toxicant group.

Table. 1: Preliminary Phytochemical Screening

Chemical constituent	CEBG
Alkaloids	A
Carbohydrates	P(***)
Glycosides	A
Saponins	P(***)
Flavonoids	P(***)
Tanins	P(***)
Proteins & Amino acids	P(*)
Fixed oils	P(***)
Steroids & Terpenoids	P(***)

P:Present; A: Absent *- Less; **-Moderate; *- High**

Table 2. Effect of leaf extracts of *barleria gibsonii* on SGPT, SGOT and SALP levels

Groups	Treatment	SGPT levels (U/L)% Reduction	SGOT levels (U/L)% Reduction	SALP levels (U/L)% Reduction
I	Normal control(10 ml/kg p.o)	-	-	-
II	Toxicant control (CCl_4 0.8 ml/kg, i.p)	-	-	-
III	Silymarin(100mg/kg, p.o) + (CCl_4 0.8 ml/kg, i.p)	99.37	91.13	91.75
IV	CEBG(250mg/kg, p.o) + (CCl_4 0.8 ml/kg, i.p)	27.64	23.11	22.44
V	CEBG (400mg/kg, p.o) + (CCl_4 0.8 ml/kg, i.p)	61.82	56.25	58.32
VI	CEBG (500mg/kg, p.o) +(CCl_4 0.8 ml/kg, i.p)	67.37	63.89	64.86

Table 3. Effect of leaf extracts of *barleria gibsonii* on Serum Bilirubin, Total protein, Albumin, Cholesterol and Triglyceride levels

Groups	Treatment	Serum Bilirubin levels % Reduction	Serum Total protein levels (mg/dl) % Increase	Serum Albumin levels (mg/dl) % Increase	Serum cholesterol levels % Reduction	Serum triglyceride levels % Reduction
I	Normal control(10 ml/kg p.o)	-	-	-	-	-
II	Toxicant control (CCl_4 0.8 ml/kg, i.p)	-	-	-	-	-
III	Silymarin(100mg/kg, p.o) + (CCl_4 0.8 ml/kg, i.p)	95.97	86.92	88.09	88.20	96.12
IV	CEBG(250mg/kg, p.o) + (CCl_4 0.8 ml/kg, i.p)	54.02	29.42	29.36	30.33	63.59
V	CEBG (400mg/kg, p.o) + (CCl_4 0.8 ml/kg, i.p)	68.96	59.43	44.44	63.13	73.90
VI	CEBG (500mg/kg, p.o) +(CCl_4 0.8 ml/kg, i.p)	78.16	70.28	84.12	79.04	85.29

Figure No 1. Effect of- leaf extracts of *barleria gibsoni* on SGPT, SGOT and SALP levels

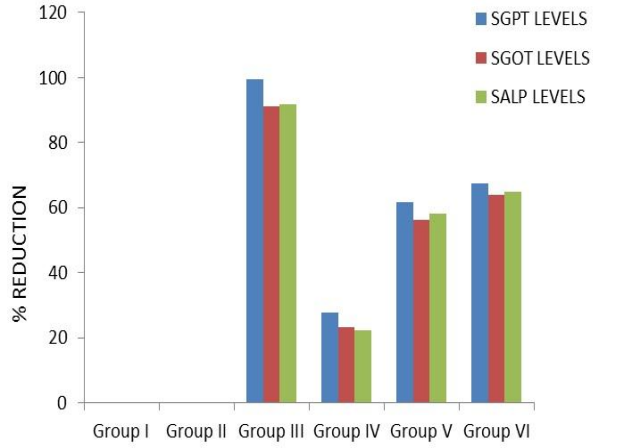


Figure No 2. Effect of- leaf extracts of *barleria gibsoni* on Serum Bilirubin, Serum Cholesterol and Serum triglyceride levels

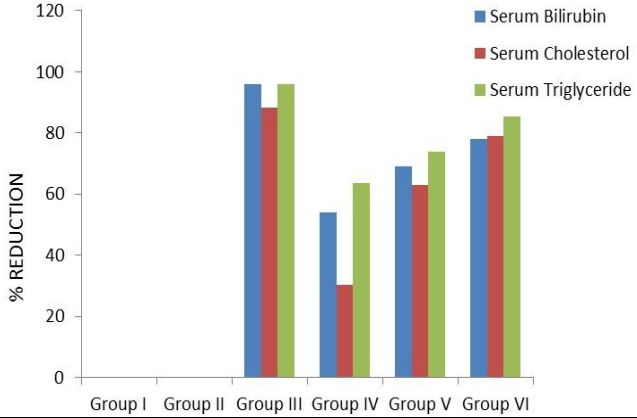


Figure No 3. Effect of- leaf extracts of *barleria gibsoni* on Serum total protein and Serum Albumin levels

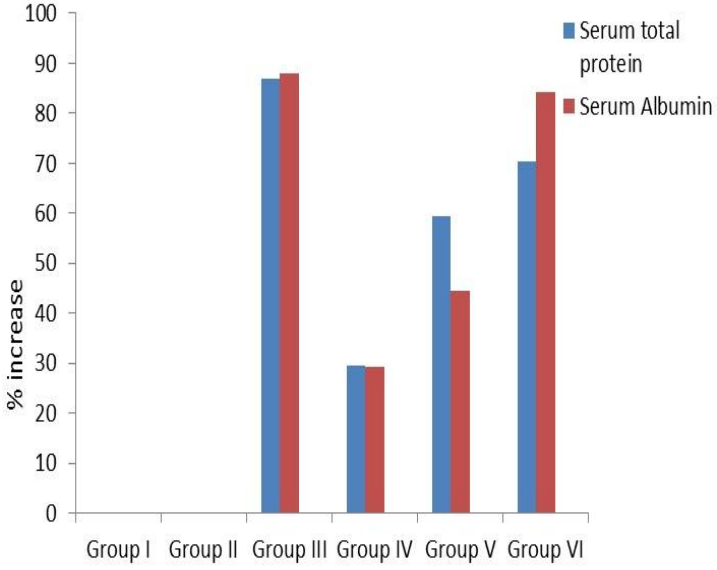
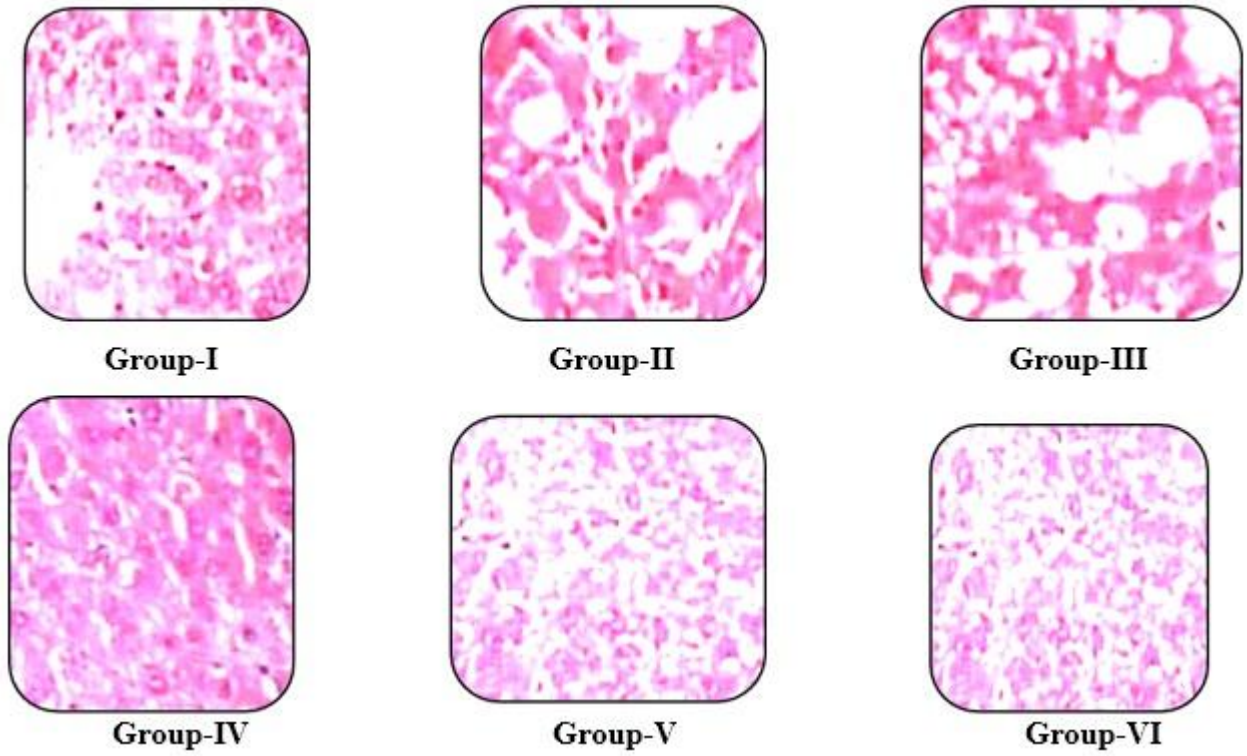


Figure no 4. Histological changes showing efficacy by CEBG intoxicated rats



DISCUSSION

The liver can be injured by many chemicals and drugs [23-27]. During hepatic damage, cellular enzyme like SGOT, SGPT, SALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration [28-31]. This decrease in elevated serum levels followed by CCl_4 treated animals in part may be due to the protective effect of *barleria gibsoni* leaf extracts on liver cells following the restoration of liver cell membrane permeability [32-34]. This protective effect indicates a reduction in enzymes present in the extra cellular milieu of the liver cell. The protective effect of the component of PHF has also been observed in several experimental studies [35-37].

Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in CCl_4 control group. Chloroform extracts of *Barleria gibsonii* (250 mg/kg, 400mg/kg and 500mg/kg, p.o) prevented these histological changes, further indicating their hepatoprotective activity. Although there is insufficient information to establish the mechanism of action of *Barleria gibsonii* protection, this could be due to its anti-oxidative of phenols.

CONCLUSION

In conclusion, the results of present study demonstrate that *Barleria gibsonii* leaf extracts (250 mg/kg, 400mg/kg and 500mg/kg,) has potent hepatoprotective activity against CCl_4 induced liver damage in rats. The results also imply that the hepatoprotective effects of *Barleria gibsonii* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepataprotective effect.

REFERENCES

1. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life science*. 74, 2004, 2157-2184.
2. Choudhary DN, Singh JN, Verma SK, Singh BP. (Antifertility effects of leaf extracts of some plants in male rats). *Indian J Exp. Biol*. 28(8), 1990, 714-6.
3. Corpinella MC, Miranda M, Almiron WR, Ferrayoli CG, Almedia FL, Palacios SM. (In vitro pediculicidal and ovicidal activity of an extract and oil from fruit of melia azedarach L). *J Am Acad Delmatol*. 56(2), 2007, 250 -6.
4. Deb AC. Fundamental of Biochemistry. 7th Ed. New Central Book Agency: Kolkata. 1998.
5. Descalzo AM, Coto C. (Inhibition of the pseudorabies virus (scis herpesvinyl) by an and vital agent isolated from the leaves of Melia azedarach. *Rev. Argent microbial*. 21(3-4), 1989, 133-40,

6. Frei B, Kim M, Ames B. Ubiquinol-10 is an effective lipidsoluble antioxidant at physiological concentrations. *Proc Natl Acad Sci U SA*. 87, 1990, 4879-83.
7. Friedman, Peter F. *GI/Liver Secrets: with STUDENT CONSULT Access*. Saint Louis: C.V. Mosby. 2006.
8. Friedman, Scott E.; Grendell, James H.; McQuaid, Kenneth R. *Current diagnosis & treatment in gastroenterology*. New York: *Lang Medical Books/McGraw-Hill.* 2003, 664–679.
9. Kalab M and Krechler T. The effect of the heptoprotective agent Liv-52 on liver damage. *Cas Lek Cesk.* 136, 1997, 758-60.
10. Kanathur N, Mathai MG, Byrd RP Jr, Fields CL, Roy TM. Simvastatin-diltiazem drug interaction resulting in rhabdomyolysis and hepatitis. *Tennessee Medicine*. 94(9), 2001, 339–41.
11. Khandelwal, K.R. Practical Pharmacognosy techniques and experiments, 16 Edition, *Nirali Prakashan*. 2006, 149-156.
12. Kokate, C.K., Purohith, A.P. &Gokhale, S.B. Pharmacognosy, *Nirali Prakashan, Pune*. 1990, 120.
13. Krajian AA. Tissue cutting and staining. In: Frankel, S., Reitman,S.(Eds.), Gradwohl's Clinical Laboratory Method and Diagnosis. *The CV. Mosby Co., Saint Louis, USA*. 1963, 1639.
14. Leo MA, Arai M. Hepatotoxicity of vitamin A and Thioacetamide. *Gastroenterology*. 82, 1982, 194-205.
15. Litchfield JT, Wilcoxon FA. Simplified method of evaluating dose effect exp eriments. *J Pharmacol Exp Ther*. 96, 1949, 99-133.
16. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin-phenol reagent. *J Biol Chem*. 193, 1951, 265-275.
17. Luna LG. Manual in histology and staining method. McGraw Hill: New York. 1999, 96.
18. Madsen HL, Nielsen BR, Bertelsen G, & Skibsted LH. Screening of antioxidative activity of spices. *Food Chemistry*. 57, 1996, 331-337.
19. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. *J. Biol. Chem*. 119, 1937, 481-490.
20. Mathur S. Role of Liv-52 in protection against beryllium intoxication. *Biol Trace Elem Res*. 41, 1994, 201-15.
21. Ostapowicz G, Fontana RJ, Schiødt FV, *et al.* Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann. Intern. Med.* 137 (12), 2002, 947–54.
22. Pang S, Xin X, Stpierre MV. Determinants of Metabolic Disposition. *Rev. Pharmacol. Toxicol*. 32, 1992, 625-626.
23. Pellegrini N, Simonetti P, Gardana C, Brenna O. Brighenti activity of Vini Novelli (Young red wines).*Journal of Agriculture andFood Chemistry*, 48, 2000, 732-735.
24. Pitchaon M, Suttajit M, Pongsawatmani R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem.* 100, 2007, 1409-1418.
25. Pokorny J, Yanishlieva N, Gordon M. Antioxidants in food, Practical Applications,Cambridge). *Woodhead Publishing Limited*. 2001, 1-3.
26. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol*. 28, 1957, 56-63.
27. Ricaurte B, Guirguis A, Taylor HC, Zabriskie D. Simvastatinamiodarone interaction resulting in rhabdomyolysis, azotemia, and possible hepatotoxicity. *Annals of Pharmacotherapy*. 40(4), 2006, 753–7.
28. Stocker R, Bowry VW, Frei B. Ubiquinone-10 protects low density lipoprotein more efficiently against lipid peroxidation than does alphatocopherol. *Proc Natl Acad Sci U SA.* 88, 1991, 1646-50.
29. Takahara S., Hamilton B.H., Nell J.V., Kobra T.Y., Ogura Y., Nishimuta E.T., *J.Clin. Invest*. 39, 1960, 610.
30. Trease GE and Evans WC. A text book of Pharmacognosy, *15th edition. Elsevier Company, Philadelphia, USA*. 2002, 191-418.
31. Vaghasiya J, Rathod S, Bhalodia Y, Manek R, Malaviya S, Jivani N. Protective effect of polyherbal formulation on simvastatin hepatotoxicity in rats. *J Young Pharmacists*. 1, 2009, 57-62.
32. Vaghasiya JD, Bhalodia YS, Manek RA, Gohil TA, Rathod SP. Oxidative stress mediated hepatotoxicity Produced by simvastatin. *Pharmacologyonline*. 3, 2008, 495-503.
33. Walter K, Schutt C. Acid and alkaline phoshatases in serum. In: Verlag Chemic Weinheim, In: Hans Ulrich Bergmeyer (Ed.), Method Enzymatic. *Anal. Academic Press Inc., New York*. 2, 1974, 856-864.
34. Wandscheer CB, Duque JE, Da Silva MA, Fukuyama Y. Wohlke JL, Adelmann J, Foutana JD. (Larvicidal action of Chloroform extracts from fruits endocarps of melia azedarach and Azadirachta indica against the dengue mosquito. *Aedes Aegypti. Toxicon*. 44(8), 2004, 829-35.
35. Ward FM, Daly MJ. Hepatic disease. In: Walker R, Edwards C, editors. *Clin. Pharm. Ther.. Churchill Livingston: New York*. 1999, 195- 212.
36. Wealth of India, raw material Vol-IV (L.M) Page no.323. [10a] *Chemical constituents*. 324-325.
37. Wolf P L, Biochemical diagnosis of liver disease, *Indian J Clin Biochem*. 14, 1999, 59.