International Letters of Natural Sciences
ISSN: 2300-9675, Vol. 14, pp 97-105
doi:10.18052/www.scipress.com/ILNS.14.97
2014 SciPress Ltd, Switzerland

Online: 2014-04-29

Effect methanolic extract of *Achyranthus aspera* on biochemical status of Albino mice

Chinnappa V. Reddy, Abhaykumar Kamble*

Department of Biotechnology, Gulbarga University, Gulbarga 585106, Karnataka, India

*E-mail address: abhay.gene@gmail.com

ABSTRACT

The effect of administration of Methanolic extract of Achyranthsu asper on biochemical parameter of mice were investigated. This drug induced significant reduction in the cholesterol level and amount of RNA. But significant increase in the amount of glycogen at 25 mg/kg and no significant reduction in the amount of glycogen at 50 mg/kg. This drug also induced increase in the level of alkaline phospatese and decrease in the level of acid phosphatase, protein and DNA, but these changes are statistically not significant. Protein 25 mg shows 4.22 ±0.112 and 50 mg shows 4.05 ±0.085. Cholesterol 25mg shows 0.5 ±0.04 and 50 mg shows 0.39 ±0.038. Glycogen 25mg shows 0.288 ±0.011 and 50mg shows 0.248 ±0.177. DNA 25mg shows 0.109 ±0.039 and 50 mg shows 0.085 ±0.035, RNA 25mg shows 0.38 ±0.036 and 50 mg shows 0.25 ±0.031

***Keywords*:** Biochemical parameter; *Achyranthus aspera*; Methanol extract

1. INTRODUCTION

Plants have been used for food, medicine, building materials and fuel for many centuries until now[1]. The World Health Organization (WHO) noted that of the 119 plants-derived pharmaceutical drugs, 74 % are used in modern medicine in ways that correlated directly with their traditional uses as Herbal plant medicine by native culture[2]. WHO estimated that about 80 % of the world population presently uses herbal medicine for some aspects of their primary health care needs while plant products also play important roles in the health care system of the remaining 20 %, who mainly reside in developed countries[3].

Millions of people in various traditional societies have resorted to the use of medicinal plants to treat their ailments. This dependence on natural products has its merits, but care must be taken not to consume harmful plants or high dose of plant extracts that could have deleterious effects on vital organs of the body either in the short term or long term[4]. There are concerns by certain medical personnel that herbal medicines may be harmful to vital organs such as liver and kidneys[5]. However, there seems to be paucity of information on safety evaluations of medicinal plants unlike the therapeutic potentials. Thus, there is need to study the effects of these plants, which have potential for therapeutic benefits, to ascertain their safety in animals using biochemical and haematological indices. Biochemical tests have immense benefits in the diagnosis and monitoring of liver diseases[6], while haematological

assays are also useful in early detection of the effects of harmful xenobiotics. The present study was therefore initiated to investigate the effect of administering high dose methanolic extract of Achyranthus aspera on the biochemical and haematological indices of albino Wistar rats, to determine their safety in animals.

2. MATERIALS AND METHODS

The present study of Methanolic extract of *Achyranthus aspera* was assessed to find the possible cumulative effect of biochemical parameters at various levels in 30 days treatment of mice, by administering drug intraperitoneally. These parameters on various organs system in mice were studied. It was considered necessary to assess its potential health hazard in man and to find the safe and effective dose.

For these studies the young and adult mice of Swiss albino strain weighing between 20-25 g were used and procured from the, CFTRI, Mysore. The mice were housed in a were mesh cages, five in a cage and at a temperature of 27 ±3 °C, pelleted food was supplied by Hindustan Lever Limited and tap water was given water adlibitum. The mice were made to acclimatize for.

3. BIOCHEMICAL STUDIES

On the 30th day different treatment groups, the mice were sacrificed and their liver was dissected out and washed with sterile distilled water and dried gently on filter paper. 50 mg of liver was taken and homogenized with solvents like 0. % NaCl for alkaline phosphates, acid phosphates, DNA and RNA, 5 % TAC 4 % TCA and 1:3 petroleum ether: alcohol, 10 % TCA separately and this filtrate was then subjected to biochemical analysis for protein, glycogen and cholesterol respectively.

3. 1. Estimation of alkaline phosphates[7]

50 mg of tissue was homogenized with 0.9 % NaCl and then centrifuged. The tissue homogenate was (0.1 ml) was mixed with 2.8 ml of a buffer (2-amino-2-methyl-1-propanol) and pH was adjusted to 10.5. It was incubated for 10 minutes at 30 °C. 4-NPP (0.1 ml) was added to the homogenate and the solution was mixed thoroughly. Change in the absorbance was recorded at 450 nm for about 3-5 minutes in spectrophotometer. Specific activity of enzyme was expressed as μ moles p-nitrophenol librated /min/100 mg of protein. Protein was estimated by the method of Lowry et al (1951)

3. 2. Estimation of acid phosphates[8]

50 % mg of tissue was homgenised with 0.9 % NaC1 and then centrifuged. In a test tube, 1 ml of buffer/substrate (4-nitrophenyl phosphate/citrate) was incubated at 37 °C for 10 min. tissue homogenate (0.2 ml) was added, mixed and incubated exactly for 30 min at 37 °C. the reaction was stopped by the addition of 4ml of 0.1 M NaOH. Blank was prepared and treated similarly like that of test sample. The yellow colour formed was read at 450 nm. Protein was estimated by the method of Lowry *et al* (1951). Results were expressed as μ moles p-nitrophenol liberated/min/100 mg of protein.

3. 3. Estimatoinof glycogen [9]

50 mg of tissue was taken and homogenized in 100 ml of 4 % TCA and then centrifuged at moderately high speed for 10 minutes. The supernatant was used for the estimation and the precipitate was discarded. To 2 ml of supernatant, 4 ml of 0.2 % anthrone reagent was added and boiled for 5 minutes on a hot water bath. Te tubes were allowed to cool. Different concentratons of standard glucose solution i.e., from 0-100 µg/1ml solution and 4 ml of 0.2 % anthrone reagent was added ad boiled for 5 minutes on a hot water bath. A blank was prepared by using distilled water. The optical density of all the solution s read on a spectrophotometer at a wavelength of 620 nm.

3. 4. Estimation of Nuceic acids [10]

5 % homogenate of the tissue was made in 0.9 % saline. It was filtered through 4 layers of cheese cloth and the filtrate was used for further isolation.

A known volume of the extract made as above was taken and treated with equal volume of 10 % TCA. The solution was shaken well, centrifuged and the supernatant was discarded. The residue was washed twice with 10 % TCA followed by washing with 5 ml of absolute alcohol twice. After this, the residue was ether extracted twice to remove the phospholipids.

The acid extraction was done as follows:

a. To the above reside 0.5 ml of 10 % TCA was added, mixed, centrifuged and saved the supernatant in a separate test tube.
b. The supernatant was treated with 5 ml of 5 % TCA. Kept at 100 °C for 15-20 minutes. Cooled. Centrifuged and 5 ml of supernatant was added to solution.
c. The final residue was treated with 2.5 ml of 4 % TCA. Heated it as above, and saved the final supernatant. From the total 10 ml of the clear acid extract, aliquots were used for DNA and RNA determination.

3. 5. Estimation of DNA [11]

A standard curve was made by pupating different aliquots of standard DNA solution, containing 50, 100, 200 and 500 µg of DNA into separate clean tubes and an 1 ml of acid extract was taken in separate test tube. To each tube 2.0 ml of freshly made dische's reagent was added and the total volume in each tube was made up to 5.0 ml with distilled water. Initially, turbidity was seen but this disappears gradually. The tubes were covered and headed in a boiling water Baath for 10-15 min. The solution was then cooled under tap water and the blue color developed was measured at 565 nm in a spectrophotometer. The blank contains 1 ml of distilled water and 2 ml of Disc he's reagent.

3. 6. Estimation of RNA[12]

The method adopted here is same as for DNA in terms of the aliquots of the acid extract µin different aliquots was taken in clean tubes. 0.5 ml of acid extract of tissue was taken in a separate test tube. In all these tubes 3.0 ml of ferric chloride reagent was added and the total volume was made to 5.5 ml with distilled water prior to the addition of ordinal reagent. Was prepared freshly in ethanol, 0.3 ml was added to all the tubes and later these tubes were kept in µa boiling water bath for 20 minutes. The green color developed was read at 75 nm against a blank treated in similar manner.

3. 7. Estimation of proteins[13]

The tissue was homogenate in 10 ml of 5 % TCA and centrifuged. 1 ml of supernatant was taken, to this 5 ml of Lowry's reagent was added and incubated at room temperature for 20 minutes. After incubation 0.5 ml of FCR was added and the solution was again incubated for 30 minutes at room temperature. Readout the optical density at 660 nm after the instrument was adjusted with blank solution. The blank was prepared by adding 1ml of distilled water with 0.5 ml of FCR was added and incubated for 30 min at room temperature. The optical density value of sample was read on the standard graph which were prepared from the different concentrations of 0-500 µg of BSA and corresponding values were calculated.

3. 8. Estimation of cholesterol[14]

The total cholesterol was estimated by Liebermann and Burckhardt reaction method. Homogenate the given tissue in 5 ml of 3:1 alcohol and petroleum ether mixture and centrifuged for 10 min. The solvent was evaporated from the supernatant by keeping it on a water bath. To this 5 ml of chloroform and 2 ml of acetic anhydride mixture was added. The mixture was kept in dark for about 30 minutes for color (green) development. The optical density was measured at 660 nm by using the blank. Blank was prepared by adding 5ml of chloroform with 2ml of acetic anhydride mixture. The standard curve was made with the different concentrations of cholesterol.

4. RESULTS AND DISCUSSION

4. 1. Biochemical studies

Biochemical studies of the liver is to understand the manifestation of its physiological status. The treatment of animals and the consequent changes in quantity of the metabolites in their organ is an indication of activity of the drug on the biochemical process either to increase or decrease (inhibit) the metabolites production this may be due to activity of the drug at the enzymatic level to bring the final compound. The liver is a vital organ performing almost all the metabolic process and the function of syntheses of plasma protein, albumin and fibrinogen. It also performs of carbohydrates, cholesterol and phospholipids. This study has shown that 25 mg/kg and 50 mg/kg body weight has brought about the decrease in protein syntheses and also syntheses of DNA but not significant enough at both the doses. Whereas RNA level has reduced significantly in both the treatment groups. It may be inferred that the drug acted during RNA syntheses, which is essential for the protein syntheses. The detailed study is required to understand at what enzymatic level the protein syntheses or RNA synthesis is inhibited.

The liver which is the site of synthesis and store house of carbohydrates such as glycogen, has decresed synthesis of glycogen at higher drug dose at 50 mg/kg of methanolic extract of achyranthus aspera, whereas at 25 mg/kg it has influenced the glycogen storage and there was an increase in the glycogen content in liver. The acid phosphatase and alkaline phosphatase protects the liver form toxicity[15]. In this study there was slight increase in alkaline phosphatase level but slight decrease in acid phosphatase at 25 mg/kg but there was further increase in the alkaline phosphates and further decrease in acid phosphates a 50 mg/kg. When the mice were treated with the drug, significant decrease in cholesterol content in the liver was also observed. Perhaps the Methanolic extract of *Achyranthus aspera* acted as hypolipidaemicat both the doses.

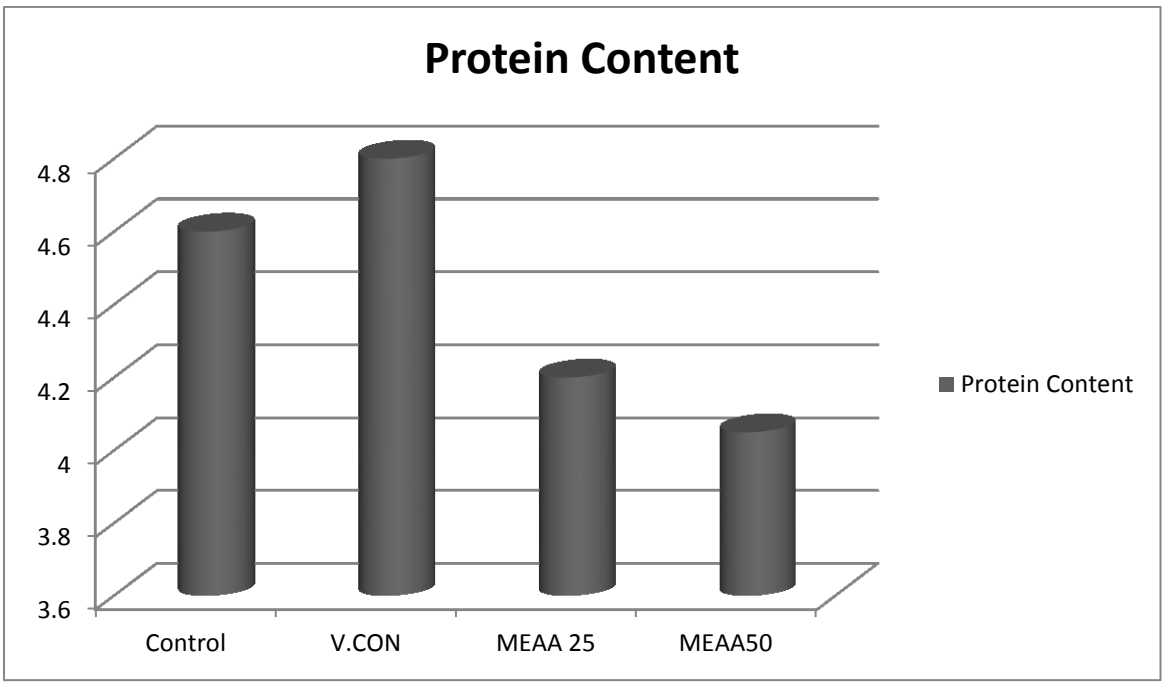

Fig. 1. Protein content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus asper.*

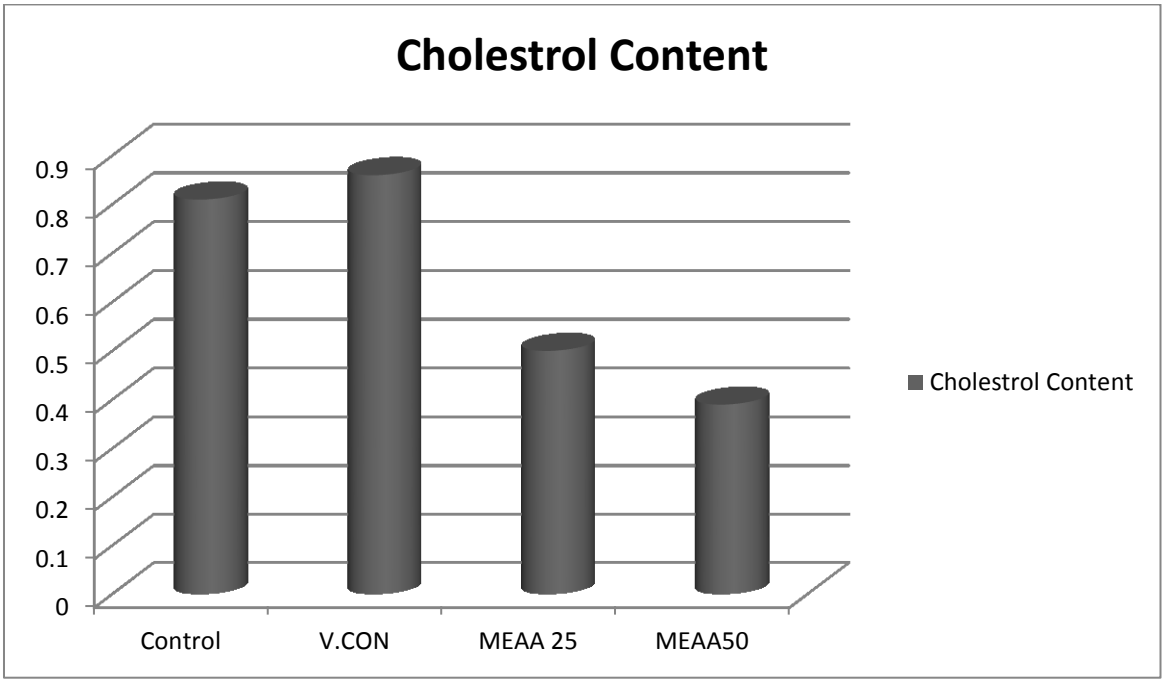

Fig. 2. Cholestrol content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus aspera.*

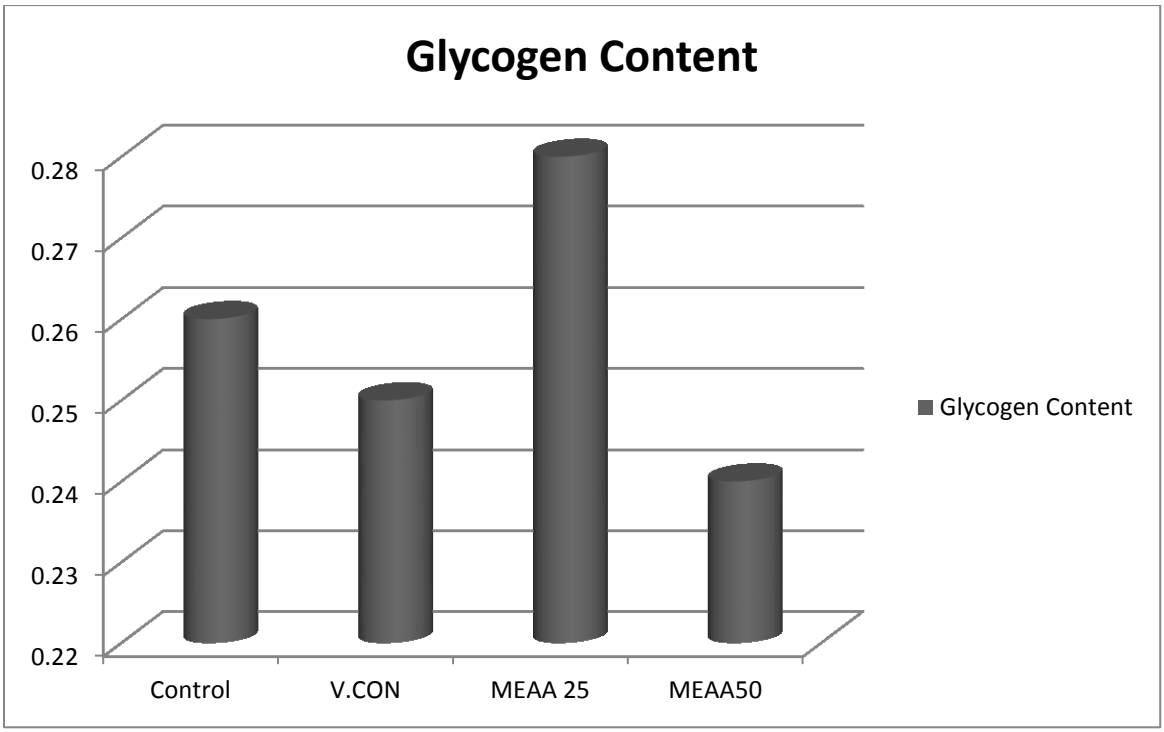

Fig. 3. Glycogen content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus aspera.*

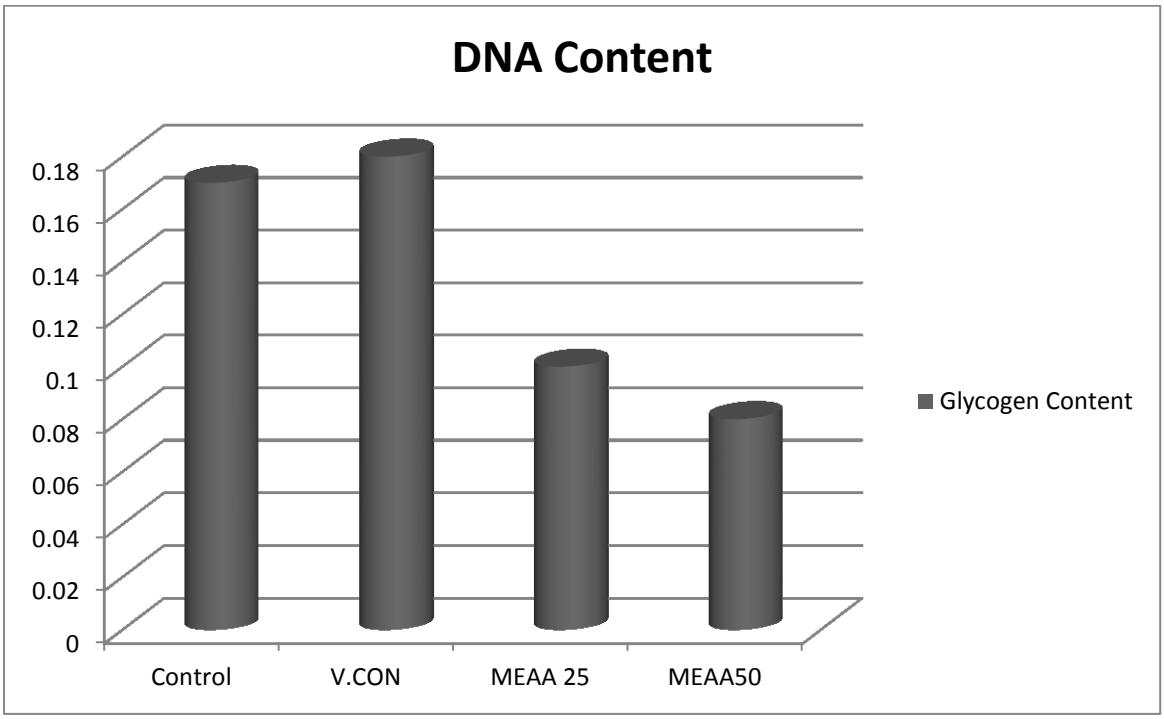

Fig. 4. DNA content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus aspera.*

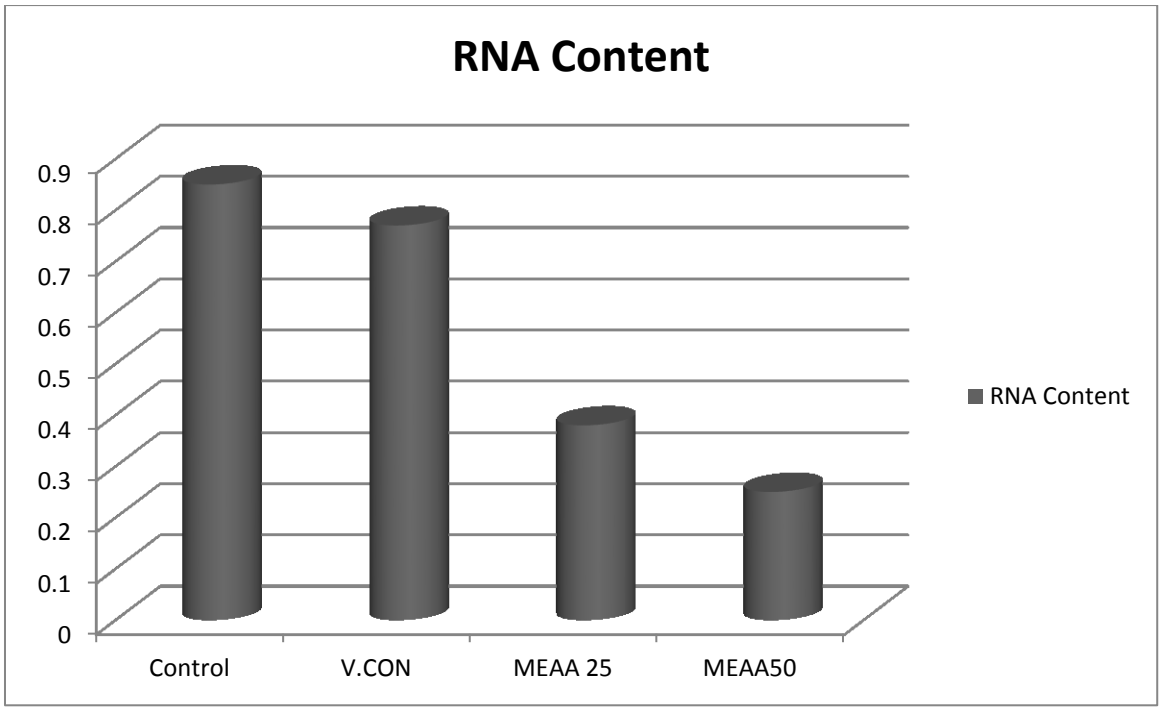

Fig. 5. RNA content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus aspera.*

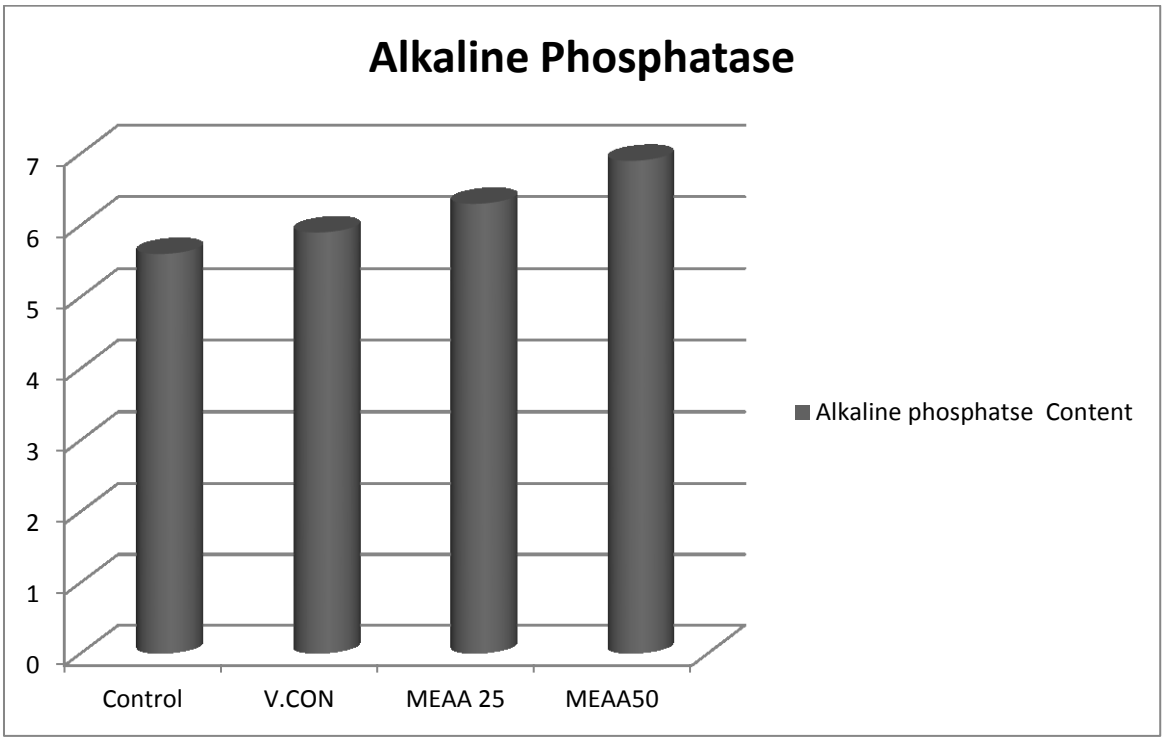

Fig. 6. Alkaline phosphatase content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus aspera.*

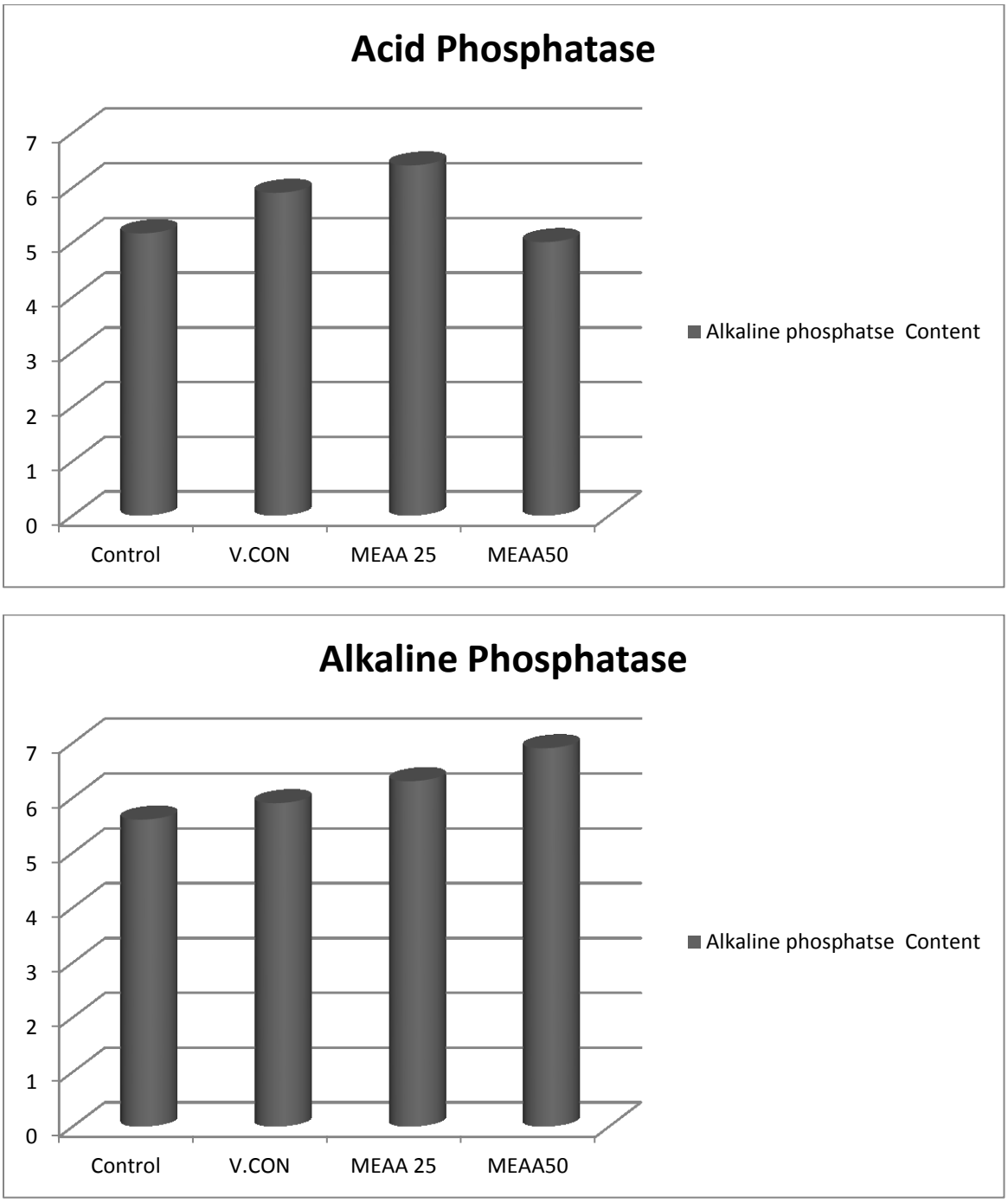

Fig. 7. Acid phosphatase content in the liver of mice after 30 days treatment with Methanolic extract of *Achyranthus aspera.*

5. CONCLUSION

This drug induced significant reduction in the cholesterol level and amount of RNA. But significant increase in the amount of glycogen at 25 mg/kg and no significant reduction in the amount of glycogen at 50 mg/kg. This drug also induced increase in the level of

alkaline phospatese and decrease in the level of acid phosphatase, protein and DNA, but these changes are statistically not significant.

References

[1] Shankar D., Ved D. K., *Indian Forester* 129 (2003) 275-287.

[2] Vijaya Kumar S, Sankar P, Varatharajan R., *Pharm Biol* 47 (2009) 973-975.

[3] The Wealth of India; a dictionary of Indian raw materials and industrial products. 1ST ed. III-C. New Delhi: CSIR; 1985, 66-67.

[4] Dwivedi S., Dubey R., Mehta K., *Ethno Leafl.* 12 (2008) 670-676.

[5] Elumalai E. K., Chandrasekaran N., Thirumalai T., *Int J Pharmtech Res* 1 (2009) 1576-1579.

[6] Goyal B. R., Goyal R. K., Mehta A. A., *A Review: Pharmacognosy Reviews* 1 (2007) 143-150.

[7] Bhosale U. A., Radha Y., Pophale P., Zambare M., Somani R. S., *Int J Phytomed.* 2 (2010) 440-445.

[8] Alam M. T., Karim M. M., Khan S. N., *J Sci Res* 1 (2009) 393-398.

[9] Vetrichelvan T, Jegadeesan M., *Phytother Res.* 17 (2003) 77-79.

[10] Khandelwal K. R., Practical Pharmacognosy. 19th ed. Pune: Nirali Prakashan; 2008, pp. 149-56.

[11] Organization for Economic Cooperation and Development (OECD). OECD guidelines for testing of chemicals test no. 423, acute oral toxicity. France: OECD Publishing; 2006, 1-27.

[12] Winter C. A., Risley E. A., Nuss G. W., *Proc Soc Exp Biol Med* 11 (1962) 544-547.

[13] Perianayagam J. B., Sharma S. K., Pillai K. K., *J Ethnopharmacol.* 104 (2006) 410-414.

[14] Sutar N., Garai R., Sharma U., Goyal P., Yadav G., *Pharmacie Globale (IJCP)* 5 (2011) 1-3.

[15] S. Palani, S. Raja, D. Venkadesan, S. Karthi, K. Sakthivel, B. Senthil Kumar, *Arch. Appl. Sci. Res* 1(1) (2009) 18-28.

(Received 22 April 2014; accepted 27 April 2014)