Journal of Sciences and Multidisciplinary Research
Volume 12, Number 2, 2020
ISSN: 2277-0135
http://www.cenresinjournals.com

LYCOPERSICON ESCULENTUM STEM AND ROOT FRACTIONS ATTENUATE ETHIDIUM BROMIDE-INDUCED BIOCHEMICAL ALTERATIONS IN RATS

[a]Sulaiman A. Faoziyat, [a]Adeoti Oluwatomiwa, [a]Sulaiman A. Afolabi, *[a]Olatunde Ahmed, [a]Abubakar F. Aluko, [a]Oseji Favor, [a]Omopupa Suliat, [a]Odetoye Omolara, [a]Bamidele B. Eniola, [a]Zakariyah R. Toyin, [a]Nurudeen A. Damilola

[a]Department of Biochemistry, Faculty of Life Sciences,
University of Ilorin, Ilorin
[b]Federal Medical Center, Ebute Metta, Lagos
[c]Department of Biochemistry
Abubakar Tafawa Balewa University, Bauchi 740272
Email: olatundebch@gmail.com

ABSTRACT

Ethidium bromide is usually applied as a marker for identifying nucleic acids bands during electrophoretic processes. The information on the ability of natural products to ameliorate the toxic effect this chemical is still scanty. However, this study investigated the ameliorative effect of Lycopersicon esculentum in ethidium bromide induced toxicity in experimental rats. Experimental animals (50) with average body weight of 95±10g were randomly assigned into 10 groups each consisting of five each. The normal group was administered with distilled water as the vehicle. The remaining groups of rats were treated with 0.5 ml (2.1 mg/kg body weight) on the skin for two weeks. The standard group was administered with 4.0 mg/kg Contiflo XL while the remaining ethidium bromide treated groups were administered with fractions (aqueous, butanol, ethyl acetate and hexane) of Lycopersicon esculentum stem and root. The results showed that the activities of AST, ALT, GGT, ALP and concentrations of total protein, albumin, bilirubin, creatinine, urea, uric acid, triglyceride, and total cholesterol were altered in the groups treated with ethidium bromide solution compared to the control group. But, Lycopersicon esculentum stem and root fractions (aqueous, butanol, ethyl acetate and hexane) administration ameliorated the alterations in ethidium bromide treated rats. The data from the study indicated that aqueous, butanol, ethyl acetate and hexane fractions of Lycopersicon esculentum stem and root attenuated the toxic effect of ethidium bromide on the liver, kidney and heart of rats.

Keywords: Lycopersicon esculentum, toxicity, biochemical indices, fractions

INTRODUCTION

Ethidium bromide (3,8-diamino-6-phenyl-5-ethylphenanthridine bromide, EtBr) is an organic compound consisting of aniline and pyridine rings. It is used in gel electrophoretic processes for staining nucleic acids. Ethidium bromide acts as a mutagen and can leads to DNA deformation through intercalation of DNA molecules resulting to altered weight, charge, flexibility and conformation of this nucleic acid [1]. It was shown that exposure of ethidium bromide caused deformed DNA molecule resulting to impaired

process of replication, translation and translation. Also, it causes the distortion of nucleic acid (RNA and DNA) synthesis in several organisms leading to impaired replication and resulting to frame shift mutation [2, 3]. In an Ames test, the chemical caused mutagenesis in bacteria [4]. Lycopersicum esculentum is an edible, red vegetable plant commonly known as a tomato. The plant belongs to the Solanaceae family and it originated in western South America [5]. Tomatoes are widely globally available and individuals of all ages and cultures consume the plant for better health [6]. Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While tomatoes are botanically berry-type fruits, they are considered culinary vegetables as an ingredient or side dish for savoury meals [7]. The leaf material of the plant was reported to contained secondary metabolites like steroidic alkaloids, and phenolic compounds such as flavonoids and hydroxycinnamic acids [8-10]. These metabolites displayed many therapeutic and nutritional activities and are involved in defence mechanisms in plants [8]. Several literatures have stated that phenolic constituents have therapeutic actions against several severe disorders which are associated with oxidative stress [11, 12]). Hence, Lycopersicum esculentum could be used in the management of oxidative stress related complications such as cancer. This study aimed to determine the effect of fractions of Lycopersicum esculentum stem and root in ethidium bromide treated rats.

MATERIALS AND METHODS

Chemicals and reagents

Ethidium bromide was obtained commercially from Sigma Aldrich Inc. (St. Louis, MO, USA). The standard drug, Contiflo XL with tamsulosin hydrochloride as the active agent was purchased from Sun Pharmaceutical industry (Europe). All other chemicals and reagents used in this study were of analytical grade.

Collection of *Lycopersicon esculentum* plant (stem and root)

Fresh whole plant parts (stem and root) were collected from Ilorin, Kwara state, Nigeria. Identification and authentication of the plant was carried out at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria, where voucher specimens were deposited. The voucher number of Lycopersicon esculentum is UILH|002|017.

Extraction procedure for *Lycopersicon esculentum* plant (stem and root)

The plant materials (stem and root) were separated thoroughly washed and air dried for a period of two week in order to remove the moisture present. The dried plant materials (stem and root) were pulverised and their weights were recorded. Aqueous extraction of the stem and roots were carried out at the ratios of 1:10 and 1:5 respectively. After 72 hours, filtration was performed using Whatmann No. 1 filter paper to separate the filtrate from the residue. The extraction using non-polar solvents of hexane, ethylacetate and butanol were performed afterward using a separating funnel. Hexane was added to the residue in certain ratios. For the stem extraction using hexane, the ratio of 1:2 was used, and for the root, the ratio of 1:4 was used. These were soaked for 72 hours after which separation was performed to remove filtrates from the residues. The filtrates were poured into separating funnel so as to obtain the hexane fraction. The hexane fraction was poured into stainless to evaporate the hexane fraction to dryness. For ethyl acetate extraction, this solvent was added to the aqueous residue in the ratio 1:1. After 72 hours, the mixture was separated using separating funnel, and the ethyl acetate fraction was collected and poured into a stainless plate and concentrated. Butanol was added to the aqueous residue in the ratio 1:1 and separated after 72 hours, the butanol fraction was collected from the separating funnel and concentrated. The final aqueous residue were also concentrated. The entire fractions (hexane, ethyl acetate, butanol and aqueous residue) were concentrated and weighed. The percentage yields were calculated as per fractions of the plant's part as shown below. The fraction concentrates were kept in sample bottles and refrigerated until require for use.

$$\text{Percentage yield of fractions} = \frac{\text{final weight (g)}}{\text{initial weight (g)}} \times 100$$

Experimental Animals

Healthy albino wistar rats (50) with an average weight of 95±10 g were obtained from the Animal House of the Department of Biochemistry of the University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in well ventilated plastics cages with saw dust as beddings, fed on standard chicken feeds and allowed free access to water ad libitum. The animals were acclimatized for two weeks before the commencement of the experiment. All rats were maintained under standard laboratory conditions (12-hrs light/dark cycle 25±2°C).

Ethidium bromide Solution Preparation

Exactly 100 ml of ethanol was mix with 1 g of ethidium bromide to form ethidium bromide solution. Experimental animal were exposed with 0.5 ml of 2.1 kg/RBW (rat body/weight) ethidium bromide on their shaven dorsal skin.

Grouping of Animals and Treatments

The experimental animals were randomly assigned into 10 groups. Each group containing 5 rat. The experimental rats in the treated groups were initially treated with 2.1 mg/kg of ethidium bromide solution. All administration were performed at 0.5 ml using a cannula. The grouping in this study is as follows:
Normal group containing rats administered with 0.5 ml distilled water (control group), ethidium bromide group treated rats administered with 4 mg/kg body weight of contiflo XL (standard group), ethidium bromide treated rats administered with 12.5 mg/kg of aqueous residue fraction of Lycopersicon esculentum stem (LEA-stem), ethidium bromide treated rats administered with 12.5 mg/kg of butanol fraction of Lycopersicon esculentum stem (LEB-stem), ethidium bromide treated rats administered with 12.5 mg/kg of ethyl acetate fraction of Lycopersicon esculentum stem (LEE-stem), ethidium bromide treated rats administered with 12.5 mg/kg of hexane fraction of Lycopersicon esculentum stem (LEH-stem), ethidium bromide treated rats administered with 12.5 mg/kg of aqueous residue fraction of Lycopersicon esculentum root (LEA-root), ethidium bromide treated rats administered with 12.5 mg/kg of butanol fraction of Lycopersicon esculentum root (LEB-root), ethidium bromide treated rats administered with 12.5 mg/kg of ethyl acetate fraction of Lycopersicon esculentum root (LEE-root), ethidium bromide treated rats administered with 12.5 mg/kg of hexane fraction of Lycopersicon esculentum root (LEH-root).

Collection of Blood Samples, Preparation of Serum and Homogenisation of Tissues

The animals were made unconscious using diethyl ether as an anaesthesia and sacrificed by a simple incision of the jugular vein, and the blood samples were collected into sterile sample tubes. Blood samples were allowed to stand at room temperature for 30 minutes to form clots, after which they were centrifuged for 10 minutes at 3500 rpm. After centrifugation, the serum sample was collected using a Pasteur's pipette into clean plain sample bottles. The serum, thus obtained were approximately labelled and stored in a refrigerator at -4 °C until required for further analysis. The rats were dissected and tissues of interest (liver, kidney and heart) were isolated, cleaned with normal saline, weighed and placed in dispensing bags before stored. Exactly 1 g of the liver, kidney and heart were cut with a clean blade, and then subjected to homogenization using mortar and pestle in ice-cold 0.25 M sucrose solution as the buffer, in a proportion of 1g of organ to 4ml of

0.25 M sucrose solution (1:4 w/v). The homogenates were stored in sterile sample tubes and kept in a – 4 °C freezer until required for further analysis.

Body Weight and Organ Body Weight Ratios

The body weights were determined throughout the experimental period using a weighing balance. The organ body weight ratios of the various organs (liver, kidney and heart) was determined at the end of the experiment prior to sacrifice of the animals, from the weight of animal and weight of organ as follows:

$$\text{organ body weight ratio (\%)} = \frac{\text{weight of organ (g)}}{\text{weight of animal (g)}} \times 100$$

Biochemical Analysis

The concentrations of total protein, albumin, bilirubin, creatinine, urea, uric acid, triglycerides, total cholesterol, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) were determined by using Randox kits (RANDOX Laboratories Limited) and UV spectrophotometer (Spectrum Lab 22pc) was used for recording absorbance at various wavelengths.

Statistical Analysis

Results were expressed as the mean ± SEM ($n = 5$). Statistical analysis was determined using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test for random comparison. The differences between groups were considered significant at $p < 0.05$. The statistical analysis was performed with GraphPad prism version 6.0 software.

Results

Table 1 shows that percentage yield of aqueous, butanol, ethyl acetate and hexane fractions of Lycopersicon esculentum stem and root. The aqueous, butanol, ethyl acetate and hexane stem fractions has percentage yield of 5.50, 1.97, 8.26 and 0.28 respectively while the aqueous, butanol, ethyl acetate and hexane root fraction has percentage yield of 3.50, 9.47, 10.39 and 0.31 respectively. From this data, ethyl acetate fraction has the highest percentage yield from the stem and root. The organ-body weight ratios of ethidium bromide treated rats administered with fractions of Lycopersicon esculentum leaves and stem is shown on Table 2. The liver, kidney and heart-body weight ratios were significantly decreased in the standard group and groups treated with Lycopersicon esculentum stem and root fraction compared to the control group. However, there was no significant difference in the LEB-root group compared to the control group (Table 2).

The effect of fractions of Lycopersicon esculentum stem and root on the activities of AST, ALT, GGT and ALP in rats treated with ethidium bromide is shown in Figure 1, 2, 3 and 4. The activities of AST, ALT, GGT and ALP in serum were significantly elevated in the liver of the standard group and Lycopersicon esculentum stem and root fractions treated groups compared to the control group. Conversely, the activities of these enzymes were significantly decreased in the serum of the standard group and groups treated with fractions of Lycopersicon esculentum stem and root compared to the control group (Figure 1, 2, 3 and 4). The concentration of protein in ethidium bromide-treated rats administered with fractions of Lycopersicon esculentum stem and root is demonstrated in Figure 5 and 6. The protein concentration was significantly increased in serum, liver, kidney and heart of standard group and the Lycopersicon esculentum stem and root fractions treated groups compared to the control group.

Figure 7 and 8 demonstrated the effect of fractions of Lycopersicon esculentum stem and root on the concentration of albumin and bilirubin in ethidium bromide treated rats. The concentration of albumin was significantly increased in the liver and serum of standard group and groups treated with fractions of Lycopersicon esculentum stem and root compared to the control group (Figure 7). Contrarily, the concentration of liver and serum bilirubin was significantly decreased in the standard group and Lycopersicon esculentum stem and root fractions treated groups compared to the control group (Figure 8).

Figure 9, 10, 11 portrayed the concentration of creatinine, urea and uric acid in ethidium bromide-treated rats administered with fractions of Lycopersicon esculentum stem and root. The concentration of creatinine, urea and uric acid in the kidney of the standard group and groups treated with Lycopersicon esculentum stem and root fractions were significantly elevated compared to the control group. Conversely, the concentration of these parameters in serum were significantly decreased in the standard group and Lycopersicon esculentum stem and root fractions treated groups compared to the control group. The concentration of triacylglycerol and cholesterol after administration of fractions Lycopersicon esculentum stem and root in ethidium bromide treated rats is demonstrated in Figure 12 and 13. The concentration of triacylglycerol and cholesterol in the serum and heart were significantly decreased in the standard group and Lycopersicon esculentum stem and root fractions treated group compared to the control group.

DISCUSSION

Exposure of many laboratories chemicals can leads to complications such as nephrotoxicity and hepatotoxicity [13]. Toxins are exogenous substances which can causes damage to tissues and they vary ranging from laboratory chemicals, natural chemicals and medicinal drugs [14, 15]. EtBr is a marker normally used for visualizing and identifying nucleic acids. The chemical act as a mutagen by abrogating replication and transcription processes [2, 4]. AST, AST, GGT and ALP are biomarkers for assessing the liver injury, damage and integrity of plasma membranes [16-18]. Studies have indicated that increase in the activities of serum ALP in ethidium bromide treated experimental animals [19, 20]. From this study, administration of Lycopersicon esculentum stem and root fractions ameliorated that altered activities of these biomarker enzymes in serum and liver of ethidium bromide-treated groups. Protein, bilirubin and albumin are bio-indicators of liver functioning capacity [21]. Ethidium bromide was reported to cause reduction in serum albumin and elevation of serum bilirubin in experimental rats [20]. From this study, oral administration of fractions of Lycopersicon esculentum stem and root at dose of 12.5 mg/kg body weight reverse the alterations in albumin, bilirubin induced by ethidium bromide in rats.

Renal function indices such as creatinine, urea and uric acid are usually used to determine the normal functioning of different parts of the kidney. In this study, the altered concentrations of creatinine, urea and uric acid in serum and kidney were attenuated after the administration of Lycopersicon esculentum stem and root fractions at dose of 12.5 mg/kg body weight. Lipid profile are biomarkers that can be used for estimating the normal functioning of the heart. From this study, the reduced concentration of triacylglycerol and cholesterol recorded in serum and heart indicated that the fractions of Lycopersicon esculentum stem and root ameliorated the ethidium bromide induced toxicity in heart. Thus the plant could be used in the management of cardiovascular-related complications

CONCLUSION

The results from the study revealed that the fractions of lycopersicon esculentum stem and root were effective in ameliorating the damage caused by ethidium bromide on the liver, kidney and heart of the experimental rats.

ACKNOWLEDGEMENT

The authors show their profound appreciation to the Department of Biochemistry, University of Ilorin, Ilorin, for their monumental contributions to the success of this work.

REFERENCES

Waring, M.J. (2005). Complex formation between ethidium bromide and nucleic acids. Journal of Molecular Biology. 13 (1): 269–282.

M.J. Waring (1965). Complex formation between ethidium bromide and nucleic acids, J. Mol. Biol. 13: 269–282.

McCann, J. Choi, E. Yamasaki, E. Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonellarmicrosome test: assay of 300 chemicals, Proc. Natl. Acad. Sci. USA 72. 1975 5135–5139.

McCann, J. Ames, B.N. (1975). "Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals". Proceedings of the National Academy of Sciences. 72 (12): 5135–5139. doi:10.1073/pnas.72.12.5135. PMC 388891. PMID 1061098.

Sato, S. Tabata, S. Hirakawa, H. Asamizu, E. Shirasawa, K. Isobe, S. Kaneko, T. Nakamura, Y. Shibata, D. (2012). "The tomato genome sequence92 provides insights into fleshy fruit evolution". Nature. 485 (7400): 635–641. doi:10.1038/nature11119. PMC 3378239. PMID 22660326.

Debjit, Bhowmik, K.P. Sampath K. Shravan, P. Shweta, S. (2012). Journal of Pharmacognosy and Phytochemistry. Tomato-A Natural Medicine and Its Health Benefits 345-350.

Parnell, J. Watt, G.R. Middleton, D. Kelly, J. Baron, M. (2004). Deformation band control on hydrocarbon migration. Journal of Sedimentary Research 74: 552–60.

Friedman, M. (2002). Tomato glycoalkaloids: Role in the plant and in the diet. Journal of Agricultural and Food Chemistry, 50, 5751–5780.

Ferreres, F. Taveira, M. Gil-Izquierdo, A. Oliveira, L. Teixeira, T. Valentão, P. Simões, N. Andrade, P.B. (2011). High performance liquid chromatography diode array detection electrospray ionization multi-stage mass spectrometric screening of an insect/plant system: The case of Spodoptera littoralis/Lycopersicon esculentum phenolics and alkaloids. Rapid Communications in Mass Spectrometry 25: 1972–1980.

Slimestad, R. Verheulb, M. (2009). Review of flavonoids and other phenolics from fruits of different tomato (Lycopersicon esculentum Mill.) cultivars. Journal of the Science of Food and Agriculture 89: 1255–1270.

Andrade, P.B. Pereira, D.M. Valentão, P. (2010). Phenolic compounds: Analysis by HPLC. In J. Cazes (Ed.), Encyclopedia of chromatography. New York: Taylor & Francis.

Taveira, M. Silva, L.R. Vale-Silva, L.A. Pinto, E. Valentão, P. Ferreres, F. Guedes de Pinho, P. Andrade, P.B. (2010). Lycopersicon esculentum seeds: An industrial by product as

an antimicrobial agent. Journal of Agricultural and Food Chemistry 58: 9529-9536.

Navarro, V.J. Senior, J.R. (2006) Drug-related hepatotoxicity. N Engl J Med 354: 731-739.

Willett, K.L. Roth, R.A. Walker, L. (2004). Workshop overview: hepatotoxicity assessment for botanical dietary supplements. Toxicol Sci 79: 4-9.

Papay, J.I. Clines, D. Rafi, R. Yuen, N. Britt, S.D. (2009). Drug-induced liver injury following positive drug rechallenge. Regul Toxicol Pharmacol 54: 84-90.

Whitfield, J.B. (2001). Gamma glutamyl transferase. Critical Reviews in Clinical Laboratory Sciences 38(4): 263-355, doi:10.1080/20014091084227.

17. Yang N.S. Rong-ze A.T. Soohyun park S.P. William, J.A. Reagan, N.H. Rick, D.E. Goldstein, R.L. Shao-Zhang, B.W. Michael, Z.K. Lawton, G.O. Francis, V.Q. Rajamoham, P.L. Kun-Qian, D.U. Li-Liu, W.J. Dawei, H.L. Gong R.Y. (2009). Alanine amino transferase Isoenzymes: Molecular cloning and quantitative analysis to tissue expression in rats and serum elevation in liver toxicity. Hepatology 49(2): 598-607.

Kuete, V. Manfeno, R.N. Beng, V.P. (2010). Toxicological evaluation of the hydrocthanol extract of tubernaemontana crassa (Apocynaceeae) steam bark. Journal of Ethmopharmacol. 130: 470-476.

Jaeschke, H. (2002). Mechanisms of Hepatotoxicity. Toxicological Sciences, 65(2), 166-176.

Sabiu, S. Sunmonu, T.O. Ajani, E.O. Ajiboye, T.O. (2015). Combined administration of silymarin and vitamin C stalls acetaminophen-mediated hepatic oxidative insults in Wistar rats. Revista Brasileira de Farmacognosia 25(1): 29-34.

Berk, P.D. Korenblat, K.M. (2007). Approach to the patient with jaundice or abnormal liver test results. In: Goldman L, Ausiello D, eds. Cecit Medicine. 23rd ed. Philadephia, Pa: Saunders Elsevier, Chapter 150.

Table 1: Percentage Yield of Lycopersicon Esculentum Fractions

Lycopersicon esculentum fractions	Stem (%)	Root (%)
Aqueous	5.50	3.50
Hexane	0.28	0.31
Butanol	1.97	9.47
Ethyl acetate	8.26	10.39

Table 2: Percentage Organ-body Weight Ratios of Rats

	Organs-body weight ratios (%)		
Groups	Liver-body weight	Kidney-body weight	Heart-body weight
Control	3.94±0.23[a]	0.53±0.03[a]	0.59±0.03[a]
Standard	3.22±0.23[b]	0.37±0.01[c]	0.40±0.02[b]

LEA-root	1.70±0.13^{a}	0.29±0.04^{ab}	0.30±0.05^{b}
LEB-root	3.10±0.17^{c}	0.34±0.01^{bc}	0.42±0.02^{c}
LEE-root	1.87±0.18^{a}	0.24±0.00^{a}	0.27±0.02^{a}
LEA-stem	1.91±0.10^{a}	0.27±0.01^{ab}	0.26±0.01^{a}
LEB-stem	3.00±0.07^{c}	0.37±0.02^{c}	0.42±0.03^{c}
LEE-stem	1.87±0.18^{a}	0.25±0.00^{a}	0.29±0.03^{a}

Values are presented as Mean ± SEM of 5 replicates. Bars with different alphabets are significantly different ($p<0.05$). LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum.

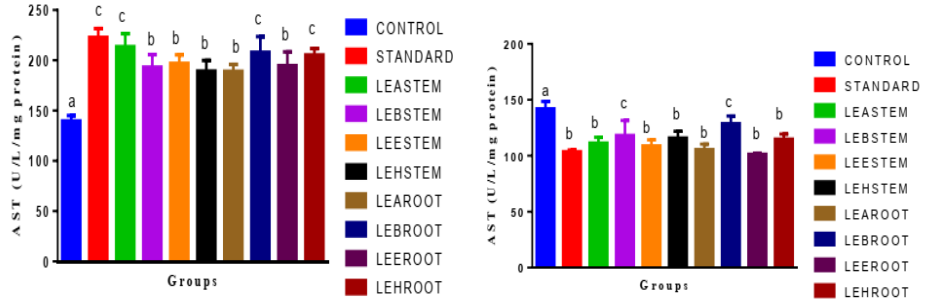
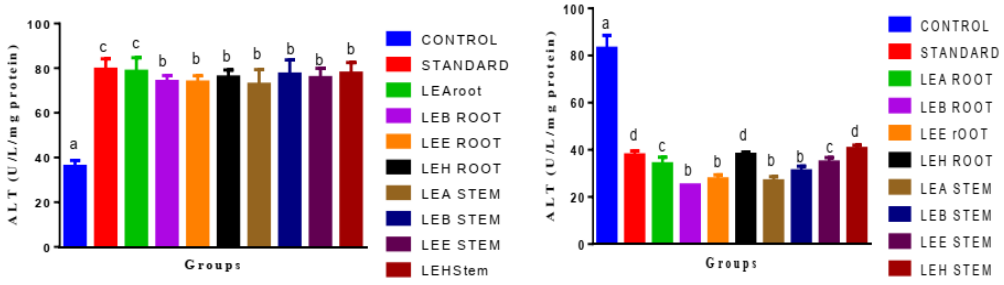


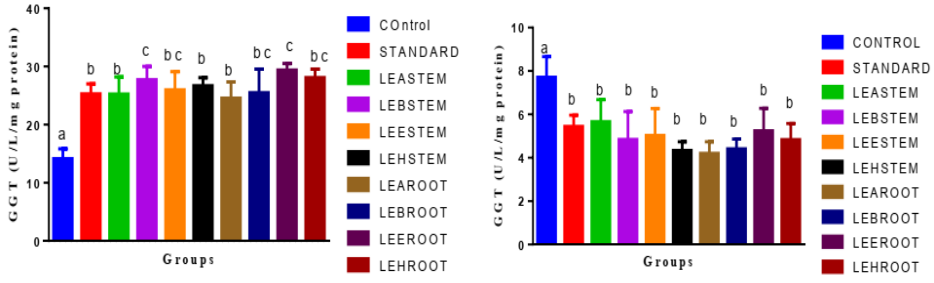
Figure 1: Activity of AST in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats +

12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Liver Serum

Figure 2: Activity of ALT in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Liver Serum

Figure 3: Activity of GGT in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$).

LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.

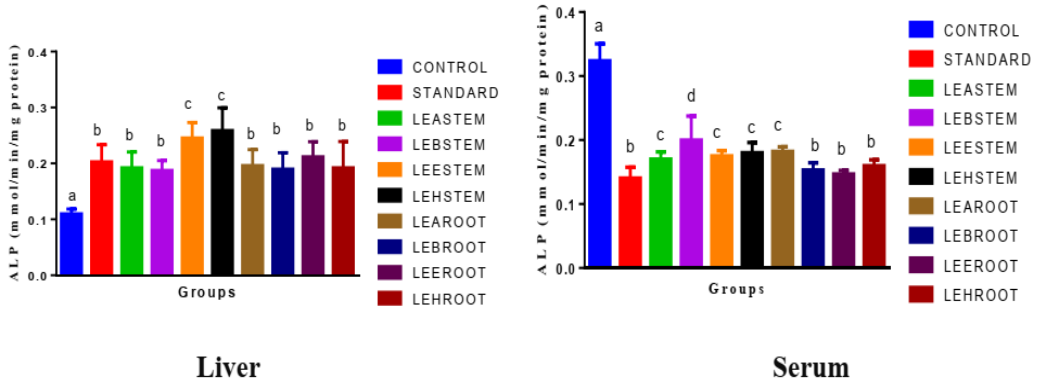
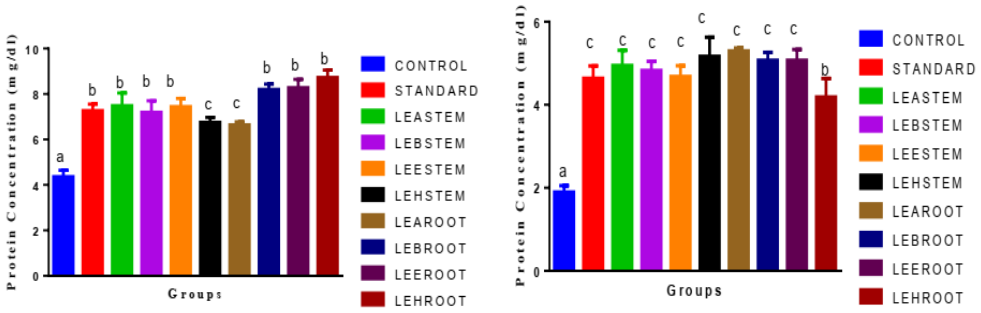
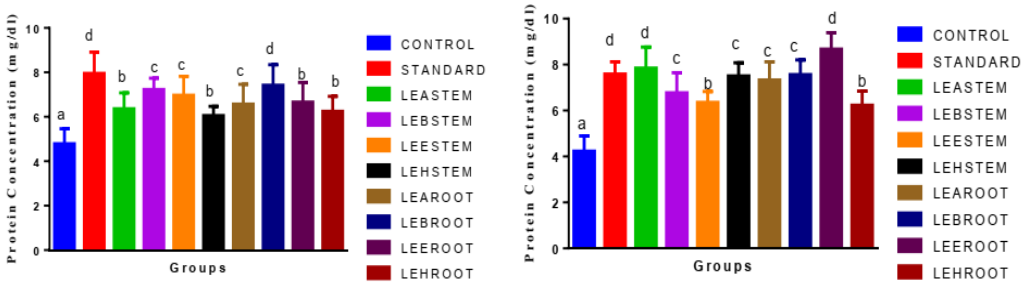


Figure 4: Activity of ALP in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Liver Serum

Figure 5: Concentration of protein in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Kidney Heart

Figure 6: Concentration of protein kidney and heart of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$).

LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.

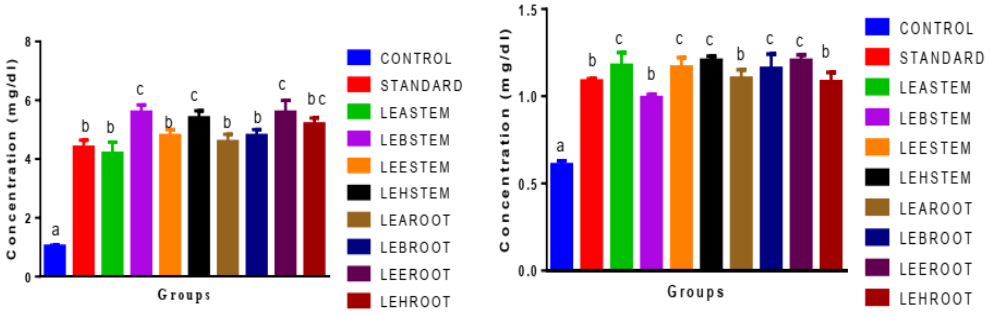
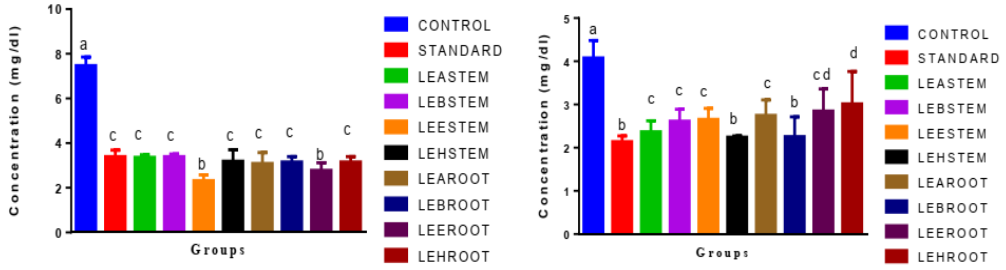
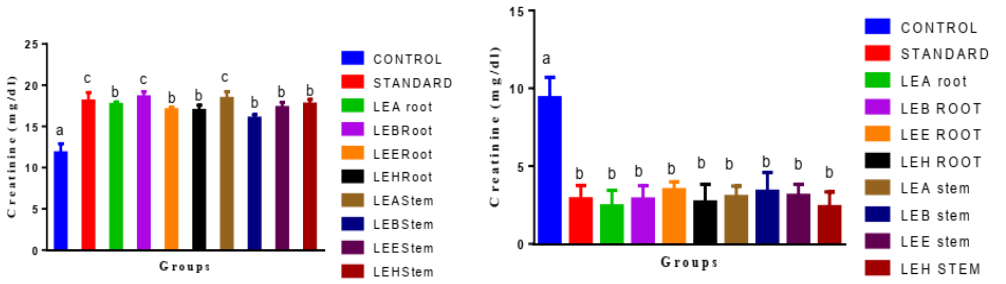


Figure 7: Concentration of albumin in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Liver Serum

Figure 8: Concentration of total bilirubin in liver and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.

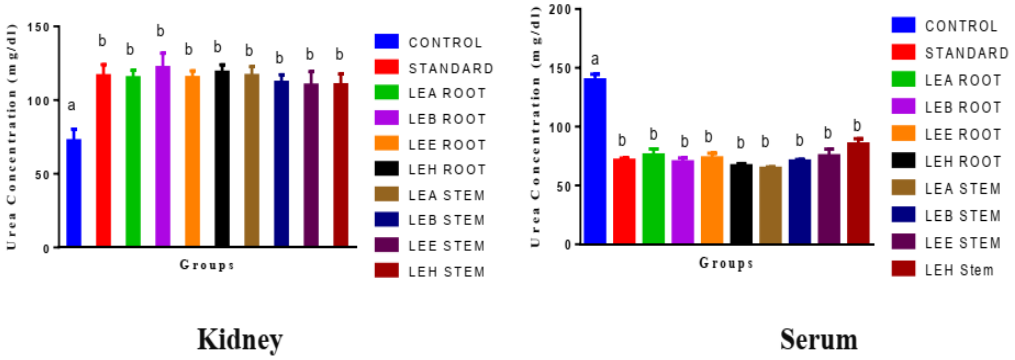


Kidney Serum

Figure 9: Concentration of creatinine in kidney and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$).

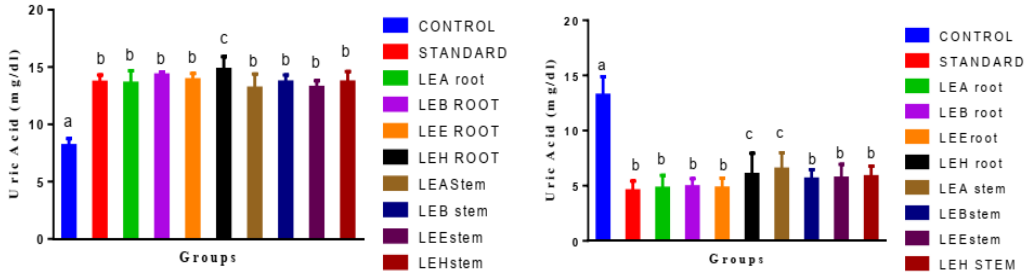
LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of

Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Kidney **Serum**

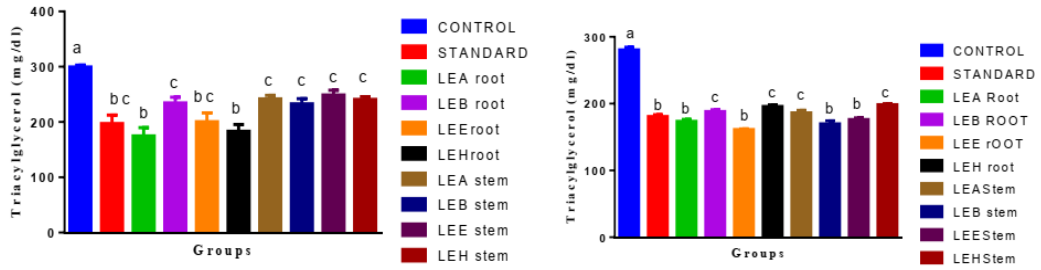
Figure 10: Concentration of urea kidney and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$). LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Kidney **Serum**

Figure 11: Concentration of uric acid in kidney and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different (p<0.05).

LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.



Heart **Serum**

Figure 12: Concentration of triacylglycerol in heart and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different (p<0.05).

LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.

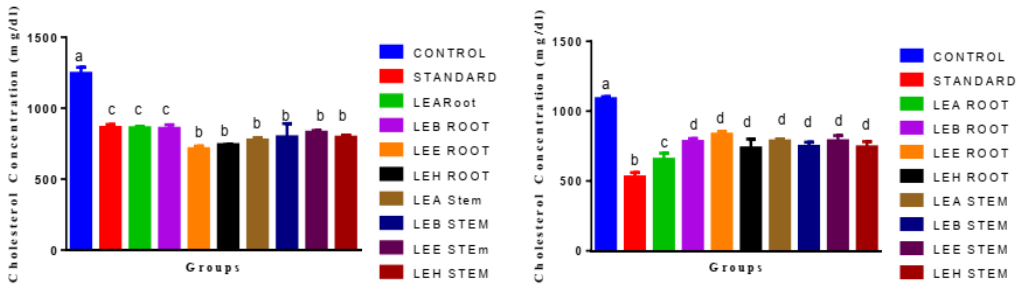


Figure 13: Concentration of cholesterol in heart and serum of ethidium bromide treated rats administered with Lycopersicon esculentum stem and root fractions. Values are shown as mean ± SEM (n = 5). Bars with different alphabets are significantly different ($p<0.05$).

LEA-stem: ethidium-treated rats + 12.5 mg/kg aqueous stem fraction of Lycopersicon esculentum, LEB-stem: ethidium-treated rats + 12.5 mg/kg butanol stem fraction of Lycopersicon esculentum, LEE-stem: ethidium-treated rats + 12.5 mg/kg ethyl acetate stem fraction of Lycopersicon esculentum, LEH-stem: ethidium-treated rats + 12.5 mg/kg hexane stem fraction of Lycopersicon esculentum. LEA-root: ethidium-treated rats + 12.5 mg/kg aqueous root fraction of Lycopersicon esculentum, LEB-root: ethidium-treated rats + 12.5 mg/kg butanol root fraction of Lycopersicon esculentum, LEE-root: ethidium-treated rats + 12.5 mg/kg ethyl acetate root fraction of Lycopersicon esculentum, LEH-root: ethidium-treated rats + 12.5 mg/kg hexane root fraction of Lycopersicon esculentum.