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Research Article

IN-VIVO CHARACTERIZATION OF TOTAL PROTEIN, ALBUMIN CONTENT, LIPID PROFILE AND ENZYMATIC PROPERTY OF *BALAJIRAKADI KVATHA CURNA* (BLJ) IN ALBINO RAT PLASMA

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ABSTRACT

The study was devised to evaluate the effect of total protein, albumin content, enzymatic property and lipid profile in rats' plasma after chronic administration of *Balajirakadi Kvatha Curna* (BLJ), a classical Ayurvedic preparation that is widely used in cough. The drug was administered per oral route at a dose of 40 ml/kg of the body weight for 45 consecutive days. Eight-week old albino rats (*Rattus novergicus* : Sprague - Dawley strain,) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University were used for the experiment. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. After the administration of BLJ preparation for a period of 45 days, the following biochemical parameters (protein, albumin, triglyceride, cholesterol, LDL, VLDL, HDL, sGPT sGOT and ALP) in the plasma of both the male and female rats were determined. An increased level of Total protein, the Albumin content and triglyceride in the plasma found in the both male and female rats, none of these changes were significantly different from their corresponding control values but noticeable. On the contrary in both female and male rats the decreased level in the total Cholesterol, VLDL, LDL and HDL was noticed and among which Total Cholesterol and VLDL are significant. Surprisingly the LDL content was almost similar to the corresponding control value and decrease in HDL was not significant. A statistically very highly significant increase in the sGPT sGOT and ALP activities in the plasma of male rats was found while in the female rats it has been showed a statistically very highly significant decrease in sGPT and sGOT but ALP activities in the plasma was statistically insignificant.

KEY WORDS: Ayurvedic, Lipid profile, Triglycerides, chronic, Albino.

INTRODUCTION

Ayurvedic medicine (also called Ayurveda) is one of the world's oldest medical systems. It originated in India and has evolved there over thousands of years. In Bangladesh, Ayurvedic medicine is considered complementary and alternative medicine (CAM) more specifically, a CAM whole medical system. Many therapies used in Ayurvedic medicine are also used on their own as CAM—for example, herbs, massage,

and specialized diets. Ayurvedic medicines are multi-components mixture containing plants, animal derived products, minerals and metals[1-4]. By using Ayurvedic medicine, expensive and extensive procedures of clinical investigations can be avoided in many cases and people in many areas have the choice to get treatment at a cheaper price depending on their choice. Considering the widespread use of Ayurveda as the popular form of treatment in Bangladesh, one cannot emphasize enough the need for establishing the safety profiles of Ayurvedic drugs[5-7]**.** Large numbers of modern medicinal agents have been sourcing from the nature in the past[8]**.** Natural plants and their constituents are giving the primary ideas about the treatment of new diseases. Hence, natural medicine like Ayurvedic drugs still remain a popular practice in the subcontinent including India, Sri Lanka and other countries like Bangladesh[9-10]**.** Ayurvedic medicines have a wide access to the large number of population in these countries. The acceptance of these medicines increased due to integrative approach for the prevention and treatment of disease through natural remedies. Traditional people are getting the benefits of this practice from ancient times. But, the uses and the safety profile of all of the Ayurvedic medicines are not ensured scientifically [11-12]**.**

Balajirakadi Kvatha Curna is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 which was approved by the Government of Bangladesh via Ministry of Health and Family Welfare. This drug is widely used in cough by the relatively economic people in our community to avoid extra expense[13-15]. The objective is to have a better understanding of the possible toxicological profile of the drug under study and, to some degree, to decide how justifiable the use of this drug is under the stated circumstances. This study was performed in an effort to evaluate the safety of this drug according to modern toxicological parameters. The present study is the continuation of the effort in which the toxicological effect of BLJ on total protein, albumin content, lipid profile and enzymatic property of rats' plasma after chronic administration.

MATERIALS AND METHODS

Chemicals and Reagents

For the evaluation of the toxicological effect of BLJ on total protein, albumin content, lipid profile and enzymatic property of rats' plasma after chronic administration, various chemicals and reagents were used. All chemicals and reagents were of analytical grade and these were collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong. These chemicals and reagents were prepared with glass-distilled water.

Preparation of drug

For the toxicological study *Balajirakadi Kvatha Curna* (BLJ) was collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong. The extract (known as kwath) was prepared from dried powder according to the procedure mentioned in Bangladesh National Ayurvedic Formulary (BNAF), 1992. The *Kwath* was prepared by adding 160ml of distilled water with 5gm of the powder and it was thoroughly mixed to make a uniform suspension, it was then boiled till the volume was reduced to 40ml and was finally filtered. This filtrate was collection I. Then residue was again boiled with 160ml of water till the volume was reduced to 40ml and was then filtered. This filtrate was collection II. The two filtrates (collection I and II) were mixed and reduced to 20ml and this mixture was known as *Kwath* and was used for the toxicological study. For the toxicological experiment, the *KWATH* was administered at a volume such that it would permit optimal dosage accuracy.

Formulary of *Balajirakadi Kvatha Curna* (BLJ)

For the preparatoon of *Balajirakadi Kvatha Curna* (BLJ), ingredients are taken as per table no -1 with their classified family and botanical name. The parts and amont used are listed in the table no-1.

Table 1: Formulary of *Balajirakadi Kvatha Curna* (BLJ)

Ayurvedic/ Traditional Name	Parts Used	Botanical Name	Family	Amount used
Bala	Root	*Sida Cordifolia*	Malvaceae	1 Part
Jiraka	Fruit	*Cuminum cyminum*	Apiaceae	1 Part
Bilva	Root	*Aegle mermelos*	Rutaceae	1 Part
Abda	Rhyzome	*Cyperus rotundus*	Cyperaceae	1 Part
Vrsa	Root	*Adhatoda vasaka*	Acanthaceae	1 Part
Visva (sunthi)	Rhyzome	*Zingiber officinalis*	Zingiberaceae	1 Part
Suradruma (devadaru)	Heart Wood	*Cedrus deodara*	Pinaceae	1 Part
Guha (salaparni)	Pulp	*Pseudarthria viscida*	Fabaceae	1 Part
Iksu	Root	*Saccharum officianarum*	Poaceae	1 Part
Laja	Stem	*Lathyrus japonicas*	Fabaceae	1 Part

Route of Administration

For the toxicological studies, the drug was administered per oral route at a dose of 40 ml/kg of the body weight. [Per oral (p.o.) route]. Ketamine were administered intra-peritoneal (500 mg/kg i.p.).

Management of Experimental Animal

Eight-week old albino rats (*Rattus novergicus* : Sprague - Dawley strain,) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University were used in the toxicological experiment. These animals were apparently healthy and weighed 50 - 70 g.

Animal Care

The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular rat prior to and after the administration could be noted separately.

Controls

A group of equal number of rat as the drug treated group was simultaneously employed in the experiment. They were administered with distilled water as placebo as per the same volume as the drug treated group for the same number of days and this group served as the control. Prior to the experiment, they were randomly divided into 4 groups of 10 animals according to sex. Thus ten rats were taken for each group for both control and the experimental group.

Toxicological experiment

After acclimatization, administration of the Ayurvedic medicinal preparation was done by intra-gastric syringe. Administration of the extract was between the hours of 10 am and noon. At the due of the 45-days treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration, the animals were anaesthetized using i.p. Ketamine (500 mg/kg i.p.). Blood samples were collected from post vena cava and transferred into heparinised tubes immediately. Blood was then centrifuged at 4,000 *g* for 10 min using bench top centrifuge (MSE Minor, England) to remove

red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection. All other reagents and chemicals that were used in this work were of analytical grade and were prepared in all glass-distilled water.

Statistical Analysis

The group data are expressed as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001. **Note:** denoted accordingly as *p<0.05, **p<0.01, ***p<0.001[16] (*,**, and *** mean noticeable, high and very high significant respectively)

Test procedure for Total Protein

Only one blank is required for a series of tests. For blank 1000µl Total Protein reagents**,** for standard 1000µl Total Protein reagents and 20µl Protein standard and for test sample 1000µl Total Protein reagents with 20µl Serum/Plasma were mixed well and then incubated at incubate at 20°C to 25°C for 10 minutes. Finally, read the result with Analyzer/Colorimeter/Spectrophotometer against Reagent Blank

Test procedure for Albumin

Only one blank is required for a series of tests. For blank 1000µl Albumin reagents**,** for standard 1000µl Albumin reagents and 10µl Albumin standard and for test sample 1000µl Albumin reagents with 10µl Serum/Plasma were mixed well and then incubated at incubate at 20°C to 25°C for 5 minutes. Finally, read the result with Analyzer/Colorimeter/Spectrophotometer against Reagent Blank.

Test procedure for Triglycerides

Only one blank is required for a series of tests. For blank 1000µl TG (Triglycerides) reagents**,** for standard 1000µl TG reagents and 10µl TG standard and for test sample 1000µl TG reagents with 10µl Serum/Plasma were mixed well and then incubated at incubate at 37°C for 5 minutes or room temperature for 10-15 minutes. Finally, read the result with Analyzer/ Colorimeter/ Spectrophotometer against Reagent Blank.

Test Procedure Total Cholesterol

Only one blank is required for a series of tests. For blank 1000µl Cholesterol reagents**,** for standard 1000µl Cholesterol reagents and 10µl Cholesterol standard and for test sample 1000µl Cholesterol reagents with 10µl Serum/Plasma were mixed well and then incubated at incubate at 37°C for 5 minutes or room temperature for 10-15 minutes. Finally, read the result with Analyzer/ Colorimeter/ Spectrophotometer against Reagent Blank.

Test Procedure Total HDL-Cholesterol

This procedure includes two steps- Precipitation step involves Precipitate reagent (PREC) and total cholesterol determination. For macro, 1000µl Precipitate Reagents and 500µl of serum are mixed and Incubated for 10 minutes at room temp. In semi micro 500µl Diluted PREC and 200µl of serum are mixed and Incubated for 10 minutes at room temp. Finally they were centrifuged for 10 minutes at 4000 g. After Centrifugation, Supernatant was collected

For total cholesterol determination only one blank is required for a series of tests. For blank 1000µl Cholesterol reagents, for standard 1000µl Total Cholesterol reagents and 10µl Cholesterol standard and for test sample 1000µl Cholesterol reagents with 10µl Supernatant were mixed well and then incubated at incubate at 37°C for 5 minutes or room temperature for 10-15 minutes. Finally, read the result with Analyzer/ Colorimeter/ Spectrophotometer against Reagent Blank.

Test procedure for GPT

For sample preparation 1000 µl of Working Reagents and 100 µl of Sample were mixed and recorded immediately after 1 minute and at the same time the stop watch started. Record the absorbance again exactly after 1, 2 and 3 minute. Finally, read the result with analyzer/Colorimeter/Spectrophotometer against Reagent Blank.

Test procedure for GOT

For sample preparation 1000 µl of Working Reagents and 100 µl of Sample were mixed and recorded immediately after 1 minute and at the same time the stop watch started. Record the absorbance again exactly after 1, 2 and 3 minute. Finally, read the result with analyzer/ Colorimeter/ Spectrophotometer against Reagent Blank.

Test procedure for ALP

For sample preparation 1000 µl of Working Reagents and 20 µl of sample were mixed and recorded immediately after 1 minute and at the same time the stop watch started. Record the absorbance again exactly after 1, 2 and 3 minute. Finally, read the result with analyzer/Colorimeter/Spectrophotometer against Reagent Blank.

RESULT AND DISCUSSION

Balajirakadi Kvatha Curna (BLJ), a classical Ayurvedic preparation which is used in cough, was studied for its toxicological aspects after chronic administrations for 45 consecutive days.

Serum protein/ Albumin (Male and Female)

In the male rats there was increase in both the Total protein and the Albumin content in the plasma. None of these changes were significantly different from their corresponding control values. In the female rats there was increase in both the Total protein and the Albumin content in the plasma. None of these changes were significantly different from their corresponding control values. On the other hand, In the female rats there was increase in both the Total protein and the Albumin content in the plasma. None of these changes were significantly different from their corresponding control values. The result was showed in Table no.2 and Figure no. 1& 2.

Table 2: Serum protein/ Albumin (Male and Female)

Control Male		***Balajirakadi Kvatha Curna* (BLJ) Male**
Total protein	4237.245 ± 67.3245	4288.2074 ± 61.3636(% incr.) (p=0.883)
Albumin	3451.7416 ± 85.3068	3495.0376 ± 65.4994(% incr.) (p=0.916)
Control Female		***Balajirakadi Kvatha Curna* (BLJ) Female**
Total protein	4608.3282 ± 113.2184	4666.0452 ± 100.9946(% incr.) (p=0.915)
Albumin	3605.9857 ± 65.7737	3674.5754 ± 68.4848(% incr.) (p=0.812)***

Lipid profile (Male and Female)

Cholesterol and triglyceride tests are blood tests that measure the total amount of fatty substances (cholesterol and triglycerides) in the blood.

Cholesterol travels through the blood attached to a protein. This cholesterol-protein package is called a lipoprotein. Lipoprotein analysis (lipoprotein profile or lipid profile) measures blood levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. A high LDL cholesterol level may increase your chances of developing heart disease. A high VLDL cholesterol level can cause the buildup of cholesterol in your arteries and increases your risk of heart disease and stroke. Having a high triglyceride level along with a high LDL cholesterol may increase your chances of having heart disease more than having only a high LDL cholesterol level[17-18].

In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis, and, by extension, the risk of heart disease and stroke. However, the relative negative impact of raised levels of triglycerides compared to

that of LDL:HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level. Another disease caused by high triglycerides is pancreatitis.

In this investigation an increase of plasma triglyceride level in the BLJ treated male rats was observed which was not statistically significant but was noticeable (p=0.066) in comparison to control. On the contrary there was decrease in the total Cholesterol, VLDL, LDL and HDL. Highly significant (p=0.004) decrease was observed with Total Cholesterol and a significant (p=0.027) decrease was observed with VLDL. Non significant decrease was observed with LDL and HDL In case of HDL though the decrease was not statistically significant but it was noticeable (p=0.066). In the female rats there was an increase in the Triglycerides content in the plasma which was not statistically significant. On the contrary there was decrease in the total Cholesterol, VLDL and HDL. The decrease in total Cholesterol was statistically significant, in case of VLDL content, the decrease was statistically highly significant. Surprisingly the LDL content was almost similar to the corresponding control value. It would be interestingly to note that the decrease in HDL was not significant. The result was showed in Table no.3 and Figure no. 3& 4.

Table 3: Lipid profile (Male and Female)

Control Male		***Balajirakadi Kvatha Curna* (BLJ) Male**
Triglycerides	85.4456 ± 1.4729	94.9688 ± 1.5324 (% incr.) (p=0.066)
Total cholesterol	61.8788 ± 1.4292	54.4066 ± 1.4775 (% decr.) (p=0.004)**
VLDL	13.7576 ± 0.5752	12.0829 ± 0.3502 (% decr.) (p=0.027)*
LDL	16.4315 ± 0.6355	16.0175 ± 0.5875 (% decr.) (p=0.792)
HDL	28.2076 ± 0.7572	25.0204 ± 0.9668 (% decr.) (p=0.068)
Control Female		***Balajirakadi Kvatha Curna* (BLJ) Female**
Triglycerides	77.4925 ± 2.5716	82.9924 ± 2.4914 (% incr.) (p=0.239)
Total cholesterol	59.1491 ± 1.2942	54.4805 ± 1.5616 (% decr.) (p=0.042)*
VLDL	13.8558 ± 0.4353	11.9178 ± 0.4923 (% decr.) (p=0.002)**
LDL	14.9734 ± 0.5224	15.0676 ± 0.5356 (% incr.) (p=0.819)
HDL	25.8685 ± 0.7505	23.4975 ± 0.7983 (% decr.) (p=0.181)

Enzymatic activity

In medicine, the presence of elevated transaminases, commonly the transaminases alanine transaminase (ALT) and aspartate transaminase (AST), may be an indicator of liver damage.[19] These levels previously were called the serum glutamate-pyruvate transaminase (SGPT) and the serum glutamate-oxaloacetate transaminase (SGOT). Elevated levels are quite sensitive for liver injury, meaning that they are likely to be present if there is injury. However, they may also be elevated in other conditions. ALT is not commonly found outside the liver; AST too is most commonly found in the liver, but also in significant amounts in cardiac (heart) and skeletal muscle. In fact, measurement of these used to be part of diagnosing heart attacks, although newer enzymes and proteins that are more specific for cardiac damage have largely replaced this usage. In general, any damage to the liver will cause medium elevations in these transaminases (usually called liver enzymes, though of course they are not the only enzymes in the liver)[20-21].

A statistically very highly significant increase in the sGPT, sGOT and ALP activities in the plasma was found in male rats. The female rats showed a statistically very highly significant decrease in the sGPT

and sGOT activities. In case of ALP activities in the plasma, a statistically insignificant decrease was noted in this rat group. The result was showed in Table no.4 and Figure no. 5& 6.

Table 4: Plasma enzymatic activity through sGPT, sGOT, ALP

Control Male		***Balajirakadi Kvatha Curna* (BLJ) Male**
sGPT	51.3336 ± 0.1096	65.6072 ± 0.3098 (% incr.) (p=0.001)***
sGOT	88.4264 ± 0.2426	101.6286 ± 1.1788 (% incr.) (p=0.001)***
ALP	36.2738 ± 0.0883	46.6092 ± 0.4054 (% incr.) (p=0.001)***
Control Female		***Balajirakadi Kvatha Curna* (BLJ) Female**
sGPT	41.3264 ± 0.1078	39.9004 ± 0.06215 (% decr.) (p=0.001)***
sGOT	70.2885 ± 0.1625	68.3105 ± 0.1505 (% decr.) (p=0.001)***
ALP	28.7365 ± 0.07946	28.6516 ± 0.09427(% decr.) (p=0.629)

CONCLUSION

Balajirakadi Kvatha Curna (BLJ), a classical Ayurvedic preparation which is used in cough by the rural and poor people in Bangladesh. By the characterization of toxicological study of *Balajirakadi Kvatha Curna* we get some significant result in both male and female rats in case of lipid profile (total cholesterol, VLDL and HDL) among the study of total protein, albumin content, lipid profile and enzymatic activity. In addition to this study it was observed that it causes few impacts on some parameters like total protein and albumin content. Although lipid profile with enzymatic activity in rats' plasma after chronic administration showed significant impacts. A statistically very highly significant increase in the sGPT sGOT and ALP activities in the plasma was found in male rats while in the female rats it has been showed a statistically very highly significant decrease in the sGPT and sGOT activities. But in case of ALP activities in the plasma, a statistically insignificant decrease was noted in this rat group. Although some recorded result is not statistically significant and noncongruent in case of both male and female rats (HDL content) which may trigger subtle further experiment to find out more valid and congruent result to claim freedom from discrepancy.

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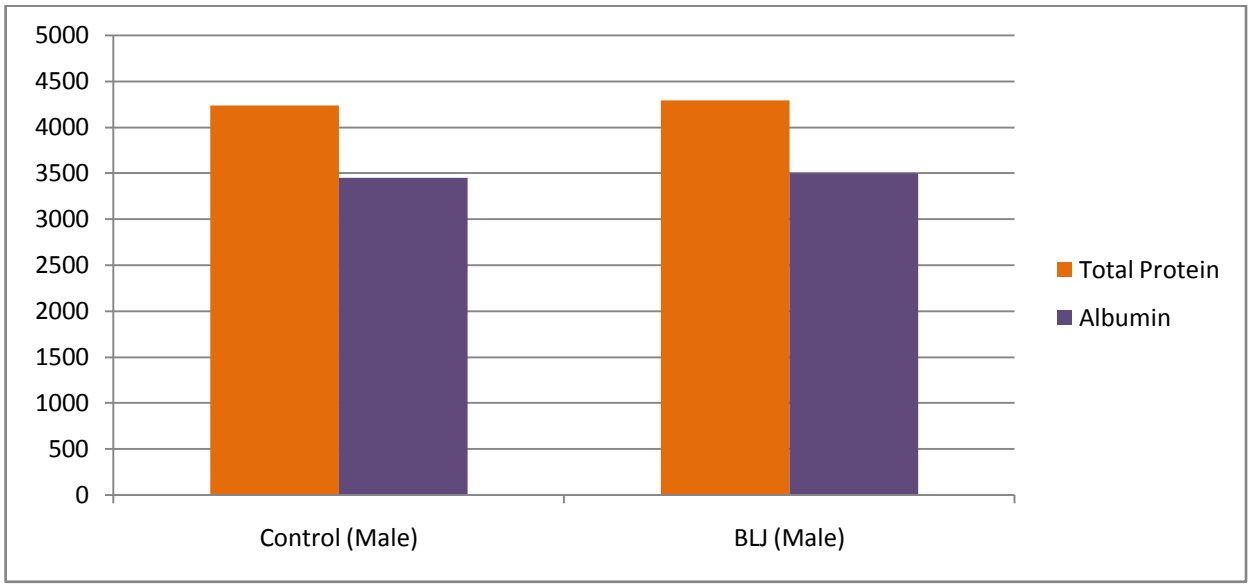

Figure 1: Comparative presentation of Effect of *Balajirakadi Kvatha Curna* (BLJ) on total serum protein and albumin between male control group and drug treated male rats.

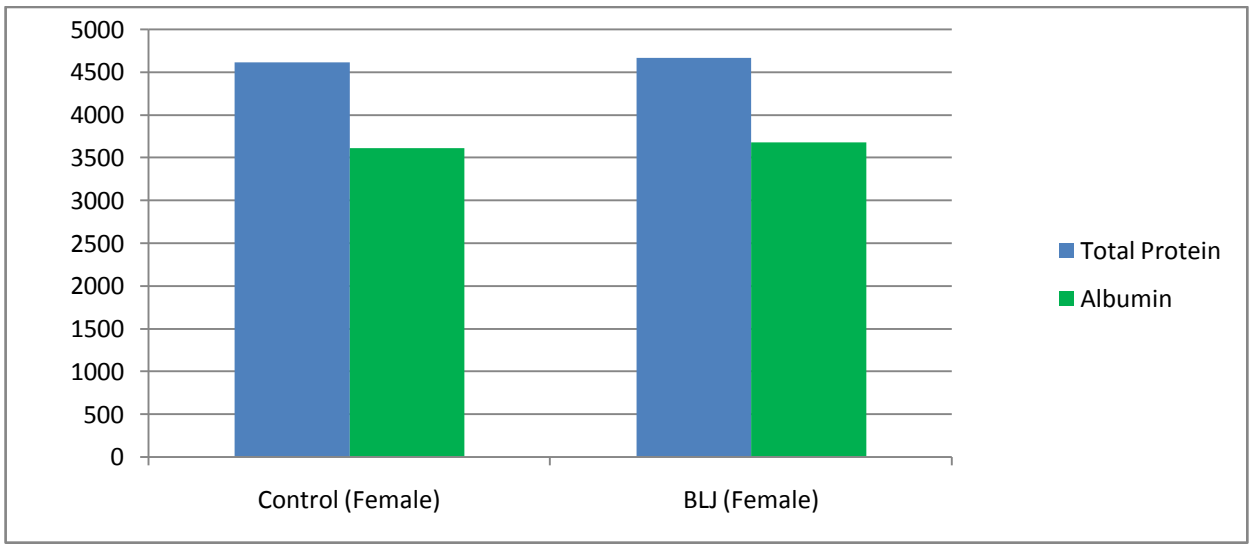

Figure 2: Comparative presentation of Effect of *Balajirakadi Kvatha Curna* (BLJ) on total serum protein and albumin between female control group and drug treated female rats.

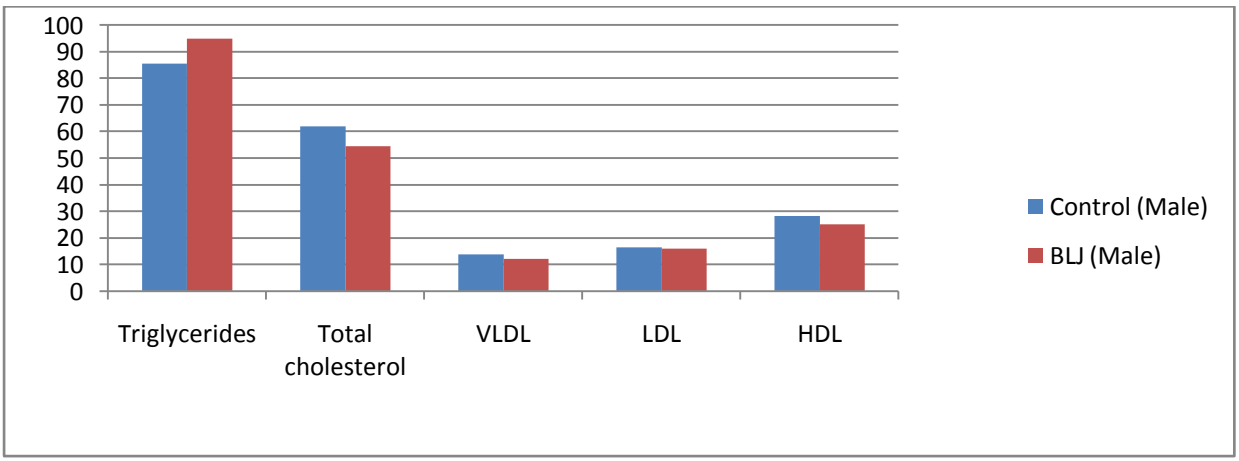

Figure 3: Comparative presentation of Effect of *Balajirakadi Kvatha Curna* (BLJ) on lipid profile (Triglycerides, Total cholesterol, VLDL=Very low density lipoprotein, LDL=Low density lipoprotein and HDL=High density lipoprotein) between male control group and drug treated male rats.

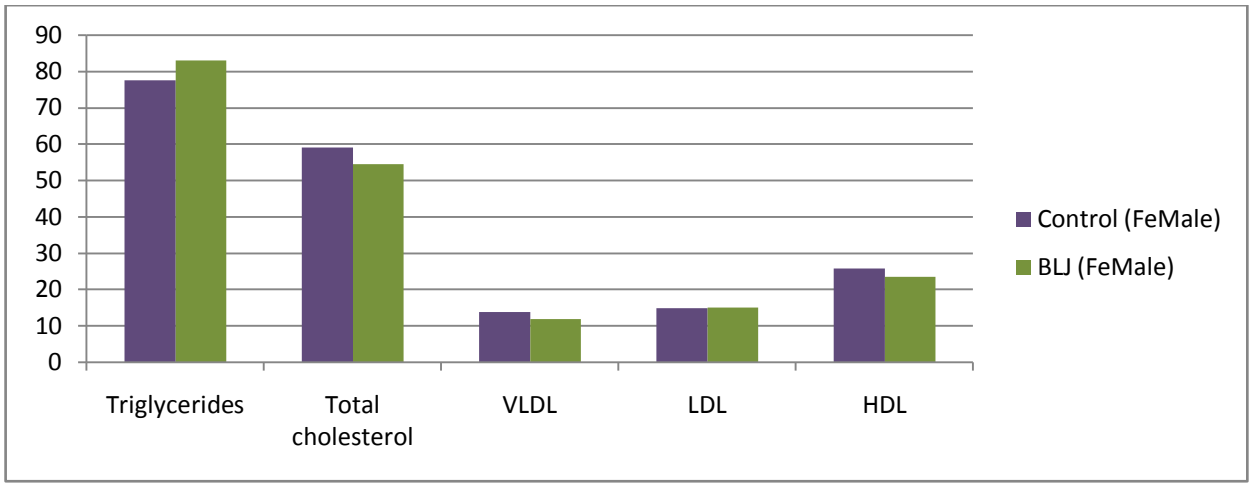

Figure 4: Comparative presentation of Effect of *Balajirakadi Kvatha Curna* (BLJ) on lipid profile (Triglycerides, Total cholesterol, VLDL=Very low density lipoprotein, LDL=Low density lipoprotein and HDL=High density lipoprotein) between female control group and drug treated female rats.

Figure 5: Comparative presentation of Effect of *Balajirakadi Kvatha Curna* (BLJ) on enzymatic property (sGPT, sGOT and ALP) between male control group and drug treated male rats.

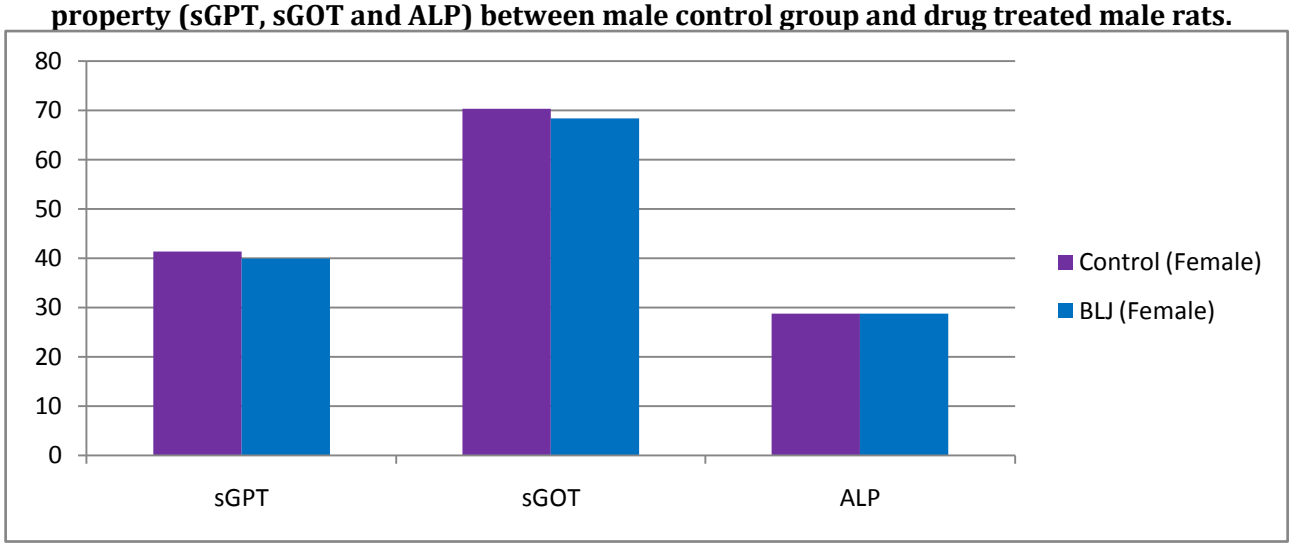

Figure 6: Comparative presentation of Effect of *Balajirakadi Kvatha Curna* (BLJ) on enzymatic property (sGPT, sGOT and ALP) between female control group and drug treated female rats.