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TOXICOLOGICAL CHRACTERIZATION OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON THE BASIS OF TOTAL PROTEIN AND ALBUMIN CONTENT, LIPID PROFILE, LIVER FUNCTION, KIDNEY FUNCTION, SERUM URIC ACID AND ENZYMATIC PROPERTY IN RATS' PLASMA AFTER CHRONIC ADMINISTRATION

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Key words:

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ABSTRACT: Brhanmanjisthadi Kvatha Curna (BRH) an Ayurvedic preparation, is used as a traditional medicine in filariasis in the northern part of the country. After 45 consecutive days of chronic administration of the BRH preparation in rats its toxicological aspects was studied. In the study the total protein content in the plasma was increased (2.80 %) in BRH treated male rats in comparison to their control male groups. The result showed no significant difference between the control and the BRH treated groups, yet the increase was noticeable (p=0.072). But the albumin content was significantly (p<0.001) increased (17.11 %) in BRH treated male rats & the increase was statistically very highly significant. In the female rats group the total protein (6.80 %) and the albumin content in the plasma were also increased in comparison to their control groups. A statistically very highly significant (p<0.001) increase was noted (15.09 %) only in the case of albumin. In this investigation, none of the changes in terms of the Lipid profile, namely Triglycerides (2.18 % decr.), total Cholesterol (0.78 % decr.), VLDL (3.77 % incr.), LDL (4.91 % decr.) and HDL (2.61 % decr.) were significantly different from their corresponding control values in male rats. Similarly, in female rats, no significant change regarding the Lipid profile (Triglycerides 2.18 % decr., total Cholesterol 1.22 % decr., VLDL 3.90 % incr., LDL 5.35 % decr. and HDL 2.61 % decr.) was found. In the male rats there was a statistically a very highly significant decrease (39.65 %) in the Bilirubin content in the plasma. In the female rats there was a statistically very highly significant increase (41.16 %) in the Bilirubin content in the plasma. In the male rats there was a decrease in both the Creatinine and the Urea content in the plasma. But a statistically a very highly significant (p=0.001) decrease (14.88 %) was noted only in the case of the Urea content in the plasma. The decrease (6.40 %) was observed non significant (p=0.152) with Creatinine. In the female rats there was a statistically very highly significant (p=0.001) decrease in both the Creatinine (25.97 %) and the Urea (16.35 %) content in the plasma. It was observed that about 6.18 % increase which is statistically significant (p<0.039) in plasma Uric acid content of BRH treated male rats in comparison to their control male rats whereas the female rats showed a comparatively insignificant (p=0.118) (3.83 %) increase in concentration of the Uric acid level. In this investigation, none of the changes in terms of the sGPT (0.10 % incr.), sGOT (0.33 % incr.) and ALP (0.58 % incr.) activities were significantly different from their corresponding control values in male rats. Similarly, in female rats, no significant change regarding the enzyme activities of sGPT (0.13 % incr.), sGOT (0.26 % decr.) and ALP (0.53 % incr.) were noted when compared to their corresponding control values in female rats.

INTRODUCTION: Alternative Medicine widely known as Ayurvedic Medicine had played note worthy role in the health care delivery system of the SAARC countries from the time immemorial.

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Government of Bangladesh in order to put into practice and institutionalize the Ayurvedic Medical System and also to support and extend the range of services in the District hospitals and Thana Health Complexes, has began the provision of Alternative Medicine in 30 selected District hospitals in 1998 under the 1998-2003 plan of HPSP (Health and Population Service Program).

World Bank has approved to bear the cost of procuring Ayurvedic Medicines for these District Hospitals. [1-3]

The research was carried out in order to characterize the chronic toxicological profile of the marketed Ayurvedic medicinal preparation, Brhanmanjisthadi Kvatha Curna (BRH) on Serum protein/albumin, Lipid profile, Liver function test, Kidney function test, Serum Uric acid and Serum enzymatic activity aspects.

Filarial parasites cause skin lesions in a condition known as filariasis or elephantiasis, and affect the lives of millions of people, especially those living in tropical countries. The spread of this disease is usually through mosquito bites. Symptoms involve a swelling and thickening of the skin and lymph glands, which can in some cases be considerable. Skin lesions usually persist for a long time. The acute infection usually causes nocturnal fever and local tenderness. Chronic lesions are usually painless.

It remains undetected for years and has flourished unchecked for many more. It is also known to cause unimaginable - and completely avoidable - pain and problems. Lymphatic Filariasis is a disease caused by parasitic worms, which can live, unnoticed in human bodies for over a decade. Over the years, Filariasis has placed at risk at least one billion people the world over - one out of every six people alive. [4-7]

The worst part is that many people do not even know that they have been infected until it is too late. Upon manifestation, Filariasis results in a grotesque swelling of limbs and that is the reason why the disease is also called Elephantiasis or the Malabar Leg.

The parasite has a life span ranging between four to six years and during this time, it releases millions of microfilariae into the blood. A person who has these microfilariae is called a 'silent' carrier, who plays host to the filarial worm. Mosquitoes transmit this disease from a silent carrier to another person. Although rarely fatal, Filariasis is debilitating and disfiguring and the patient has to endure considerable pain. While 25 million men suffer from genital disease (one of the possible outcomes), a majority of the 15 million, who have elephantiasis of the leg, are women. With approximately 120 million people in tropical and subtropical areas of the world affected by this disease, the World Health Assembly in 1997 adopted a resolution for the elimination of Lymphatic Filariasis as a global health problem.

Evidence indicates that herbal formulae may be helpful in filariasis. Using this set of treatments, a significant reduction in lymphoedema was observed.

By using Ayurvedic medicine costly and extensive procedures of clinical investigations can be avoided in many cases and people in these selected areas have the choice to get treatment at a cheaper price depending on their choice.

Bearing in mind the widespread use of Ayurveda as the popular form of TM in Bangladesh, one cannot emphasize enough the need for establishing the safety profiles of Ayurvedic drugs. Keeping in mind the present scenario, this research work on Ayurvedic formulation, Brhanmanjisthadi Kvatha Curna (BRH) explores a spectrum of its toxicological aspects utilizing experimental animals. The objective is to have a better understanding of the potential toxicological profile of the drug under study and, to some degree, to decide how justifiable the use of this drug for the indicated diseases or symptoms. The project will eventually result in supplementing and complementing the existing health care facilities and, in the long run, will ensure total coverage of the population in terms of public health.

MATERIALS AND METHODS:

Chemicals and Reagents:

For the evaluation of the toxicological effect of BRH 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, Brhanmanjisthadi Kvatha Curna (BRH) 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 Brhanmanjisthadi Kvatha Curna (BRH):

For the preparatoon of Brhanmanjisthadi Kvatha Curna (BRH), ingredients are taken as per **Table 1** with their classified family and botanical name. Which parts and what amont were used are listed in the table.

TABLE 1: FORMULARY OF BRHANMANJISTHADI KVATHA CURNA (BRH)

Ayurvedic/ Traditional Name	Parts Used	Botanical Name	Family	Amount used
Manjistha	Stem	*Rubia Cordifolia*	Rubiaceae	1 Part
Musta	Rhyzome	*Cyperus rotundus Linn.*	Cyperaceae	1 Part
Kutaja	Stem,bark	*Wrightia antidysenterica*	Apocynaceae	1 Part
Guduci	Stem	*Tinospora cordifolia*	Menispermaceae	1 Part
Kustha	Root	*Saussuria lappa*	Asteraceae	1 Part
Nagara (sunthi)	Rhyzome	*Zingiber officinalis*	Zingiberaceae	1 Part
Bharngi	Root	*Clerodendrum serratum*	Lamiaceae	1 Part
Ksudra (kantakari)	Pulp	*Solanum xanthocarpum*	Solanaceae	1 Part
Vaca	Rhyzome	*Acorus calamus*	Acoraceae	1 Part
Nimba	Stem,bark	*Azadirachta indica*	Meliaceae	1 Part
Haridra	Rhyzome	*Curcuma longa*	Zingiberaceae	1 Part
Daruharidra	Stem	*Berberis aristata*	Berberidaceae	1 Part
Haritaki	Fruit	*Terminalia Chebula*	Combretaceae	1 Part
Bibhitaka	Fruit	*Terminalia belerica*	Combretaceae	1 Part
Amalaki	Fruit	*Emblica Officinalis*	Euphorbiaceae	1 Part
Patola	Leaf	*Trichosanthes dioica*	Cucurbitaceae	1 Part
Katuki	Rhyzome	*Luffa amara*	Cucurbitaceae	1 Part
Murva	Root	*Marsedenia tenacissima*	Asclepiadaceae	1 Part
Vidanga	Fruit	*Embelia ribes*	Myrsinaceae	1 Part
Asana	Heart wood	*Pterocarpus marsupium*	Papilionaceae	1 Part
Citraka	Root	*Plumbago indica*	Plumbaginaceae	1 Part
Satavari	Root	*Asparagus racemosus*	Liliaceae	1 Part
Trayamana	Pulp	*Gentiana kurroo*	Gentianaceae	1 Part
Krsna (pippali)	Fruit	*Piper longum*	Piperaceae	1 Part
Indrayava	Seed	*Holorrhena antidysenterica*	Apocynaceae	1 Part
Vasaka	Root	*Adhatoda vasica*	Bignoniaceae	1 Part
Bhrnga raja	Pulp	*Eclipta prostrata*	Asteraceae	1 Part
Maha daru (deva daru)	Heart wood	*Cedrus deodara*	Pinaceae	1 Part
Patha	Root	*Cyclea peltata*	Menispermaceae	1 Part
Khadira	Heart wood	*Acacia catechu*	Mimosoideae	1 Part
Candana	Heart wood	*Santalum album*	Santalaceae	1 Part
Trivrt	Root	*Ipomoea turpethum*	Convolvulaceae	1 Part
Varuna	Stem, Bark	*Crataeva nurvala*	Capparidaceae	1 Part
Kairata (kiratatikta)	Pulp	*Swertia chirata*	Gentianaceae	1 Part
Bakuci	Seed	*Psoralea corylifolia*	Papilionaceae	1 Part
Krtamalaka (aragvadha)	Fruit	*Cassia fistula*	Fabaceae	1 Part
Sakhotaka	Stem, Bark	*Streblus asper*	Moraceae	1 Part
Mahanimba	Stem, Bark	*Ailantus malabarica*	Xanthoxylaceae	1 Part
Karanja	Stem, Bark	*Pongamia glabra*	Papilionaceae	1 Part
Ativisa	Root	*Aconitum heterophyllum*	Ranunculaceae	1 Part
Jala (hrivera)	Root	*Coleus vettiveroides*	Labiatae	1 Part
Indra varunika	Root	*Cucumis trigonus*	Cucurbitaceae	1 Part

Ananta (ananta mula)	Root	*Hemidesmus indicus*	Apocynaceae	1 Part
Sariva	Root	*Cordia wallichii*	Boraginaceae	1 Part
Parpata	Pulp	*Fumaria indica*	Fumariaceae	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-peritoneally (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. [8]

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 duke 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.

Determination of total protein, albumin content, lipid profile, liver and kidney fuction, serum uric acid and enzymatic property:
Biochemical analysis was carried out on serum, to assess the state of the lipid profile, liver and kidney, serum uric acid and enzymatic property. Biochemical studies involved analysis of parameters such as total protein, serum albumin, blood urea nitrogen (BUN), bilirubin (total and direct), creatinine, and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).Total protein content of the samples was assayed by the Biuret method (Plummer, 1978). Serum albumin concentration was determined using the method of Doumas et al (1971).

Triglycerides and total cholesterol concentration as well as protein content were evaluated using assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). Serum total cholesterol and high-density lipoprotein cholesterol were determined using Randox Laboratory kit reagents. Serum triacylglycerol level was estimated using Randox Laboratory test kit and VLDL-cholesterol was

calculated using the formula TG/2.2 mmol/l. Low density lipoprotein (LDL) cholesterol was determined by differential substraction of the sum of the cholesterol fractions from the total cholesterol. The method of Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples. The procedure of Tietz et al (1994) was used to determine serum creatinine concentration while the serum urea concentration was determined by the method of Kaplan (1965). Alkaline phosphatase activities were determined using the method as described by King and King (1954). The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC.).[9-17]

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[18]

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.[19]

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.[20-21]

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.[22]

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 Total Bilirubin:
For sample blank 1000µl blank reagents , for tests 500µl DCA and 500µl NIT are mixed and allowed to Wait at least 15 minutes at room temperature

protected from light. Then 100µl of Serum / Plasma for sample Blank and 100µl of Serum/Plasma for test sample were mixed well and then incubated at 20°C to 25°C for 10 minutes. Finally, read the result with Analyzer / Colorimeter / Spectrophotometer against Reagent Blank.

Test Procedure for Creatinine:
For standard 500µl of Picric acid and diluted NaOH 500µl, for test sample 500µl of Picric acid and diluted NaOH 500µl were mixed well and allowed to wait for 5 minutes at 37°C . 100µl of standard and 100µl of Serum/Plasma were mixed well and read immediately after 30 sec, recorded the absorbance, A_1. Then read and recorded the absorbance A_2 exactly after 2 min. Finally, read the result with Analyzer / Colorimeter / Spectrophotometer against Reagent Blank.

Test Procedure for Urea:
Only one blank is required for a series of tests. Enzyme Reagent 1a is the Enzyme Concentrating 1ml + 100ml Reagent-1 But, For one test Enzyme concentrating 10 µl + 1 ml Reagent -1(1 ml = 1000 µl). For blank 1000µl Enzyme Reagent - 1a (Above Proportionate to enzyme + Reagent - 1) and for standard 1000µl of Enzyme Reagent - 1a with 10µl of standard reagent were mixed and incubated for 5 minutes at Room temperature or 3 minutes at 37° C. For sample 1000 µl Enzyme Reagent - 1a and 10 µl of Sample(Serum) were mixed and and incubated for 5 minutes at Room temperature or 3 minutes at 37° C. Then 1000µl (1ml) of reagent -2 was mixed and incubate for 10 minutes at room temperature and 5 minutes at 37°C for each blank, standard and sample. Finally, read the result with analyzer / Colorimeter / Spectrophotometer against Reagent Blank (Normal Range: 10 - 50 mg/dl).

Test Procedure for Uric Acid:
Only one blank is required for a series of tests. For blank 1000µl total protein reagents, for test sample 1000µl of total protein reagents and 20µl of Serum/Plasma and for standard 1000µl total protein reagents with 20µl of protein standard are mixed and incubated at 20°C to 25°C for 10 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. [23]

RESULT AND DISCUSSION:

Brhanmanjisthadi Kvatha Curna (BRH) an Ayurvedic preparation, is used as a traditional medicine in filariasis in the northern part of the country., was studied for its toxicological aspects after chronic administrations for 45 consecutive days.

Serum protein/ Albumin (Male and Female):
After 45 consecutive days of chronic administration of the BRH preparation, the total protein and albumin content in plasma were determined in both male and female rats group.

In the study the total protein content in the plasma was increased (2.80 %) in BRH treated male rats in comparison to their control male groups. The result showed no significant difference between the control and the BRH treated groups, yet the increase was noticeable ($p=0.072$). But the albumin content was significantly ($p<0.001$) increased (17.11 %) in BRH treated male rats & the increase was statistically very highly significant. In the female rats group the total

protein (6.80 %) and the albumin content in the plasma were also increased in comparison to their control groups. A statistically very highly significant ($p<0.001$) increase was noted (15.09 %) only in the case of albumin. (**Table 2** and **Fig. 1 & Fig. 2**).

TABLE 2: SERUM PROTEIN/ ALBUMIN (MALE AND FEMALE)

	Control Male	Brhanmanjisthadi Kvatha Curna (BRH) Male
Total protein	4237.245 ± 67.3245	4355.7975 ± 57.3945 (2.80 % incr., p=0.072)^{NS}
Albumin	3451.7416 ± 85.3068	4042.1916 ± 69.7356 (17.11 % incr., p=0.001)***
	Control Female	**Brhanmanjisthadi Kvatha Curna (BRH) Female**
Total protein	4608.3282 ± 113.2184	4921.6788 ± 132.2136 (6.80 % incr., p=0.238)^{NS}
Albumin	3605.9857 ± 65.7737	4150.2397 ± 62.9509(15.09 % incr., p=0.001)***

For both cases (male and female rats), in each group 10 male/female rats were taken. P values were calculated using unpaired 't'-test in comparison to control: NS = Not significant, ***$p<0.001$

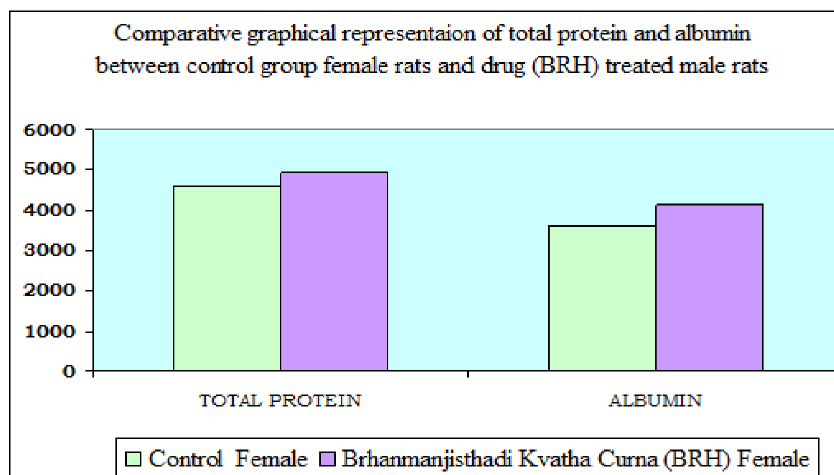

FIG. 1: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON TOTAL SERUM PROTEIN AND ALBUMIN BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

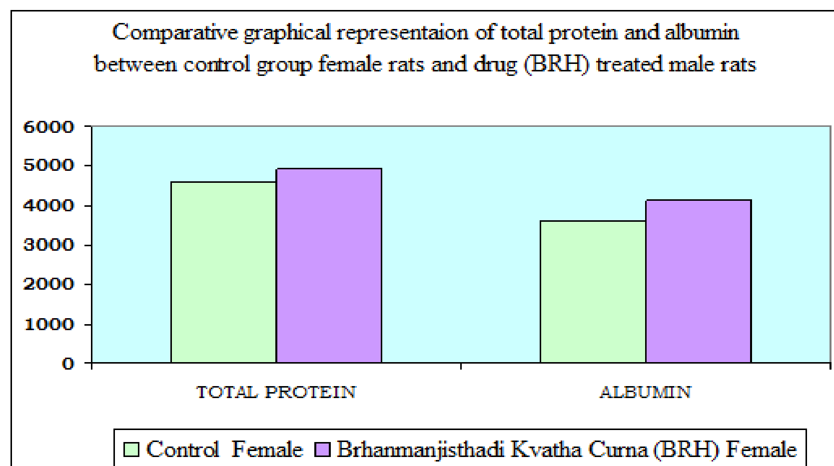

FIG.2: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON TOTAL SERUM PROTEIN AND ALBUMIN BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

Lipid profile (Male and Female):

In this investigation, none of the changes in terms of the Lipid profile, namely Triglycerides (2.18 % decr.), total Cholesterol (0.78 % decr.), VLDL (3.77 % incr.), LDL (4.91 % decr.) and HDL (2.61 % decr.) were significantly different from their corresponding control values in male rats (**Table 3** and **Fig.3**). Similarly, in female rats, no significant change regarding the Lipid profile (Triglycerides 2.18 % decr., total Cholesterol 1.22 % decr., VLDL 3.90 % incr., LDL 5.35 % decr. and HDL 2.61 % decr.) was found. (**Table 3** and **Fig. 3** and **Fig. 4**)

TABLE 3: LIPID PROFILE (MALE AND FEMALE)

Control Male		Brhanmanjisthadi Kvatha Curna (BRH) Male
Triglycerides	85.4456 ± 1.4729	83.5748 ± 1.5708 (2.18 % decr., p=0.693)^{NS}
Total cholesterol	61.8788 ± 1.4292	61.3951 ± 1.5097 (0.78 % decr., p=0.711)^{NS}
VLDL	13.7576 ± 0.5752	14.2764 ± 0.5747 (3.77 % incr., p=0.652)^{NS}
LDL	16.4315 ± 0.6355	15.6245 ± 0.6805 (4.91 % decr., p=0.684)^{NS}
HDL	28.2076 ± 0.7572	27.4696 ± 0.9176 (2.61 % decr., p=0.884)^{NS}
Control Female		**Brhanmanjisthadi Kvatha Curna (BRH) Female**
Triglycerides	77.4925 ± 2.5716	75.8004 ± 2.6562 (2.18 % decr., p=0.723)^{NS}
Total cholesterol	59.1491 ± 1.2942	58.4266 ± 1.3234 (1.22 % decr., p=0.903)^{NS}
VLDL	13.8558 ± 0.4353	14.3961 ± 0.4384 (3.90 % incr., p=0.776)^{NS}
LDL	14.9734 ± 0.5224	14.1724 ± 0.4948 (5.35 % decr., p=0.816)^{NS}
HDL	25.8685 ± 0.7505	25.1925 ± 0.8403 (2.61 % decr., p=0.987)^{NS}

For both cases (male and female rats), in each group 10 male/female rats were taken. P values were calculated using unpaired 't'-test in comparison to control: NS = Not significant, ***p<0.001

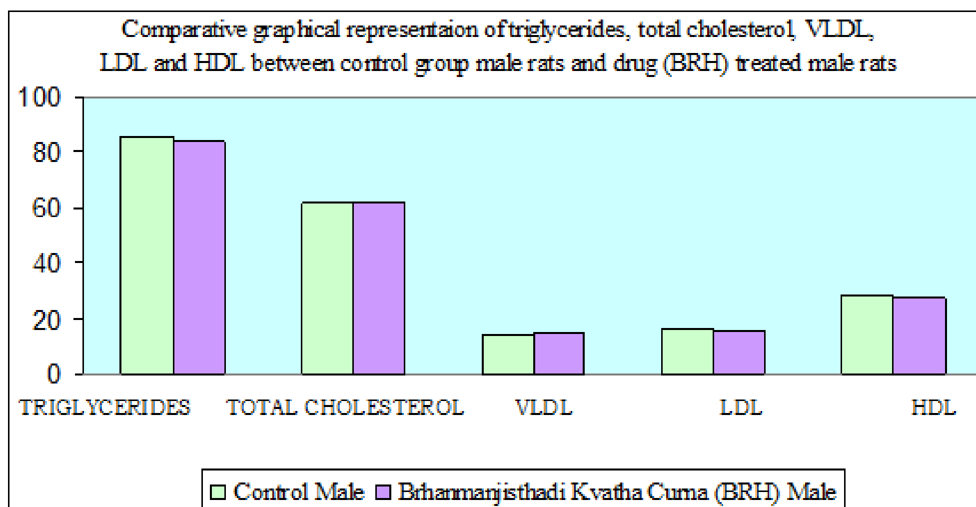

FIG.3: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON LIPID PROFILE (TRIGLYCERIDES, TOTAL CHOLESTEROL, VLDL=VERY LOW DENSITY LIPOPROTEIN, LDL=LOW DENSITY LIPOPROTEIN AND HDL=HIGH DENSITY LIPOPROTEIN) BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

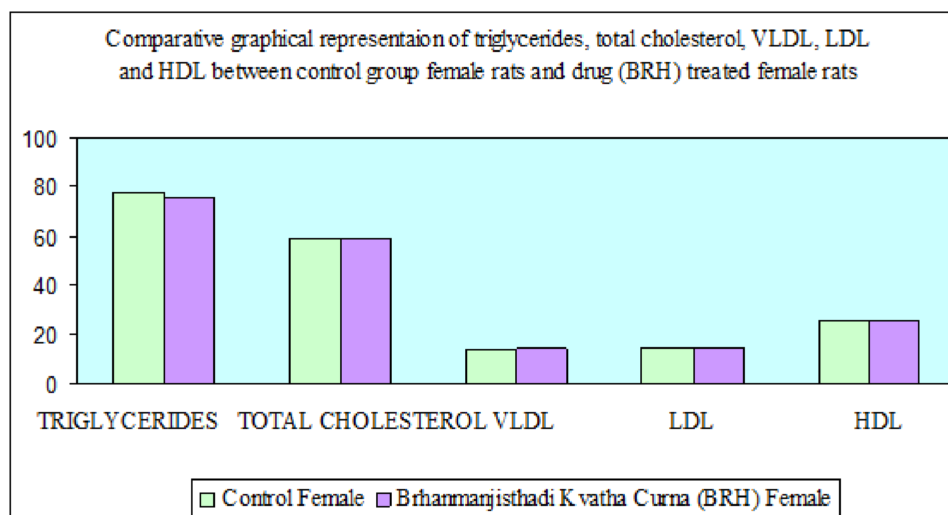

FIG.4: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON LIPID PROFILE (TRIGLYCERIDES, TOTAL CHOLESTEROL, VLDL=VERY LOW DENSITY LIPOPROTEIN, LDL=LOW DENSITY LIPOPROTEIN AND HDL=HIGH DENSITY LIPOPROTEIN) BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

Effect on Liver Function Test (Male and Female):

The liver function test was performed to assess the state of the liver by the determination of plasma bilirubin level in the rats. In the male rats there was a statistically a very highly significant decrease (39.65 %) in the Bilirubin content in the plasma. In the female rats there was a statistically very highly significant increase (41.16 %) in the Bilirubin content in the plasma. (**Table 4** and **Fig. 5** and **Fig. 6**)

TABLE 4: BILIRUBIN (MALE AND FEMALE)

	Control Male	Brhanmanjisthadi Kvatha Curna (BRH) Male
Bilirubin	0.0926 ± 0.002312	0.05588 ± 0.002025 (39.65 % decr., p=0.001)***
	Control Female	**Brhanmanjisthadi Kvatha Curna (BRH) Female**
Bilirubin	0.07771 ± 0.003352	0.1097 ± 0.008338 (41.16 % incr., p=0.001)***

For both cases (male and female rats), in each group 10 male/female rats were taken. P values were calculated using unpaired 't'-test in comparison to control: NS = Not significant, ***p<0.001

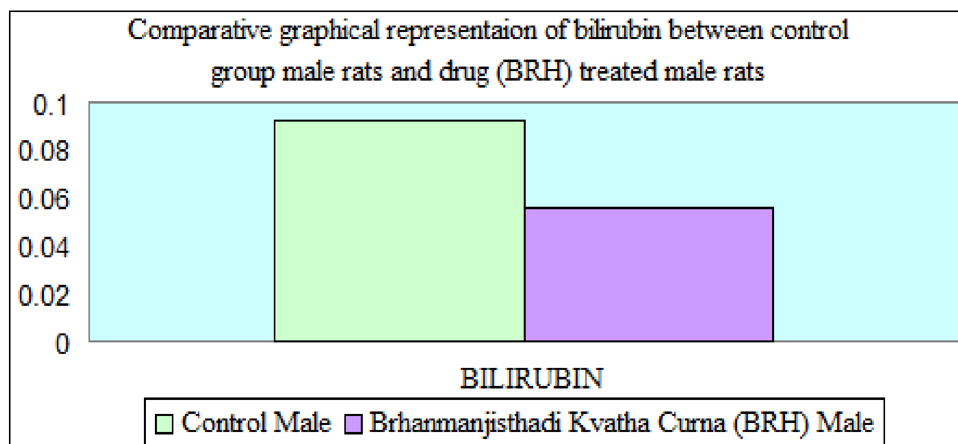

FIG.5: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON BILIRUBIN BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

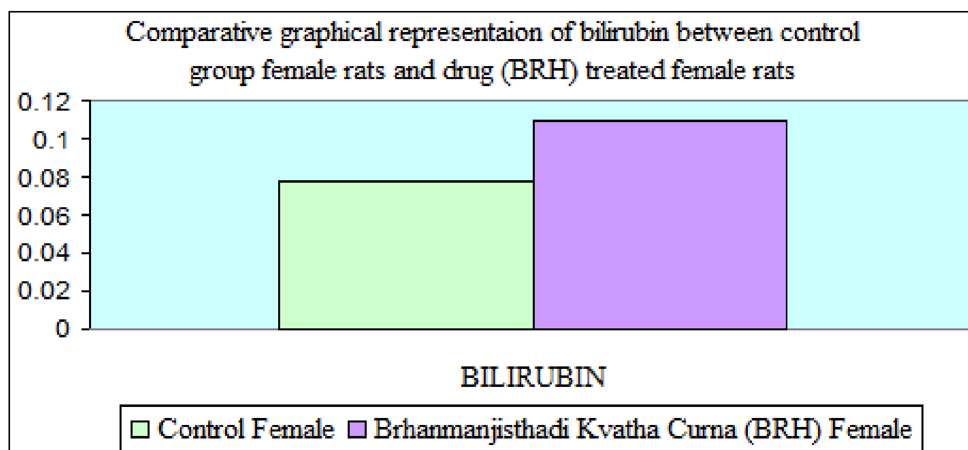

FIG. 6: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON BILIRUBIN BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

Effect on Kidney Function Test:

Kidney function test performed to measure the creatinine and urea content in the plasma. These two contents can provide information about how effective the kidney function is. In the male rats there was a decrease in both the Creatinine and the Urea content in the plasma. But a statistically a very highly significant (p=0.001) decrease (14.88 %) was noted only in the case of the Urea content in the plasma. The decrease (6.40 %) was observed non significant (p=0.152) with Creatinine. In the female rats there was a statistically very highly significant (p=0.001) decrease in both the Creatinine (25.97 %) and the Urea (16.35 %) content in the plasma. (**Table 5** and **Fig.7, 8, 9** and **10).**

TABLE 5: KIDNEY FUNCTION (MALE AND FEMALE)

Control Male		Brhanmanjisthadi Kvatha Curna (BRH) Male
Creatinine	0.8302 ± 0.02058	0.7771 ± 0.03446 (6.40 % decr., p=0.152)[NS]
Urea	56.5725 ± 0.9185	48.1545 ± 1.2707 (14.88 % decr., p=0.001)***
Control Female		Brhanmanjisthadi Kvatha Curna (BRH) Female
Creatinine	0.7216 ± 0.02643	0.5342 ± 0.02854 (25.97 % decr., p=0.001)***
Urea	44.5328 ± 0.8147	37.2505 ± 0.7726 (16.35 % decr., p=0.001)***

For both cases (male and female rats), in each group 10 male/female rats were taken. P values were calculated using unpaired 't'-test in comparison to control: NS = Not significant, ***p<0.001

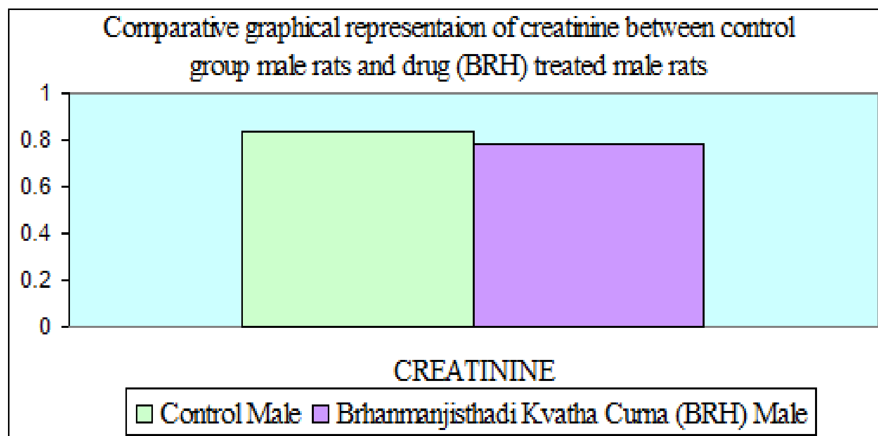

FIG.7: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON CREATININE BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

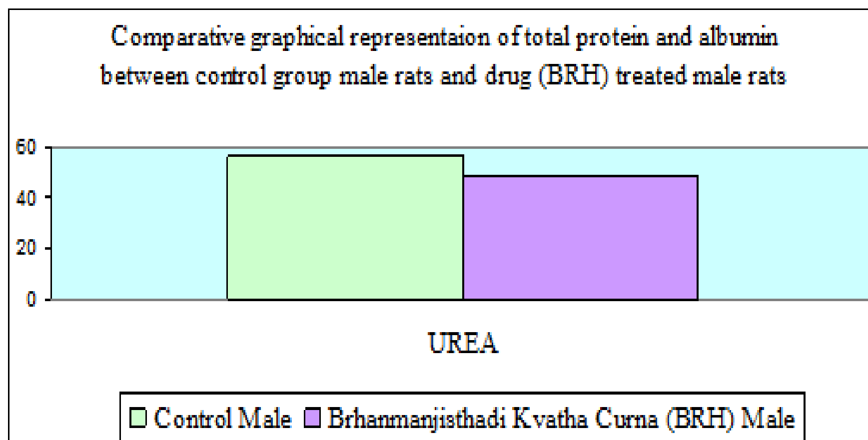

FIG. 8: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON UREA BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

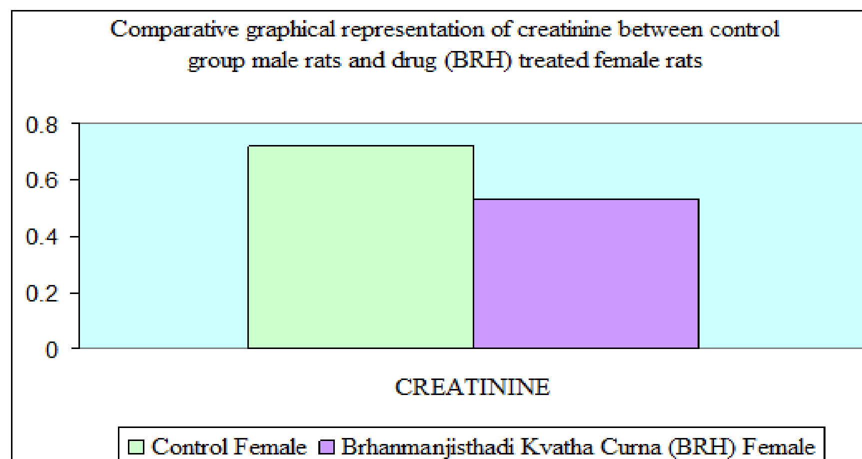

FIG. 9: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON CREATININE BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

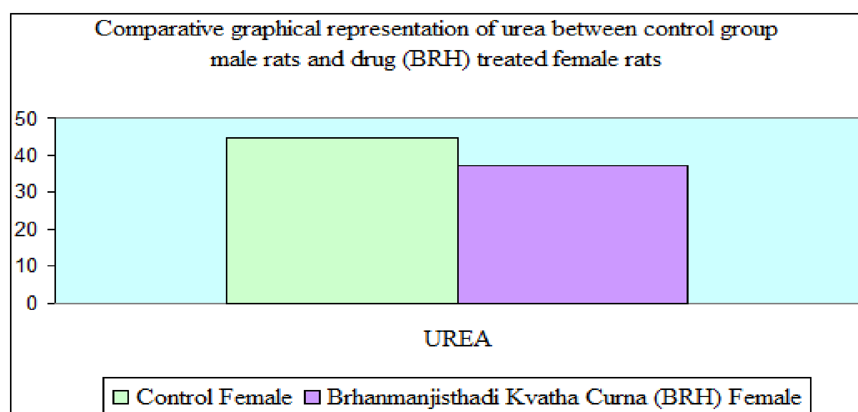

FIG.10: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON UREA BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

Effect on Serum Uric Acid:

If body produces too much uric acid or doesn't remove enough of it, a person becomes sick. So the test was performed to measure the effect of BRH on serum uric acid level. From the study, it was observed that about 2.2412 % increase which is statistically significant ($p<0.039$) in plasma Uric acid content of BRH treated male rats in comparison to their control male rats whereas the female rats showed a comparatively insignificant ($p=0.118$) (3.83 %) increase in concentration of the Uric acid level. (**Table 6 and Fig. 11** and **12**)

TABLE 6: SERUM URIC ACID (MALE AND FEMALE)

	Control Male	Brhanmanjisthadi Kvatha Curna (BRH) Male
Uric acid	2.1108 ± 0.05405	2.2412 ± 0.04656 (6.18 % incr., p=0.039)*
	Control Female	**Brhanmanjisthadi Kvatha Curna (BRH) Female**
Uric acid	2.4175 ± 0.07563	2.5101 ± 0.07861 (3.83 % incr., p=0.118)^{NS}

For both cases (male and female rats), in each group 10 male/female rats were taken. P values were calculated using unpaired 't'-test in comparison to control: NS = Not significant, ***p<0.001

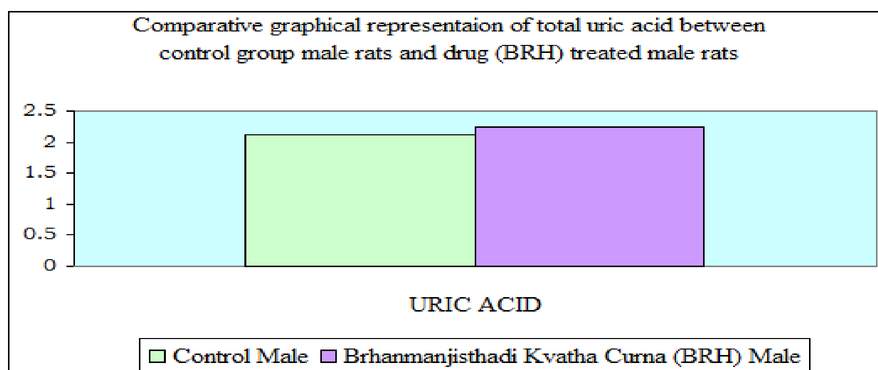

FIG. 11: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON URIC ACID BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

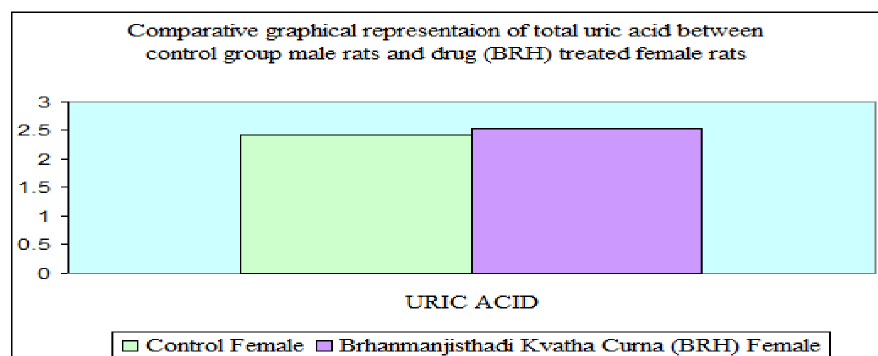

FIG. 12: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON URIC ACID BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

Enzymatic activity:

The test was done to find out the effect of Brhanmanjisthadi Kvatha Curna on liver enzymes like- serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT), and on alkaline phosphatase.

In this investigation, none of the changes in terms of the sGPT (0.10 % incr.), sGOT (0.33 % incr.) and ALP (0.58 % incr.) activities were significantly different from their corresponding control values in male rats (Table-11 and Figure-11). Similarly, in female rats, no significant change regarding the enzyme activities of sGPT (0.13 % incr.), sGOT (0.26 % decr.) and ALP (0.53 % incr.) were noted when compared to their corresponding control values in female rats (**Table 7** and **Fig. 13** and **14**).

TABLE 7: PLASMA ENZYMATIC ACTIVITY THROUGH sGPT, sGOT, ALP

Control Male		Brhanmanjisthadi Kvatha Curna (BRH) Male
sGPT	51.3336 ± 0.1096	51.3832 ± 0.1152 (0.10 % incr., p=0.792)^{NS}
sGOT	88.4264 ± 0.2426	88.7207 ± 0.2398 (0.33 % incr., p=0.874)^{NS}
ALP	36.2738 ± 0.0883	36.4842 ± 0.07708 (0.58 % incr., p=0.806)^{NS}
Control Female		**Brhanmanjisthadi Kvatha Curna (BRH) Female**
sGPT	41.3264 ± 0.1078	41.381 ± 0.1256 (0.13 % incr., p=0.883)^{NS}
sGOT	70.2885 ± 0.1625	70.104 ± 0.1623(0.26 % decr., p=0.681)^{NS}
ALP	28.7365 ± 0.07946	28.8896 ± 0.0946 (0.53 % incr., p=0.754)^{NS}

For both cases (male and female rats), in each group 10 male/female rats were taken. P values were calculated using unpaired 't'-test in comparison to control: NS = Not significant, ***p<0.001

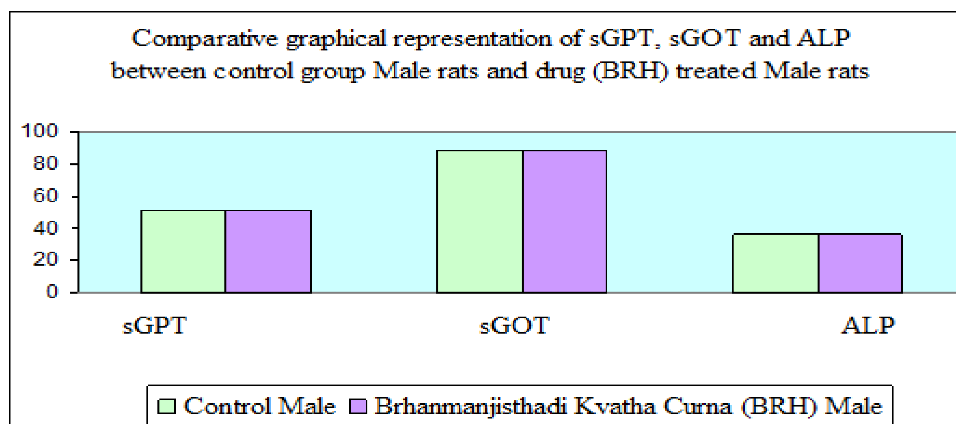

FIG.13: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON ENZYMATIC PROPERTY (sGPT, sGOT AND ALP) BETWEEN MALE CONTROL GROUP AND DRUG TREATED MALE RATS.

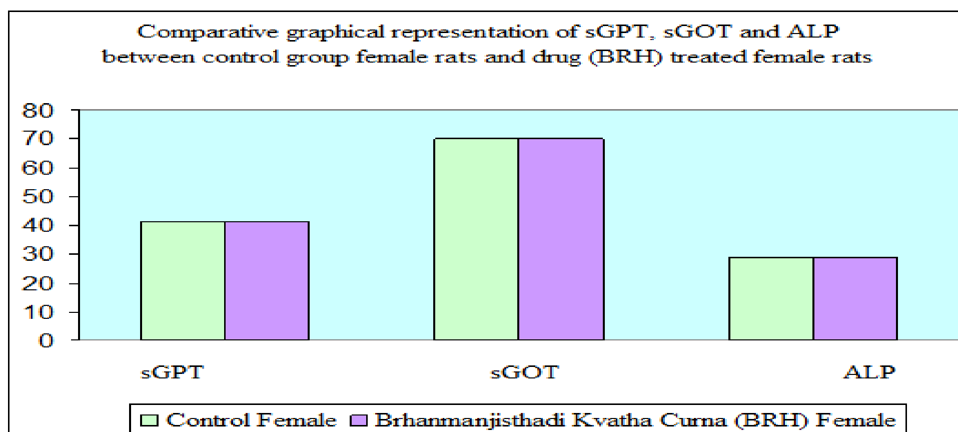

FIG.14: COMPARATIVE PRESENTATION OF EFFECT OF BRHANMANJISTHADI KVATHA CURNA (BRH) ON ENZYMATIC PROPERTY (sGPT, sGOT AND ALP) BETWEEN FEMALE CONTROL GROUP AND DRUG TREATED FEMALE RATS.

CONCLUSION: Brhanmanjisthadi Kvatha Curna (BRH) an Ayurvedic preparation, is used as a traditional medicine in filariasis in the northern part of the country, Bangladesh. By the characterization of toxicological study of Brhanmanjisthadi Kvatha Curna (BRH) we get some significant and

insignificant impacts on different parameters. A statistically very highly significant increase result in both male and female rats in case of albumin content was found. But in case of Bilirubin content in the plasma, a statistically very highly significant decrease was noted in male rat group while in case of female group a statistically very highly significant increase was noticed.

During evaluating effect on Kidney function test a statistically a very highly significant decrease was noted only in the case of the Urea content in the plasma in case of male rats but in case of female rats there was a statistically very highly significant decrease in both the Creatinine and the Urea (16.35 %) content in the plasma. Again a statistically significant increase was observed in plasma Uric acid in male rats. In this study it was observed that it causes few impacts on some parameters like total protein, enzymatic activity and lipid profiles. 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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