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Effect of Ardhabilva Kvatha Curna, an ayurvedic formulation, on liver and kidney function parameters of rat plasma after chronic administration

Kaiser Hamid*[1], Md. Sohel Kabir[2], M Obayed Ullah[3], Israt Jahan Bulbul[1], Masuma Siddiqua[1], MSK Choudhuri[2]

[1] Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh.
[2] Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.
[3] School of Chemistry and Molecular Biosciences, University of Queensland, QLD, Australia.

***Corresponding Author:** kaiserpharm_1134@yahoo.com

Abstract
Ardhabilva Kvatha Curna (ADR), a widely used Ayurvedic formulation of Bangladesh, is the preparation of five important medicinal plants. In the present study, the liver and kidney function parameters of rats' plasma were studied after chronic administration of ADR usually used in the treatment of Malabandha *(obstructed feces)*. The animal used was albino rats (*Rattus novergicus*: Sprague-Dawley strains) and the drug was administered per oral route at a dose of 40 ml/kg body weight, once daily, up to 41 days for all the experiments. Forty rats, equally of both sexes, were randomly grouped into four where one male and one female group were used as control and other groups were used as test. In both of the male and female rats, there was a statistically very high significant increase in Bilirubin and Creatinine (p=0.001***). Except ALP and total protein, the other parameters that demonstrates liver function also increased significantly in both male and female rats as sGOT (male, p=0.283, female, p=0.029*), sGPT (male, p=0.028*, female, p=0.008**). A decreased concentration of both ALP and total protein was observed in both male and female rats and it was not statistically significant. The increase in albumin content was statistically very high significant in both sexes of the animal (p=0.001***). A decreasing trend of both Urea and Uric acid content was noticed in male and female rats and incase of female rats, it was statistically significant (p=0.038* and p=0.048* respectively).

Keywords: Liver and kidney function parameters; Ardhrbilva Kvatha Curna; ADR; Ayurvedic formulation; rat plasma.

Introduction

"Ardhabilva Kvatha Curna" is an Ayurvedic formulation that is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health- 1/Unani-2/89/ (Part-1) 116 dated 3-6-1991). It is indicated in the treatment of Malabandha *(obstructed feces)*. In fact ADR is a preparation of five important medicinal plants that are used in particular amount in the formulation (Table 1).

Zingiber officinale (Ginger) belongs to the family Zingiberaceae and is widely used around the world as a spice or food additive and medicine. Several pungent elements of ginger are the oleoresin-gingeroles, shogaols and zingerone. These are credited for various pharmacological effects which include anti-nauseant or antiemetic, abortifacient, antimicrobial, anti-inflammatory (Verma et al., 1994), antioxidant (Shobana et al., 2000), anticoagulant, antihypercholesterolemic, antihypertensive, antihyperglycaemic, anti-spasmodic, aperient (Bradley, 1992), alexeteric, circulatory stimulant, counter irritant, sialagogue and vasodilator effect.

Achyranthes aspera Linn belongs to the family Amaranthaceae is an annual herb that grows throughout India (Nadkarni and Nadkarni, 1976). In the indigenous system of medicine, the whole plant is reported to be used for the treatment of renal dropsy, bronchial affections and leprosy (Kirtikar and Basu, 1935; Ojha et al., 1966). *A. aspera* has also been attributed with abortifacient, contraceptive, cardiac stimulant, astringent, diuretic and purgative properties (Nadkarni and Nadkarni, 1976; Satyavati et al., 1976; Akhtar and Iqbal, 1991).

Fagonia cretica L belongs to the family Zygophyllaceae is a small spiny under shrub (Chopra et al., 1992, Hooker and Thomson, 1881). It is astringent, febrifuge and prophylactic against small-pox. The plant is bitter and used for the treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver trouble, typhoid, toothache, stomach troubles and skin diseases (Baquar, 1989).

Boerhaavia diffusa L belongs to the family Nyctaginaceae, commonly known as "Red hogweed". It is abundantly available in India, Nigeria and many other countries. Indigenous people use various plant parts of the plants to get relief from diabetes mellitus. The plant has also been extensively used in Ayurvedic and Unani practice in the Indian subcontinent (Chopra et al., 1958).

Solanum xanthocarpum belonging to the family Solanaceae, commonly known as the Indian night shade or Yellow berried night shade, is a prickly, diffusely bright-green, perennial shrub which grows abundantly in arid areas of India. Traditionally different parts of this plant have been used for curing various ailments. Fruit juice is useful in sore throats and rheumatism; decoction of the plant is used in gonorrhea; paste of leaves is applied to relieve pains; seeds act as expectorant in cough and asthma; roots are expectorant and diuretic, useful in the treatment of catarrhal fever, coughs, asthma and chest pain (Ghani, 1998).

Previously, our research group reported the effect of various Ayurvedic formulations on lipid profile (Obayed et al., 2010) liver and kidney function parameters (Obayed et al., 2008) after chronic administration. The aim of the present study was the continuation of our effort and to explore the effect of ADR on liver and kidney function parameters of rat plasma after chronic administration.

Materials and Methods

Chemicals and Reagents:

All the reagents and chemicals that were used in this work were of analytical grade and were prepared in all glass-distilled water. To evaluate the effect of Ardhabilva Kvatha Curna (ADR) on liver and kidney function parameters of rat plasma, it was collected from Sree Kundeshawri Aushadhalaya Ltd, Chittagong.

Dose and route of administration:

The liquid "Ardhabilva Kvatha Curna" was administered to the animals at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For investigating the liver and kidney function parameters, the drug was administered per oral route at a dose of 40 ml/kg body weight. For all the studies, the drug was administered orally. [per oral (p.o.) route]. Ketamine was administered intra-peritoneally (500 mg/kg i.p.).

Experimental animals and their Management:

Forty 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 this experiment. These animals were apparently healthy and weighed 50-70 g. The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the whole experiment period. 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 the 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. 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 par 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 / sex. Thus ten rats were taken for each group for both control and the experimental group

Preparation of Plasma for the Test:

At the end of the 41-day 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.

Determination of Liver and kidney function Parameters:

To assess the state of the liver and kidney function, biochemical analyses was carried out on plasma. These 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 Serum glutamic oxaloacetic transaminase (sGOT), Serum glutamic pyruvic transaminase (sGOT) and alkaline phosphatase (ALP). Total protein content of the samples was assayed by the Biuret method (Plummer, 1971). 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 Kind and King (1954).

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.

Results

Liver function:

In case of assay of liver function parameter, similar trend of result was noticed in both sexes of the animal. There was marked increase in the Bilirubin content in both male and female rats and it was statistically highly significant (p=0.001***) [Table 2, graph 1 and 2]. The similar result was observed incase of albumin content and there was remarkable increase in both male and female rats which was statistically highly significant (p=0.001***). Except ALP and total protein, the concentration of other parameters as sGPT, sGOT was increased. The increase in sGPT was statistically significant in male (p=0.028*) and female rats (p=0.008**). The concentration of ALP and total protein was decreased in both male and female rats and it was not statistically significant. [Table 2, graph 3 and 4]

Kidney function:

All the kidney function parameters assayed during the experiment decreased in both male and female rats (table 2, graph 5, 6, 7 and 8) except Creatinine. The increase in Creatinine, the major kidney function parameter was remarkable and it was statistically very highly significant (p=0.001***). Incase of Uric acid, the decrease was statistically significant in the female rats (p=0.048*) but in male rats this decrease was not statistically significant. The similar phenomenon was observed incase of urea. It was decreased significantly in female rats (p=0.038*) but it was not statistically significant in male rats (p=0.093).

Discussion

The biochemical indices monitored in the liver and kidney is useful 'markers' for assessment of tissue damage. The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis (Malomo, 2000); assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Yakubu et al., 2003b). Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Akanji, 1986). According to Naganna (1989), increase in bilirubin is indicating the abnormal liver function which may be the results of higher synthetic function of the liver. On the other hand hyperbilirubinaemia is often the first and sometimes the only manifestation of liver disease. Impaired hepatic Bilirubin clearance is specifically due to reduced uptake or possible competition for binding to 2-protein or ligandin (Lee and Garner, 1983).

The elevation in the serum activities of transaminases (sGPT and sGOT) suggested leakage from hepatocytes and possible damage which might have resulted from change in membrane permeability (Latha et al., 1998). This may have a consequential effect on the metabolism and regulation of amino acids in the liver. The increased level of serum sGOT and sGPT may be also related to a number of factors including increased enzyme release due to an increase in the number of liver cells that are

injured and are undergoing turnover (Johnson, 1995) or a normal turnover of cells whose aminotransferase content has increased (Olagunju, 1992; Olagunju et al., 2000). Increased liver sGPT and sGOT activities could also lead to increased formation of amphibolic materials which could be channeled to the biosynthesis of lactate, glucose and fatty acids (Lehninger et al., 1993).The reduction in total protein concentration following treatment of ADR in the rats could also be attributed to a decrease in functional activity of the liver caused by components of the extracts (Bruneton, 1999). In this study, plasma creatinine was increased significantly after the chronic administration of ADR which could be due to muscular dystrophy, or a loss of function of the kidneys. Also, a significant increased Creatinine level in the serum beyond normal values could be associated with increased mortality (Gibson et al., 2003). Urea is the major nitrogen-containing metabolic product of protein catabolism. The significant reduction in the serum urea concentration following the administration of ADR may be attributed to impairment in the urea cycle leading to reduced production of the metabolic product (Yakubu et al., 2003).

Conclusion

"Ardhabilva Kvatha Curna" is an Ayurvedic formulation that is used in the treatment of Malabandha *(obstructed feces)* altered all the assayed parameters of rat plasma after chronic administration. Incase of liver function parameters, all of them increased except ALP and total protein and in kidney function parameters, all of the parameters decreased except Creatinine in both sexes of the animal. Thus, it necessitates further close investigation to find out the reason of this discrepancy incase of different parameters that represent the proper functioning of both liver and kidney.

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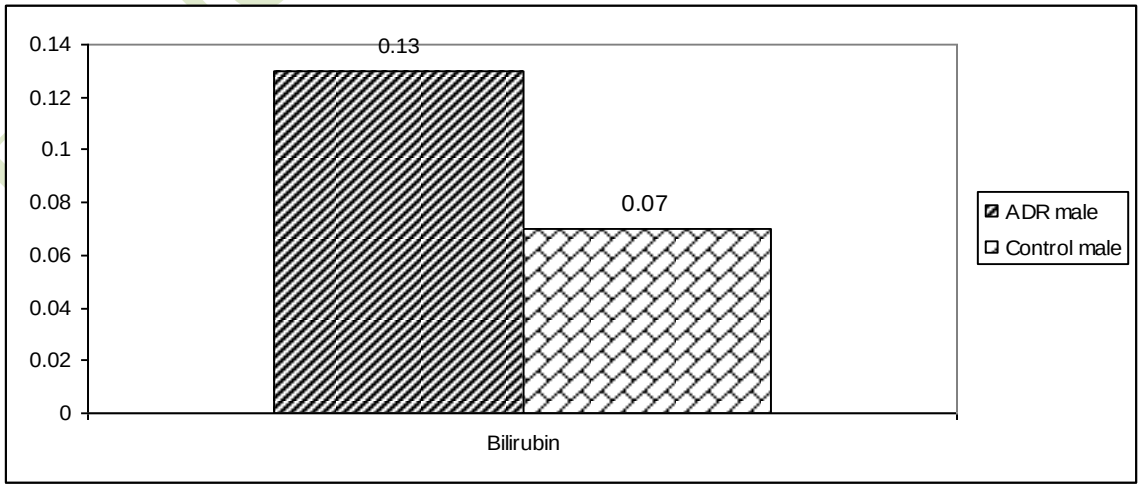
Table 1: Formulary of ADR

Name of Plants / Ingredients	Used Parts	Botanical Name	Family	Amount Used
Cukku (sunthi)	Rhizome	*Zingiber officinale*	Zingiberaceae	24 g
Katalati mula (apamarga)	Root	*Achyranthes aspera linn*	Amaranthaceae	24 g
Cunda mula (brhatibheda)	Root	*Solanum Xanthocarpum*	Solanaceae	24 g
Tuva mula (duralabha)	Root	*Fagonia cretica Linn*	Zygophyllaceae	24 g
Tazutama mula (punarnava)	Root	*Boerhaavia diffusa*	Nyctaginaceae	24 g

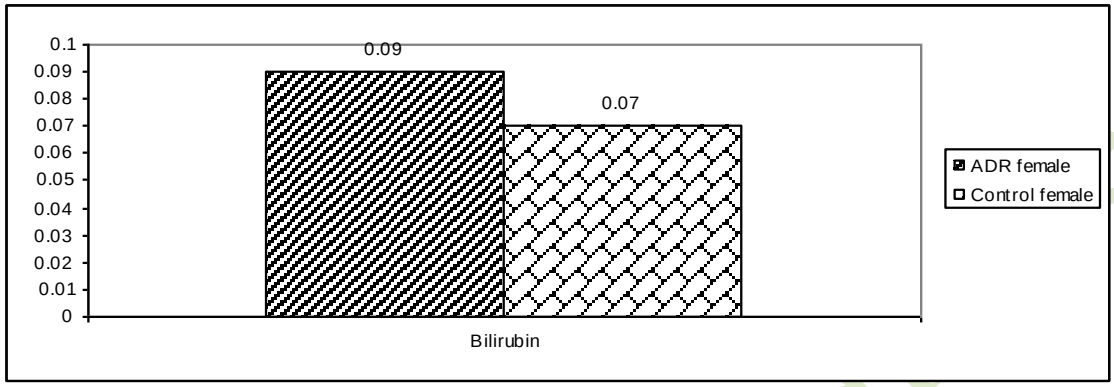
Table 2: Effect of ADR on liver and kidney function parameters

Parameters	Male rats			Female rats		
	Control (n=10)	ADR (n=10)	P value	Control (n=10)	ADR (n=10)	P value
Bilirubin	0.0657 ± 0.01	0.1318 ± 0.01	p=0.001***	0.06534 ± 0.01	0.09058 ± 0.001	p=0.001***
sGPT	59.8897 ± 0.12	60.1288 ± 0.12	p=0.028*	56.0077 ± 0.15	57.1568 ± 1.04	p=0.008**
sGOT	103.342 ± 0.28	103.9861 ± 0.40	p=0.283	95.5919 ± 0.22	96.9194 ± 0.35	p=0.029*
ALP	42.4660 ± 0.11	42.3062 ± 0.11	p=0.289	39.2208 ± 0.11	39.0892 ± 0.12	p=0.511
Total protein	6251.0896 ± 84.52	6045.1490 ± 96.01	p=0.173	5238.6616 ± 50.28	5102.0477 ± 125.35	p=0.354
Albumin	4814.2776 ± 118.94	5800.8444 ± 100.94	p=0.001***	3996.996 ± 72.91	4226.6276 ± 67.18	p=0.001***
Creatinine	0.7536 ± 0.03	0.8788 ± 0.02	p=0.001***	0.9718 ± 0.06	1.0924 ± 0.03	p=0.001***
Urea	59.9005 ± 0.97	58.1814 ± 0.85	p=0.093	67.7140 ± 1.24	65.2797 ± 1.26	p=0.038*
Uric acid	2.9494 ± 0.07	2.8317 ± 0.05	p=0.193	2.8956 ± 0.12	2.5006 ± 0.08	p=0.048*

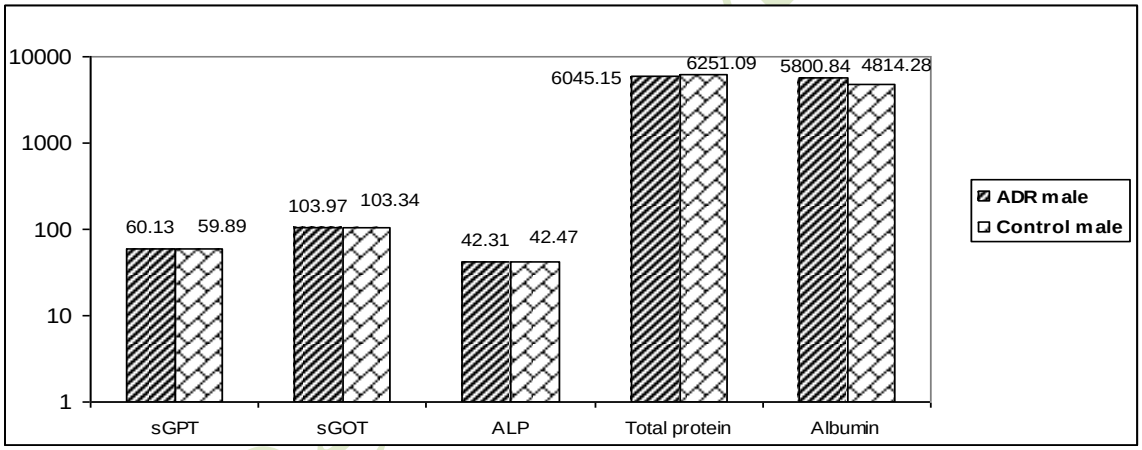
Graph 1: Comparative graphical representation of Bilirubin content between control and drug (ADR) male rats.



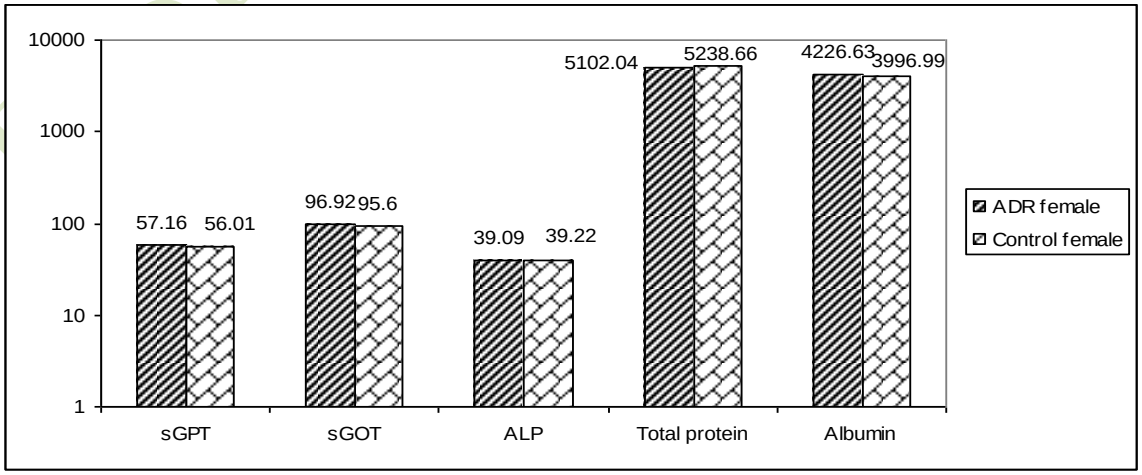
Graph 2: Comparative graphical representation of Bilirubin content between control and drug (ADR) female rats.



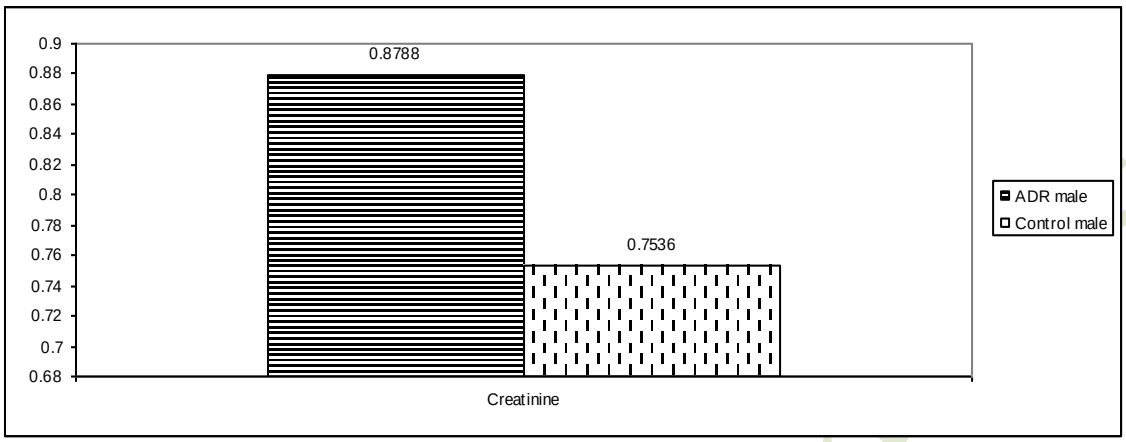
Graph 3: Comparative graphical representation of sGPT, sGOT, total protein, albumin and ALP content between control and drug (ADR) male rats.



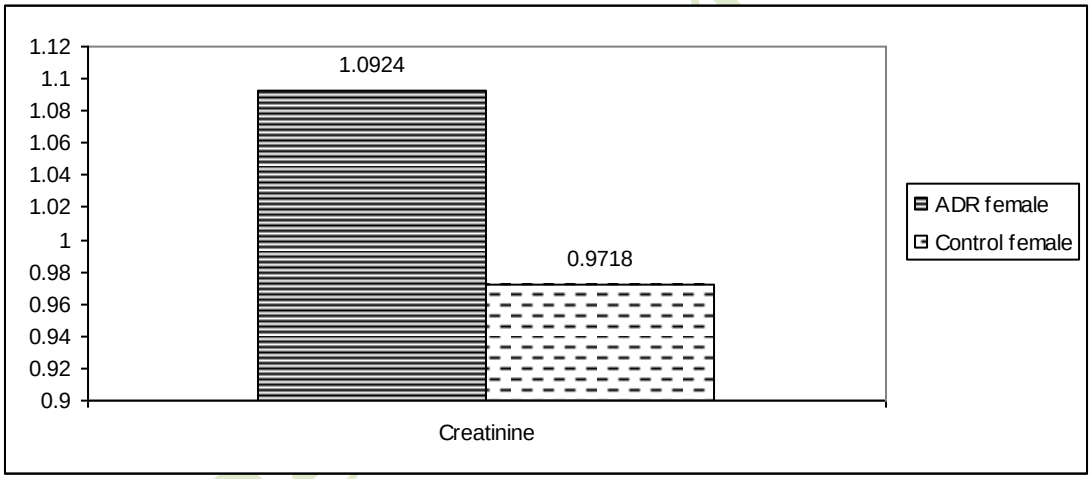
Graph 4: Comparative graphical representation of sGPT, sGOT, total protein, albumin and ALP content between control and drug (ADR) female rats.



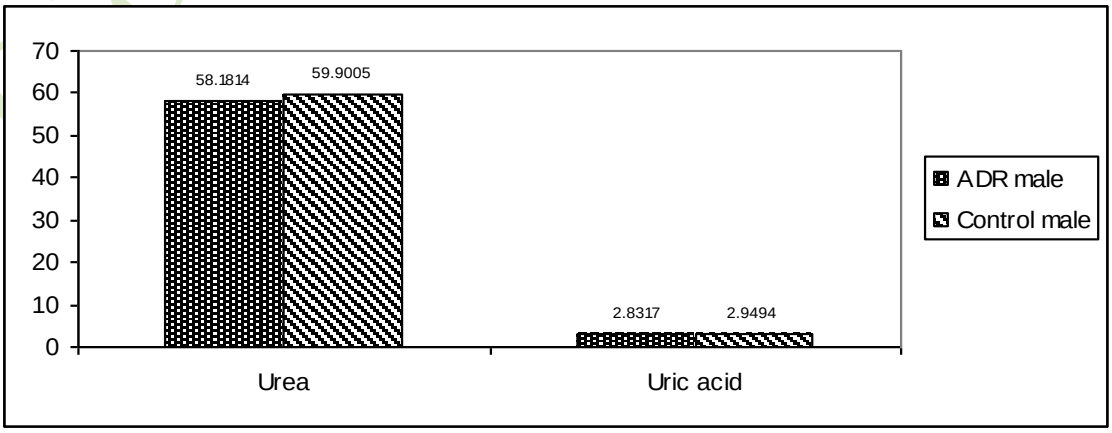
Graph 5: Comparative graphical representation of Creatinine content between control and drug (ADR) male rats.



Graph 6: Comparative graphical representation of Creatinine content between control and drug (ADR) female rats.



Graph 7: Comparative graphical representation of urea and uric acid content between control and drug (ADR) male rats.



Graph 8: Comparative graphical representation of urea and uric acid content between control and drug (ADR) female rats.