EFFECTS OF THE ROOT BARK EXTRACT OF Monotheca buxifolia (FALC.) A. DC ON ANTIOXIDANT POTENTIAL AND SERAL PROPERTIES OF RABBIT (Oryctolagus cuniculus)

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ABSTRACT

This activity was aimed to examine the phytomedicinal effects of the crude extract of the bark of the root of Monotheca buxifolia (Falc.) A. DC on the serum biochemistry and antioxidant potential of the in-vitro lipids present in various tissues of the rabbits and the overall correlation of the extract and serum lipids. The extract was administered orally to the rabbits in different doses (0, 25, 75 and 150 mg) in triplicate daily for 10, 20 and 30 days respectively. The data showed that the weights of rabbits and their different organs were reduced. Serum cholesterol level of the experimental animal decreased, while no variation in the serum glucose was observed. The histopathology results showed that the central vein had normal endothelial lining with no evidence of pericentral fibrosis. The hepatic cords in almost all cases were well formed. The portal tracts showed branches of hepatic artery, portal veins and cholengioles with normal morphology. A significant ($P < 0.05$) reduction was observed in the RSA values of the brain, liver and muscles of the subject animals. Increasing the dose reduced the RSA values in all the tissues. It was concluded that this plant has the potency for decreasing weight of the whole body and also decrease the cholesterol level without significantly affecting other parameters. Hence, there are implications for further detailed studies on this plant.

Keywords: Anti-Oxidant, Monotheca buxifolia (Falc.) A. DC, LDL-c level, HDL-c level, total cholesterol.

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INTRODUCTION

Monotheca buxifolia (Falc.) A. DC. Vern. Gurgurah belongs to family Sapotaceae. It has short thorns either axillary or terminal in position and alternate leaves. Pedicelate flowers of about 5 mm in diameter are produced in April to May. The filaments of the flower are longer than the petals. The fruit of M. buxifolia are round in shape and of about 1 cm in diameter. The fruits are eaten by local people and are famous for their delicious taste. M. buxifolia is one of the numerous species of wild fruits and nuts found in the different areas of Pakistan (Nasir and Ali, 1972), Afghanistan, Oman, North of Somalia and Southern Ethiopia (Ahmed et al., 2010).

It has been investigated that it contains hydrocarbons (heptacosane, penta triacontane) and alcoholic sterols (Nasir & Ali, 1972). Different parts of M. buxifolia contains high quantity of antioxidant compounds like anthraquinones, flavonoids, terpenoids, tannins, saponins, and cardiac glycosides (Rehman et al., 2013; Ullah et al., 2016). The antioxidants from these natural sources have strong effect on serum lipid profile (HDL, LDL, and cholesterol), body weight (Georgiev et al., 2011), atherosclerosis (Peng et al., 2011) and against the liver diseases (Fontanari et al., 2012).

Medicinally, fruits and other parts of the plant are laxative, used against gastric and in urinary tract diseases (Rashid and Marwat, 2006). Extracts of the various portions of plant are used extensively in conventional medicine, especially for the preparation of different remedies like treatment of cough, laryngitis and liver diseases. In South Asia, plant extracts are used as a separate branch of medicine from the very ancient time (Dahanukar et al., 2000; Habib ul Hassan et al., 2014).

This activity was aimed to examine the phytomedicinal effects of the crude extract obtained from the bark of the root of Monotheca buxifolia on the serum biochemistry and antioxidant potential of the in-vitro lipids present in various tissues of the rabbits and the overall correlation of the extract and serum lipids.

MATERIALS AND METHODS

Extraction of Metabolic contents of M. buxifolia: The plant was collected from the Hindu Kush range of lower Dir, Khyber Pakhtunkhwa Pakistan. After complete shade drying the bark was grinded to a fine powder. The powder was then put in 50 % aqueous methanolic solution in a beaker and was placed on a shaker for five days. After complete shaking of the methanolic solution was filtered out and then put on rotary vacuum evaporator. This activity was continued till all the methanolic and aqueous portion got separated from the crude extract. The extract was then reserved in an oven for some time at a high temperature to make it completely dry (Chellan et al., 2008).

Total Phenolic Contents Assay

The total phenolic content (TPC) of the extracts was determined by using Folin-Ciocalteu's reagent. Rendering to the method of Slinkard and Singleton (1977) Gallic acid was used as a reference. After incubation at 27℃ for 1 hr. the absorbance was measured at 765 nm with a Shimadzu UV/Vis-1700 spectrophotometer (Shimadzu, Japan).

Animals Feeding

Twelve numbers of rabbits of the local strains were selected as experimental animals. The animals were classified into four groups (S, P, Q and R) triplicate in each. All the rabbits were kept in uniform conditions of temperature and fodder feeding. Group S were control hence no extract was given, group P was fed with 25 mg, group Q with 75 mg of extract and group R were fed 150 mg extract given orally for one month on daily basis.

Blood Biochemical and hematological Analyses

At the end of 10^{th}, 20^{th} and 30^{th} day, 3 mL blood was taken from each rabbit and was stored in gel and clot activator tubes (Guangzhou Medical Instrument Co. Ltd Guangzhou, China) for

biochemical analysis. The same were then subjected to centrifugation to get the serum. The serum was then analyzed for HDL-c, TC, LDL-c, TG, Glucose (G) contents and ALT values using Shimadzu UV-vis-1700 spectrophotometer (Shimadzu, Japan). Likewise blood was also taken in EDTA tube (Guangzhou Medical Instrument Co. Ltd Guangzhou, China for hematological analysis) and were analyzed for Haemoglobin level, TLC (total leucocytes count), Total RBCs (red blood cells), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelet Count.

Liver Histopathology

After completion of the one month trial the rabbits were slaughtered to obtain the different organs (kidney, brain and liver) for further analyses. Liver from each triplicate was taken. Sections were prepared by using microtome and stain on microscopic slides. The images were analyzed for any possible change produced by the extract using CC TV fitted binocular microscope.

Lipid Extraction from Tissues

Liver, muscles, and brain were collected from the rabbits on autopsy. These were preserved in formalin and used for the lipid extraction. A 10 gram of each tissue was used. The tissues were homogenized separately and chloroform-methanol mixture (2:1) was added. After complete shaking for 72 h the homogenized was separated by using various instruments like filter paper and separating funnel. The obtained filtrate was then subjected to rotary vacuum evaporator for the separation of lipids from the filtrate (Folch et al., 1957).

Radical Scavenging Assay (RSA)

The Radical Scavenging Assay of the extracts and lipids obtained from different tissues like brain, muscles and liver of the rabbits were calculated using Shimadzu UVvis-1700 spectrophotometer (Shimadzu, Japan).

Data Analysis

For data analysis Graph Pad Prism 5 for windows version 5.03 (Graph Pad Software, Inc, 2009) was used. Analysis of variance (ANOVA) and multiple comparisons were carried out. The necessary data are presented in Tables and Figures.

RESULTS AND DISCUSSION

Effects on the Rabbits Body and organs Weight

Increase in body weight or obesity are related to a number of diseases including cardiovascular, diabetic, hypertension, gall bladder and cancer (Akil and Ahmad, 2011). This result showed that the root bark extracts of the plant reduced the total body weight of the rabbits. Similarly the weight of liver, kidney and brain were also reduced due to the intake of extracts after feeding for one month. In contrast to the varying treatments, no change in total body weight was observed in control group (S). Increasing the dose decreased the total weight effectively to 1.05 ± 0.022 at the end of 4^{th} week in R group as shown in Table-1b. Similarly a reduction in weight of liver, kidney and brain were also observed upon feeding of M. buxifolia root extracts (Table-1a). Comparable results were also communicated by Eweka and Enogieru (2011). The researchers in their work concluded that natural extracts reduce the lipid per-oxidation in the tissues and thus decreasing the weight of the subject animals and the tissues related.

Blood Biochemical Analyses

Significant reductions ($P < 0.05$) in total cholesterol (TC) contents were detected when rabbits were fed with root extracts of M. buxifolia. It was found that increasing the dose decreased the TC. At the end of 30^{th} day the lowest value of TC (128.3 ± 18.9) was observed. However, no significant change was observed on glucose concentration at 20^{th} day. But at the end of 30^{th} day a significant change in glucose was investigated, increasing the value to 56.76 ± 2.8 when compared with

10th day of group S. No significant change in serum triglycerides (TG) was recorded in the present findings. All the values were almost at the same range as shown in Table 2. However, a significant increase in HDL-c level was observed when rabbits were fed with M. buxifolia extracts. Comparing the values of 10th, 20th and 30th day it was found that the extracts had the ability to elevate the level of HDL-c effectively) [Table-2]. It is concluded from the current data that M. buxifolia root extract decreased the TC level, while increased glucose and HDL-c level. But there was no or less effect in case of serum TG level.

Higher level of total Cholesterol (TC) in blood is often related to many pathological conditions, including cardiovascular diseases (CVD) and arthrosclerosis (Pearson et al., 2003). Similarly any abnormality in the blood glucose may lead to the condition of hypoglycemia or hyperglycemia. The most prominent example of hyperglycemia is Diabetes mellitus, which is related to failure of blood sugar regulation. The blood also contains TG that is mainly responsible for energy requirement of the body. The HDL cholesterol is a type of lipoproteins. The higher concentrations of LDL are atherogenic, whereas HDL high concentrations are negatively related with the risk for CVD (Kannel, 1992).

The current results showed that M. buxifolia root extract decreased the TC level, while increased glucose and HDL-c level. But, there was no or less effect in case of serum TG level (Table-2). The results are in support with Uhl et al. (1992) who also recorded a significant reduction in TC level. But the study of Meral et al. (2001) deviates from the current findings, they showed that plant extracts have no effect on serum glucose concentration. However the study of Murugaiah et al. (1999) and Yokozawa et al. (1996) show that plant root extracts increased HDL-c level and thus reducing the TC burden of the body.

Blood hematological Analyses

Blood hematology is another important parameter to study the effect of a particular compound on animal physiology. In this activity the M. buxifolia root extract decreased the TLC level significantly ($P < 0.05$). The dose of 150 mg decreased the TLC value to 6950.0 ± 464.2 cmm as compared to control group (13600.3 ± 3200.9 cmm). But there was no significant change in Hb, TRBC, HCT, MCH and MCHC concentration. However, a significant change ($P < 0.05$) in platelets count was observed. The dose of 75 mg increased the platelets up to 538166.7 ± 40707.6 cmm, but 150 mg dose again reduced the value to 478166.7 ± 6604.3 cmm as shown in Table-3. The current results show that the root extract has a significant effect ($P < 0.05$) on some hematological indices like the level of TLC decreased, while the platelets level was increased. However no significant change in Hb, TRBC, HCT, MCH and MCHC was deciphered (Table-3).

These results are in agreement with the findings of Rashid and Marwat (2006), who evaluated that plant extracts significantly influenced many of the hematological indices. In their study they found that blood can be used as a good sign of body health and pathological reflect of the entire body. In this way Owoyele and his coworker in 2011 studied the hematological and biochemical studies on Parquetina nigrescens root extract in albino rats. In their study they investigated the hematological, lipid, and antioxidant effects of Parquetina nigrescens (PN) plant. In their study they finally observed that this plant possesses erythropoietic potentials at minimal dose.

Table-1a. Change in organ weight with the oral administration of extract.

Group	Dose (mg day^{-1})	Organs weight (g)		
		Liver	Kidney	Brain
S	0	0.0533± 0.0097	0.0130± 0.0014	0.0067± 0.0012
P	25	0.0417± 0.0016	0.0090± 0.0014	0.0067± 0.0019
Q	75	0.0367± 0.0123	0.0100± 0.0007	0.0057± 0.0002
R	150	0.0423± 0.0040	0.0090± 0.0000	0.0057± 0.0012

Table-1b. Change in body weight with the oral administration of extract.

Group	Dose	Weight Change (kg)			
	(mg day^{-1})	1st week	2nd week	3rd week	4th week
S	0	1.41± 0.09	1.47± 0.13	1.25± 0.04	1.35± 0.14
P	25	1.39 ± 0.11	1.36 ± 0.06	1.20 ± 0.06	1.20 ± 0.05
Q	75	1.56 ± 0.08	1.50 ± 0.14	1.34 ± 0.12	1.22 ± 0.12
R	150	1.15 ± 0.001	1.13 ± 0.022	1.10 ± 0.047	1.05 ± 0.022

Table-2. Effect of M. buxifolia root extracts on serum biochemistry.

Parameter	Days	Control	25 mg (p group)	75 mg (q group)	1_{50} mg (r group)
TC	10	220.7 ± 31.8 (a)	203.9 ± 1.4 (a)	203.0 ± 17.6 (a)	164.2 ± 25.1 (a)
	20	216.8 ± 30.5 (a)	143.1 ± 31.8 (a)	180.5 ± 56.2 (a)	245.6 ± 23.3 (a)
	30	218.4 ± 30.3 (a)	153.7 ± 41.3 (b)	129.7 ± 15.6 (c)	128.3 ± 18.9 (d)
GL	10	16.9 ± 0.2 (a)	53.8 ± 1.4 (b)	35.1 ± 7.8 (a)	32.8 ± 19.8 (a)
	20	49.0 ± 20.6 (a)	48.1 ± 16.6 (a)	49.1 ± 19.8 (a)	24.3 ± 10.6 (a)
	30	40.2 ± 1.9 (a)	34.0 ± 6.6 (a)	26.7 ± 11.2 (a)	56.76 ± 2.8 (b)
TG	10	244.5 ± 5.5 (a)	251.3 ± 13.8 (a)	255.8 ± 6.2 (a)	250.4 ± 17.0 (a)
	20	253.3 ± 2.8 (a)	157.9 ± 2.1 (b)	192.7 ± 1.4 (c)	143.8± 22.0 (d)
	30	288.5 ± 1.4 (a)	266.5 ± 3.5 (b)	251.3 ± 5.5 (c)	244.0 ± 4.8 (d)
HDL	10	119.1 ± 9.3 (a)	64.0 ± 37.5 (b)	87.4 ± 22.9 (a)	196.1 ± 12.2 (c)
	20	124.3 ± 4.29 (a)	42.1± 13.7 (b)	67.8 ± 37.8 (c)	33.7 ± 18.3 (d)
	30	129.2 ± 5.5 (a)	86.1 ± 10.1 (b)	91.3 ± 19.2 (c)	83.5 ± 17.4 (d)

LIVER HISTOLOGY

Liver is an important organ of the body and hence plays enormous roles in energy and nutrient metabolism. Feeding different compounds to subject animal changes the Liver functions (Tres et al., 2009). The current result in Figure 1 showed that S group has normal endothelial lining with no evidence of pericentral fibrosis and showing normal hepatocytes architecture. Similar results were also investigated for Q and R groups. However in P group there was few eosinophils along with mild infiltrate of lymphocytes. Some zenoparasites at the interface of portal tracts showed hepatocytes degeneration in the same group. Similarly in R group the supporting tissues of the portal tracts showed mild infiltrate of lymphocytes and scattering of a few neutrophils is shown. In this connection the study of Bartels et al. (2007) is quite important. They showed that plant extracts contains natural antioxidants that has beneficial role on liver histology

Table-3. Effect of M. buxifolia plant extracts on the hematological indices of the rabbit.

Parameter	Groups			
	S	P	Q	R
Hb (g/dl)	11.3 ± 2.2	12.3 ± 2.6	12.8 ± 1.0	12.9± 1.0
TLC (cmm)	13600.3±3200.9	13358.3 ± 3520.9	6733.3 ±1000.3	6950.0 ± 464.2
TRBC (mil/cmm)	6.2 ± 0.1	6.6 ± 0.3	6.9 ± 1.9	6.3 ± 0.3
HCT	42.5 ± 1.2	46.5 ± 1.6	46.5 ± 1.6	42.9 ± 1.1
MCV (fl)	62.3 ± 1.1	72.3 ± 1.6	69.3 ± 1.5	69.4 ± 2.4
MCH (pg)	19.1 ± 0.1	19.1 ± 0.1	19.5 ± 0.1	19.0 ± 0.1
MCHC (g/dl)	27.1 ± 0.1	27.1 ± 0.1	27.5 ± 0.9	27.8 ± 0.5
Platelets (cmm)	401166.7 ± 19388.2	391166.7±112388 .2	538166.7± 40707.6	478166.7±660 4.3

Alanine amino transferase (ALT)

Administration of root bark extracts significantly reduced ($P < 0.05$) the ALT level in P, Q and R groups. The effect was most dominant in R group because of high dose (150 mg) of the test plant root extracts. At $t30^{th}$ day the ALT value was 60.4 ± 52.91 mg/dL for P group, 52.7 ± 11.07 mg/dL for Q and 51.5 ± 10.03 mg/dL for R group, respectively as shown in Table-4. Thus it is concluded from the current findings that root extracts of the plant under study reduce the ALT level and have a pronounced effect on liver biochemistry.

Alanine Aminotransferase (ALT) is an enzyme found in many tissues of the body in minor traces like kidney, skeletal muscle, heart, pancreas, spleen, and red blood cells but the largest concentration resides in the liver. Its main role is to catalyze the transferring of amino groups between L Alanine and glutamate to meet physiological requirements of the body. The largest amount of ALT in bloodstream shows that the liver has been damaged due to certain reasons like hepatic cirrhosis, liver tumors, obstructive jaundice. Therefore the measurement of ALT is used in the diagnosing of some sorts of hepatic diseases. (Sherman,

1991). The present results of ALT level in the serum of rabbits administered with M. buxifolia root extracts significantly reduced the ALT level in P, Q and R groups ($P < 0.05$). Increasing the dose effectively decreased the ALT level in the subject animals. Similar results were investigated by Zahra et al. (2012). They analyzed various enzymes of the blood serum including ALT to find out the hepatocurative and hepatoprotective effects of Momordica charantia. They concluded that natural extracts significantly reduce the ALT level.

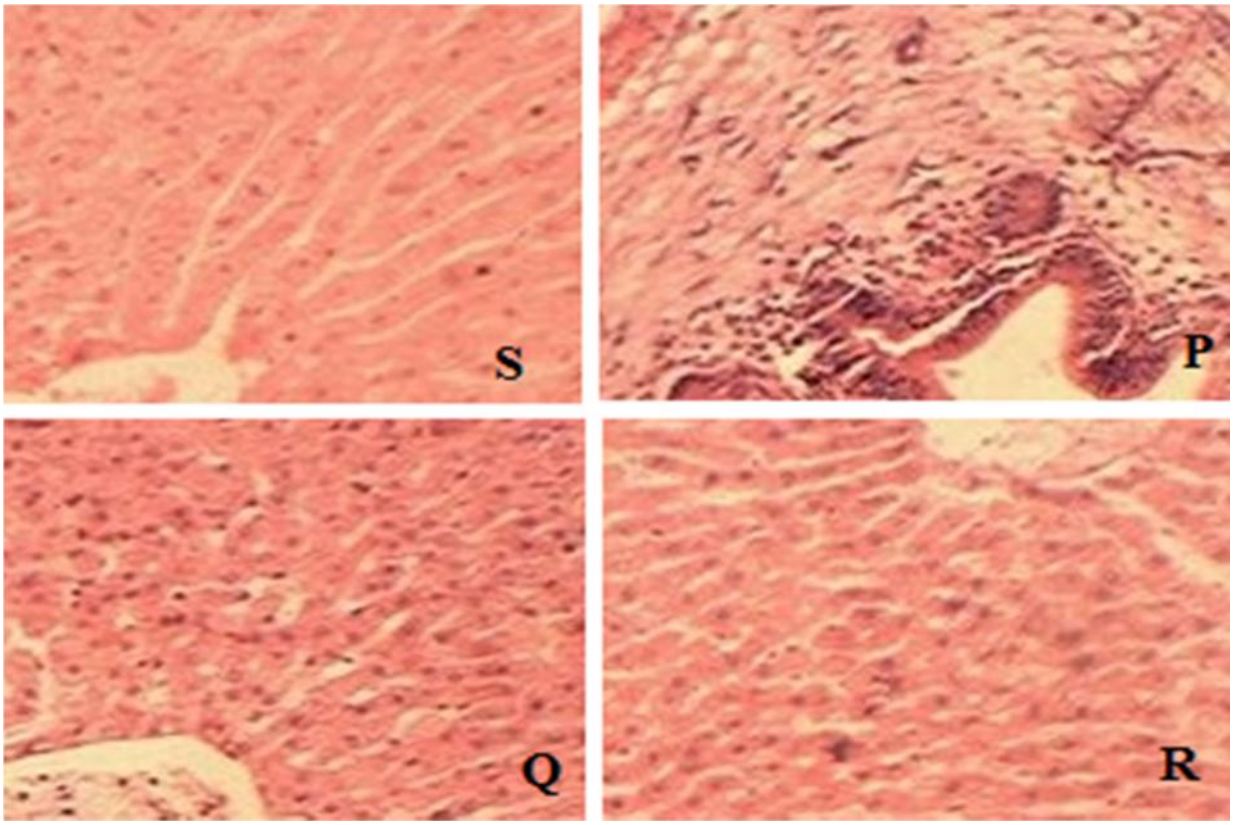

Figure 1. Liver histology of subject rabbits.

Table-4. ALT value of the serum and RSA in brain of the blood after extract administration.

Treatment	Treatment days			Brain RSA
	10	20	30	
Control	70.1 ± 0.06	70.09 ± .074	70.1 ± 0.05	6.79 ± 3.42
P	112.8 ± 50.4	71.91 ± 12.31	60.4 ± 52.91	6.60 ± 3.48
Q	59.6 ± 13.6	55.74 ± 10.21	52.7 ± 11.07	4.27 ± 2.03
R	83.6 ± 3.18	61.29 .± 32.74	51.5 ± 10.03	0.28 ± 0.50

Radical Scavenging Assay (RSA)

In this work a significant reduction ($P < 0.05$) was observed in the RSA values of the brain, liver and muscles tissues of the subject animals. Increasing the dose reduces the RSA values in all the tissues. A dose of 150 mg reduced the RSA value to 0.28 ± 0.50 in brain, 1.23 ± 0.68 in liver and 1.66 ± 0.79 in muscles respectively as shown in Table-4. This result showed that brain was most vulnerable to the effect of M. buxifolia root extracts.

An antioxidant is naturally used to prevent the oxidation of other compounds and molecules. In Oxidation reaction free radicals are produced that lead to a chain reactions. This chain reaction finally leads to cell damage (Halliwell, 1994). Antioxidants from natural sources fight free radicals and protect us from various diseases. They act upon either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Similar results were observed by Gonzalez-santiago et al. (2006). They concluded that free radicals in the body reduce the RSA activity of the subject animals significantly.

CONCLUSIONS

It is concluded from the current study that M. buxifolia root extract decreased the body and organs weight of the subject animals. A significant decrease in TC and ALT was also observed. However, there was increase in HDL-c level, thereby decreasing the risks of CVD. The extracts have also beneficial effect on liver morphology and biochemistry. Similarly the Administration of root bark extracts significantly reduced the ALT value. That needs further investigation. However, there is no significant effect in RSA value. Finally it can be concluded that the body of the animals got improvement in some of the physiological functions upon the administration of root extracts.

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