

Effect of Methanol Extract of Brassica Oleracea on Body Weight and Hematological Parameters in Rabbits

Tahira Zamir¹, Ishrat Younus², Talea Hoor,³ Rabia Arshad⁴

ABSTRACT:

Objective: To evaluate the effect of methanol extract of Brassica oleracea var. capitata on body weight and hematological parameters in rabbits.

Materials and Methods: The study was conducted on 28 healthy white rabbits of either sex. All animals were equally divided into four groups. Animals of test groups were administered methanolic extract of Brassica oleraceae in three different concentrations example 100, 300 and 500 mg/kg body weight respectively for 30 days. Whereas animals of normal control group received normal saline 1 ml/day equivalent to the volume of doses given to test animals. Body weights were recorded weekly on weighing machine. Hematological assay was performed twice once at 16th day and then at the end of dosing that is at 31st day.

Results: Administration of methanolic extract of Brassica oleraceae for a period of 30 days resulted in dose dependent gradual decrease in mean body weight. The reduction was significant (6% P value <0.05) at the dose of 300 mg/kg body weight while highly significant (14% P value < 0.01) at the dose of 500 mg/kg body weight. Hematological assay performed at 16th day showed significant decrease in platelet count as compared to the normal control group at the doses of 300 and 500 mg/kg body weight. Whereas the decrease in platelet count was significant (P<0.05) as compared to the control group at the dose of 300 mg/kg body weight while highly significantly (P<0.01) at the dose of 500 mg/kg body weight at 31st. day on hematological examination.

Conclusion: Methanolic extract of Brassica oleraceae exhibited weight reducing effect in rabbits and decreased the number of platelets in blood.

Keywords: Brassica oleraceae, Body weight, Hematological assay, Rabbits.

INTRODUCTION:

Research on medicinal plants is increasing worldwide showing their vast potential in the field of pharmacology.¹ Various plants have been studied using modern scientific techniques.² Presently, these are reported to be used against a wide range of health problems.³ Natural products have always played a crucial role in the discovery of new biologically active molecules and drug development. It is documented that 25% of all medicines prescribed today are developed from plant source.⁴ Brassica oleracea L. var. capitata (Cruciferae) commonly called cabbage

is a specie of Brassica. It is an inhabitant of Coastal Southern and Western Europe, near limestone sea cliffs and is similar to other Brassica vegetables in constituents.⁵ It is available in wide range of colors like green, red and purple. The most popular types are green, red, savoy and Chinese.⁶ Brassica oleracea is commonly used as a vegetable as well as in the therapy of different diseases all around the world.⁷ It is stated in the literature to have anticancer, antioxidant, antiplatelet and cholesterol lowering activities.⁸ Compounds involved in these actions of Brassica oleracea include isothiocyanates, glucosinolates, phenolics including flavonoids.⁹ Brassica oleracea is rich in antioxidant compounds, which regulate immune system and provide protection against various diseases such as cardiac diseases and cancer. Furthermore, it contains carotene, lutein, and zeaxanthin.¹⁰ Increased body weight can cause many complications in the form of chronic heart disease, diabetes, and stroke.¹¹ Prior studies have suggested that low-fat, plant-based diets reduce body weight, improve cardiovascular risk factors, provide glycemic control, and, in combination with other lifestyle interventions reverse atherosclerosis.¹² Many plants are recommended in the literature for weight loss. Research should be conducted to find out scientific evidence of these claims on plants.¹³ Fresh cabbage juice, prepared either separately or mixed with other vegetables such as carrot and celery, is often included in many commercial weight-loss diets.¹⁴ Hematological assays can be used to determine the effect of the bioactive compounds in the extract on the blood.¹⁵ There is a dire need to investigate these effects of Brassica oleracea, so as to ascertain its safe use. With this background, present study was specifically designed to evaluate the effect of Brassica oleracea L. var. capitata on body weight and hematological parameters in rabbits.

✉ Dr. Tahira Zamir

Assistant Professor
Department of Pharmacology
Bahria University Medical and Dental College
Karachi
Email: drtahiraasad@yahoo.com □

✉ Ishrat Younus

Assistant Professor
Faculty of Pharmacy
Hamdard University
Karachi □

✉ Dr. Talea Hoor

Associate Professor
Department of Pharmacology
Bahria University Medical and Dental College
Karachi □

✉ Dr. Rabia Arshad

Assistant Professor and Head
Department of Pharmacology
Altamash Institute of Dental Medicine
Karachi

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MATERIALS AND METHODS:

This study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi after getting approval from Board of Advance Study and Research (BASR).

Plant material and preparation of extract: Fresh cabbages were purchased from local market in Karachi and identified at Director Centre for Plant Conservation Herbarium and Botanic Garden, University of Karachi. The voucher specimen (H.No.BO-09-12) was deposited in the Department of Pharmacognosy, University of Karachi. The crude extract was prepared through cold extraction process.¹⁶ After thorough washing, 5 kg of Brassica oleracea leaves were chopped into small pieces and dried under shade for about a week. The dried material was ground to coarse powder. This powder was soaked in 80% methanol for 10 days with occasional shaking and stirring. The solvent was filtered through cotton and then through filter paper (What-mann No.1). After filtration, the methanol extract was evaporated under reduced pressure in a rotary evaporator at 40°C - 45°C and then followed by freeze drying at -30°C. The extract so obtained was kept at -20°C until further use. The resultant yield of extract obtained was 19.3% of dry weight.

Animals: The study was conducted on 28 healthy white rabbits of both sexes (1600g to 2200g), housed at the animal house of Department of Pharmacology, University of Karachi, under controlled condition of temperature (22 ± 2C) and humidity (50 to 60%) in an alternating 12-hour of light/dark cycle. The animals were kept in separate cages and were given standard diet and water regularly. The use of animals in this experiment was in accordance with the “National Institute of Health (NIH) Guide for the care and use of laboratory animals.”¹⁷

Preparation of dosage of plant extract: Brassica oleracea extract was given in sterilized water in such a concentration that each 1 ml contained the required dose of the extract in three different concentrations i.e. 100, 300 and 500 mg/kg of the body weight respectively.

Dosing: All animals were uniformly divided into four groups, each, consisting of 7 animals. Three groups were treated as test animals and were given methanolic extract of Brassica oleracea extract in three different concentrations that is 100mg, 300mg, and 500mg. While one group served as normal control, and received normal saline 1ml/day equivalent to the volume of doses given

to test animals. Extract and test drugs were administered continuously for 30 days through oral route once daily at the same time.

Weight measurement: Initially base line body weight (in gm) of all the animals was recorded on weighing machine. Thereafter, it was recorded weekly till the end of dosing

Sample collection: Blood samples of about 5 ml were collected twice, once at 16th day and the other at the end of dosing that is at 31st day through ear veins of animals in all groups in vacuum blood collection tubes that is Ethylene-Diamine-Tetra Acetic acid Vacuette (EDTA, K3) for hematological examination.

Hematological assay: Huma count plus ,a fully automated hematology analyzer (Human Germany) was used to analyze the hematological parameters which includes hemoglobin concentration (Hb), packed cell volume (PCV) mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration (MCHC), red blood cell count (RBCs) ,white blood cell count (WBCs) and ,platelet count (PLT).

Statistical analysis: All values were compared with the control by taking mean and standard error to the mean. Values of P<0.05 were considered as significant and P<0.01 as highly significant following student t test. All statistical methods were performed using SPSS version 17.0.

RESULTS:

Effect on body weight: Results shown that animals received methanolic extract of Brassica Oleraceae in a dose of 100 mg/kg body weight showed insignificant decrease (P value > 0.05) in mean body weight (1783±2.4)at the end of fourth week in comparison to the control animals (1957±4.92). Whereas animals received methanolic extract of Brassica Oleraceae in a dose of 300 mg/kg body weight showed significant decrease in mean body weight (1799±1.2* P value <0.05) at the end of fourth week in comparison to the control animals (1957±4.92). While the administration of methanolic extract of Brassica Oleraceae in a dose of 500 mg/kg body weight showed significant decrease in mean body weight (1800±6.3* P value <0.05) at the end of 3rd week and highly significant decrease in mean body weight (1733±1.02** P value <0.01) at the end of fourth week in comparison to the control animals (1957±0.1 and 1957±4.92 P value > 0.05) respectively.(Table 1)

Table: 1
Mean body weight during one month period in different groups

	Time interval (Weeks)	Control Group	Brassica oleraceae extract in three different concentrations		
		NS1ml/kg body weight	100 mg/kg body weight	300 mg/kg body weight	500 mg/kg body weight
Mean	First week	1958±3.2	1829±6.7	1900±3.2	2015±1.7
Body	Second week	1958±2.9	1823±3.2	1897±1.2	1983±9.2
Weight	Third week	1957±0.1	1800±9.2	1850±2.8	1800±6.3*
(gm)	Fourth week	1957±4.92	1783±2.4	1799±1.2*	1733±1.02**

n = 7 , Average value ± S.E.M , *P < 0.05 as compared to control , **P < 0.01 as compared to control

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Animals at low, moderate and high doses showed 46gm, 101gm and 282 gm decrease in mean body weight respectively at the end of fourth week in comparison to

the control animals (1gm). While the percent decrease in mean body weight was 3%, 6% and 14% respectively in same treated groups in comparison to the control animals (0%).(Table 2)

Table: 2
Effect of methanolic extract of Brassica oleraceae on body weight of rabbits in one month

Effect on body weight	Control Group	Brassica oleraceae extract in three different concentrations		
	NS1ml/kg body weight	100mg/kg body weight	300mg/kg body weight	500mg /kg body weight
Initial Mean body weight	1958±3.2	1829±6.7	1900±3.2	2015±1.7
Final Mean body weight	1957±4.92	1783±2.4	1799±1.2	1733±1.02
Difference (gm)	1gm	46gm	101gm	282gm
Difference (percentage)	0%	3%	6%	14%

n = 7, Average value ± S.E.M, *P < 0.05 as compared to control, **P < 0.01 as compared to control

Table: 3
Base line values of hematological parameters in rabbits of all experimental groups

Hematological parameters	Control Group	Experimental Groups		
	NS 1ml/kg body weight	Group 1 (100mg/kg body Weight)	Group 2 (300mg/kg body weight)	Group 3 (500mg/kg body weight)
Hb g/dl	12.66±0.71	12.81±0.66	12.41±0.21	12.99±0.33
PCV (%)	36.76±2.77	36.43±2.81	38.34±3.21	37.27±3.28
MCV (fl)	63.68±2.83	63.15±1.08	63.67±2.89	62.19±1.67
MCH (Pg)	20±0.04	20±0.01	20±1.32	20±0.08
MCHC (g/dl)	32±0.04	32±0.18	32±0.20	32±0.09
RBC × 10 ⁶ /ul	4.13±0.20	4.13 ±0.18	4.10±0.07	4.17±0.31
WBC × 10 ⁹ /ul	4.8±0.05	4.3±0.04	4.00±0.09	4.7±0.01
PLT × 10 ⁹ /ul	223.51±33	223±9.21	217±12.32*	199.03±3.21*

n = 7, Average value ± S.E.M, *P < 0.05 as compared to control, **P < 0.01 as compared to control

Table: 4a
Effect of Brassica oleaceae on hematological parameters after 15 days

Hematological parameters	Control Group	Treated Groups		
	NS 1ml/kg body weight	100mg/kg body weight	300mg/kg body weight	500mg/kg body weight
Hb g/dl	12.66±0.71	12.77±0.66	12.49±0.21	12.99±0.77
PCV (%)	36.76±2.77	36.46±2.81	38.39±3.21	37.27±3.22
MCV (fl)	63.68±2.83	63.15±1.08	63.67±2.77	62.19±1.67
MCH (Pg)	20±0.04	20±0.06	20±1.66	20±0.09
MCHC (g/dl)	32±0.04	32±0.19	32±0.00	32±0.12
RBC × 10 ⁶ /ul	4.13±0.20	4±0.18	4.10±0.06	4.17±0.37
WBC × 10 ⁹ /ul	4.8±0.05	4.3±0.09	4.00±0.07	4.7±0.00
PLT × 10 ⁹ /ul	223.51±33	223±9.00	222±12.44	224.03±3.0

n = 7, Average value ± S.E.M, *P < 0.05 as compared to control, **P < 0.01 as compared to control

Table: 4b
Effect of Brassica oleaceae on hematological parameters after 30 days

Hematological parameters	Control Group	Treated Groups		
	NS 1ml/kg body weight	100mg/kg/ body weight	300mg/kg body weight	500mg/kg/ body weight
Hb g/dl	12.91±0.56	12.88±0.43	12.98±0.78	11.87±0.3
PCV (%)	36.93±1.2	36.88±2.81	37.31±3.21	37.19±1.00
MCV (fl)	63.15±1.52	63.29±1.00	63.67±1.32	62.93±0.02
MCH (Pg)	20±1.3	20±0.05	20±0.02	20±1.4
MCHC (g/dl)	32±1.2	32±0.19	32±0.18	32±1.3
RBC × 10 ⁶ /ul	4.07±0.29	4.13 ± 0.19	4.08±0.32	4.19±1.2
WBC × 10 ⁹ /ul	4.6±0.02	4.3±0.03	4.7±0.01	4.3±0.03
PLT × 10 ⁹ /ul	221.48±31	220±11.99	193±8.82*	187.31±3.12**

n = 7, Average value ± S.E.M, *P < 0.05 as compared to control, **P < 0.01 as compared to control

Effect on hematological parameters: Base line values of haematological parameters in rabbits of all experimental groups is given in Table 3. Table 4a reveals the comparison of hemoglobin concentration (Hb), packed cell volume (PCV) mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell (RBC) count, white blood cell (WBC) count and platelet (PLT) count in animals of control group and animals received Brassica olerace in three different concentrations i.e 100, 300 and 500 mg/kg after 15 days while a similar comparison between the same groups of animals after 30 days is shown in Table 4b. Animals received methanolic extract of Brassica olerace in the dose of 100 mg /kg body weight for a period of 15 days did not show any change in any parameter. While the animals received methanolic extract of Brassica olerace in the dose of 300 mg/kg body weight for a period of 15 days showed significant decrease in platelet count ($217 \pm 12.32^*$ P value < 0.05) in comparison to the control animals (223.51 ± 33 P value > 0.05). Similarly, the effect on all the other parameters was insignificant. The animals received methanolic extract of Brassica olerace in the dose of 500 mg /kg body weight for a period of 15 days showed significant decrease in platelet count ($199.03 \pm 3.21^*$ P value < 0.05) in comparison to the control animals (223.51 ± 33 P value > 0.05). The effect on all the other parameters was insignificant. Administration of methanolic extract of Brassica olerace extract in the dose of 100 mg/kg for a period of 30 days did not show any change in any parameter. While the administration of methanolic extract of Brassica olerace in the dose of 300 mg/kg body weight for a period of 30 days showed significant decrease in platelet count ($193 \pm 8.82^*$) in comparison to the control animals (221.48 ± 31). The effect on all the other parameters was insignificant. Similarly, the animals received methanolic extract of Brassica olerace in the dose of 500 mg for a period of 30 days showed highly significant decrease in platelet count ($187.31 \pm 3.12^{**}$) in comparison to the control animals (221.48 ± 31). The effect on all the other parameters was insignificant.

DISCUSSION:

Obesity is the fifth leading cause of death worldwide. At least, 2.8 million adults die each year as a result of being overweight. In addition, 44% of the diabetes burden, 23% of the Ischemic heart diseases and between 7% to 14% of some cancers occur due to obesity.¹⁸ Increase in body weight occurs due to imbalance between calories consumed and calories stored. The increased intake of animal's meat, partially hydrogenated fats, refined carbohydrates and lower intake of fiber diet has resulted in increased incidence of obesity and degenerative diseases.¹⁹ On the other hand, the populations that consume pre dominantly plant based diets that is high in whole plant foods, rich in fiber and protective phytochemicals are reported to have less incidence of these diseases.²⁰ Several studies have demonstrated the

effectiveness of plant based dietary patterns in the management of obesity and cardiovascular risk factors.²¹ A variety of natural products including natural extracts and isolated compounds from plants have been reported to cause weight loss.²² Moreover, a nutritional based remedy is an inexpensive solution to weight management.²³

Administration of methanolic extract of brassica olerace caused dose dependent gradual decrease in mean body weight in all the treated animals. However, the reduction was significant at 300 and 500 mg/kg body weight of doses. In literature, Brassica olerace is documented to have hypolipidemic effect in animals. It can be assumed that the weight lowering potential of brassica olerace can be due to its hypolipidemic effect.⁸

Antioxidant compounds are able to reduce the blood levels of glucose, triglycerides and LDL levels, increase energy utilization, fat oxidation, as well as lower body weight and adiposity.²⁴ They are also able to inhibit enzymes involved in lipid metabolism like pancreatic lipase, lipoprotein lipase and glycerophosphate dehydrogenase.²⁵ It can be ascertained that the weight lowering potential of Brassica olerace can be due to its antioxidant activity as mentioned earlier. In the present study, administration of methanolic extract of Brassica olerace resulted in decrease in platelet count. The reduction was highly significant at 500 mg/kg body weight of dose at the end of 30 days of dosing. Flavonoids are reported to have antiplatelet activity.²⁶ Brassica olerace is rich in flavonoids. The antiplatelet effect of Brassica olerace might be due to its flavanoid content as reported for other plants of Brassica family.²⁷ Various mechanisms have been proposed for antiplatelet activity of flavonoids, that is, by lowering intracellular Ca levels, alteration in the metabolism of cAMP and thromboxane A₂.²⁸ Thus it may be inferred that the antiplatelet activity of Brassica olerace might be mediated by these mechanisms. Platelet aggregation leads to pathogenesis of various thrombotic disorders. Moreover, platelets play an important role in the initiation as well as stability of atherosclerotic plaques. Antiplatelet agents are prescribed to patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction.²⁹ The extract therefore can be useful in the management of cardiovascular diseases.

The non-significant effect of the extract on the RBC and WBCs is an indication that the balance between their production and destruction is not disturbed. MCH and MCHC are related to individual's red blood cells while Hb, RBC and PCV are correlated to the total population of red blood cells. The non significant effect of extract on all these parameters could mean that neither the incorporation of hemoglobin into red blood cells, nor the morphology and osmotic fragility of red blood cells were altered.³⁰

CONCLUSION:

Methanolic extract of Brassica olerace exhibited weight reducing effect in rabbits. Moreover, it decreased the total number of platelets in blood. Reduction in platelet

count can be helpful in the prevention of cardiovascular diseases. This study should be conducted on humans also for the development of new herbal drug for the above mentioned effect. Brassica oleraceae can be a nutritive alternative for weight management. Whereas reduction in platelet count produced by brassica oleraceae can be helpful in the prevention of cardiovascular diseases and in other conditions where low platelet levels are required.

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