ORIGINAL ARTICLE:

Effect and Toxicity of Methanolic Extract of Brassica Oleracea on Body Weight of Rabbits

Tahira Zamir¹, Rabia Arshad², Talea Hoor³

ABSTRACT:

Objective: To evaluate the effect and toxicity of methanolic extract of Brassica Oleracea var. capitata on body weight of rabbits.

Materials And Methods: This experimental study was conducted onl4 healthy white rabbits of either sex at department of pharmacology, faculty of pharmacy, university of Karachi in 2011. All animals were equally divided in two groups i.e. control and test group received methanolic extract of Brassica Oleracea in a concentration of 100 mg/kg of body weight for 30 days (4 weeks) through oral route once daily. While control group received normal saline lml/day equivalent to volume of dose given to test animals. Gross toxicity was observed during whole period in animals of both groups. Body weight of animals was recorded weekly on weighing machine.

Results: The animals who received methanolic extract of Brassica Oleracea in a dose of 100 mg/kg body weight showed significant decrease in mean body weight of (1783±2.4)as compared to control animals (1957±4.92). Difference in mean body weight recorded on day 31 (at the end of 4 weeks) was 46gm as compared to animals of control group where decline was only 1 gm. While the percent decrease in mean body weight was found to be (3%) as compared to control animals (0%). Features of gross toxicity such as loss of hair, change in hair color, aggressiveness in behavior. diarrhea, and haematuria, were not found in animals of both groups during whole experimental period.

Conclusion: Methanolic extract of Brassica Oleracea exhibited weight reducing effect in rabbits without any gross toxicity. Keywords: Brassica Oleracea, Methanolic extract, Body weight, Rabbits, Toxicity

INTRODUCTION:

Plants based research is increasing worldwide revealing the immense potential of medicinal plants in the area of Pharmaco-therapeutics. Various medicinal plants have been studied using modern scientific approaches.^{1,2}

Plants as medicinal agents were cited in history dating back many thousands of years.3Presently, these are reported to be used against a wide range of health problems. Plant-derived remedies have been estimated by the World Health Organization (WHO) to be the most frequently used therapies worldwide⁵. Therapeutic agents derived from plants include pure chemical entities available as prescription drugs (e.g., digitoxin, morphine, and taxol) standardized extracts, herbal teas, and food plants; containing phytochemicals with potent pharmacological and toxicological properties⁶. Screening for new drugs in plants implies the screening of their extracts for the presence of novel compounds and investigation of their biological activities.

Over 100, 000 secondary metabolites are known in nature, but only small percentage of all plant species have been studied to some extent for the presence of these secondary metabolites. It is currently estimated that approximately 4, 20, 000 plant species exist in nature but less than 5% of known plants

☑ Dr. Tahira Zamir

Senior Lecturer

Department of Pharmacology

Bahria University Medical and Dental College

Karachi.

Email: drtahiraassad@yahoo.com

☑ Dr. Rabia Arshad

Assistant Professor

Department of Pharmacology

Sir Syed College of Medical Sciences

Karachi.

☑ Dr. Talea Hoor

Assistant Professor Department of Pharmacology

Bahria University Medical and Dental College

Received: April 10, 2014 Revised: April 16, 2014 Accepted: May 02, 2014

only have been screened for one or more biological activities⁷.

Brassica Oleracea L. var. capitata (Cruciferae) commonly called cabbage (figure 1) is a species of Brassica native to Coastal Southern and Western Europe, naturally occurring near to limestone sea cliffs, similar in composition to other Brassica vegetables. It is available in various shades of green, red or purple. The most popular varieties are green, red, savoy and Chinese.

Figure 1 Brassica Oleracea34



Bras Oleracea is widely used as a vegetable and remedy for different diseases all around the world¹⁰ It is found to have anticancer, antioxidant, antiplatelet and antihypercholesterolemic activities11, 12. It has been found to attenuate bronchoconstriction and inflammation by virtue of its anti anaphylactic activity¹³. Compounds responsible for these activities of Brassica Oleracea include isothiocyanates and their cognate glucosinolates, phenolics including flavonoids and other non-nutrients.14 Brassica Oleracea is a rich source of antioxidant nutrients, which regulate immune system and protect against various diseases such as heart disease and cancer. Furthermore, it contains 13 caroteine, lutein and zeaxanthine¹⁵. Increase in body weight leads to many complications in the form of chronic heart disease, diabetes, and stroke16. Every year millions of people diet to reduce

weight but the continuing response of this is poor as in reaction to food deprivation drive to eat increases and the metabolism slows down there by neutralizing the effects of dieting¹⁷.Plants have achieved significant position in health care system all over the world not only in the diseased condition but also as promising agent for maintaining proper health18 Many plants are documented in the literature for weight loss. 19Studies have recommended that ow-fat, plant-based diets decrease body weight, improve cardiovascular risk factors, provide glycemic control, and, in combination with other lifestyle modifications reverse atherosclerosis.20 With this background, present study was specifically designed to evaluate the effect of Brassica Oleracea L. var. capitata on body weight of rabbits along with any gross toxicity.

MATERIALS AND METHODS:

This experimental study was conducted in the Department of Pharmacology, Faculty of Pharmacy and University of Karachi after approval from Board of Advance Studies and Research (BASR) in 2011 as per fulfillment of requirement for M-Phil degree. Plant Material And Preparation Of Extract: Fresh cabbages were purchased from local market in Karachi and identified by Prof. Anjum Parveen, Director Centre for Plant Conservation Herbarium and Botanic Garden, University of Karachi, Karachi-75270. 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 process21 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 (Whatmann 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 325 g.

Animals:

The study was conducted on 14 healthy white rabbits of either sex (1500-2200g), housed at the animal house of Department of Pharmacology, University of Karachi, under controlled condition of temperature (22±2°C) and humidity (50to60%) in an alternating 12-h 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 Animals²².

Preparation Of Dosage Of Plant Extract: Methanolic extract of Brassica Oleracea was given in sterilized water such that each 1 ml contained the 100 mg /kg body weight dose of the extract. Dosing:

All the animals were equally divided in two groups i.e. control and test groups. Test group received methanolic extract of Brassica Oleracea in a concentration of 100mg/kg body weight. Extract was administered to animals of test group continuously for 30 days (4 weeks) through oral route once daily. While the animals of control group received normal saline per orally, imi/day equivalent to the volume of dose given to test animals.

Body Weight Measurement:

Initially base line body weight of all the animals of both groups was recorded. Thereafter, it was recorded daily and mean body weight was calculated at the end of each week till four weeks on weighing machine till the end of dosing. The difference in mean body weight of animals was calculated by subtracting the final mean body weight of animals from initial mean body weight of animals. Formula for calculating difference in mean body weight and percent decrease in mean body weight was adapted from the study of Hisham.²³

Gross Toxicity:

During the whole experimental period, all the animals used were subjected to a detailed gross examination that included careful examination of the external surface of the body and all orifices. Features of gross toxicity like loss of hairs, change in hair color, behavioral changes and loss of activity, diarrhea, hematuria and sedation were also observed.

STATISTICAL ANALYSIS:

All values were compared with the control by taking mean and standard error to the mean (Mean \pm S.E.M) using one sample t-test. P- value <0.05 in comparison to the control were considered significant.

RESULTS:

The animals who received methanolic extract of Brassica Oleracea in a dose of 100 mg/kg body weight showed significant decrease in mean body weight of (1783±2.4) in comparison to the control animals (1957±4.92).(Table 1) The difference in mean body weight from initial mean body weight of animals recorded on day 31 (at the end of four weeks) was 46gm in comparison to animals of control group where decline was only 1 gm.(Table 2) While the percent decrease in mean body weight was found to be (3%) in comparison to the control animals where it was (0%).(Table 3a, 3b). No gross toxicities were observed in any animal including control during the total period of experiment.

Table 1
Baseline Body Weight of Animals
N=14

No.of Animals	Control group n=7 Body Weight (gm)	Test group n=7 Body Weight (gm)
1	2000	2200
2	2100	2000
3	1800	1500
4	2000	1600
5	1700	1503
6	2100	2000
7	2000	2000

Table 2
Mean Body Weight of Animals per Week
N=14

Number of Weeks	Control group n=7 Mean body weight (gm)	Test group n=7 Mean body weight (gm)
First week	1958±3.2	1829±6.7
Second week	1958±2.9	1823±3.2
Third week	1957±0.1	1800±9.2
Fourth week	1957±4.92	1783±2.4

Table 3a
Comparison of Mean Body Weigh
N=14

Mean body weight (gm)	Control gruup	Test group	P-value
Initial mean body weight	1958±3.2	1829±6.7	2.37
Final mean body weight	1957±4.92	1783±2.4*	0.04

n=7

Average value \pm S.E.M

Table 3b
Difference in Mean Body Weigh
N=14

Mean body weight (gm)	Control gruup	Test group
Difference in gram (gm)	1gm	46 gm
Difference in percentage (%)	0%	3%

DISCUSSION:

The World Health Organization describes the "escalating global epidemic" of obesity as "one of today's most blatantly visible yet most neglected public health problem". According to WHO, globally 1.5 billion people were reported to be overweight in the year 2008. Among which, over 200 million people were males and nearly 300 million were females. There have been two major reasons for increased body weight. Firstly, an increased intake of energy rich foods that are high in fat, salt and sugar content but low in vitamins, minerals and other micronutrients, and secondly, decrease in physical activity due to the increasingly sedentary life style, changing modes of

transportation and increasing urbanization.²⁴
Obesity is the fifth risk factor for death worldwide
Annually approximately 2.8 million people die due
to obesity. Moreover, 44%cases of diabetes, 23 %
cases of ischemic heart diseases and 7 to 41% cases
of cancers are caused by increase in body weight.
It is a major threat to human health. Increased body
weight put stresses on almost every individual part
of human body. It can lead to a variety of health
problems like bone and joint disorders, gall stones,
liver problems, coronary heart disease, congestive
heart failure, stroke, increased blood pressure
increased blood lipid levels, increased blood suga
and sleep problems. Overall work efficiency of
human being is suffered due to being overweight.

^{*}P<0.05 as compared to control

Worldwide change in dietary habits by taking more animal diet, partially hydrogenated fats, refined carbohydrates and less consumption of fibers has resulted in increased obesity and degenerative diseases. 200n the contrary the populations that consume plant diet are reported to have less incidence of these diseases. 27 Several studies have confirmed the usefulness of plant based diet in the management of obesity and cardiovascular risk factors. 28,29

Common treatment measures for weight loss include dietary changes, increase physical activity, behavioral changes, and anti-obesity medications. The preferred treatment modality for reducing weight is to change the dietary habits and increase the physical activity.³⁰

Anti-obesity drugs are generally effective, but severe adverse toxicities limit their usefulness. Herbal products are being extensively utilized because of their less side effects in comparison to chemically synthesized drugs. Many studies have documented that herbal products are less likely to cause toxicity, are effective in reducing appetite and promoting significant weight loss. 22

In our study, administration of methanolic extract of Brassica Oleracea in a dose of 100 mg/kg body weight decreased the mean body weight in all the test animals in comparison to the control animals. The difference in mean body weight from initial mean body weight of animals recorded on day 31 (at the end of 4 weeks) was 46gm (3%) in comparison to animals of control group where decline was only 1 gm (1%). Decrease in body weight of animals indicates that Brassica Oleracea reduces body weight.

In literature, it is documented that Brassica Oleracea has hypolipidemic effect in animals. It can be assumed that the weight lowering potential of Brassica Oleracea might be due to its hypolipidemic effect¹¹.

As mentioned earlier, Brassica Oleracea is a rich source of protective phytochemicals. In one of the studies, it is stated that the weight lowering potential of plants is not merely due to their hypolipidemic effect but also due to the presence of phytochemicals in them. Therefore, weight lowering potential of Brassica Oleracea might also be contributed by the presence of phytochemicals¹².

Antioxidant compounds have the ability to decrease the levels of glucose, triglycerides and LDL, increase fat oxidation and lower body weight. They can also inhibit enzymes associated with fat metabolism.³³1t can be ascertained that the weight lowering potential of Brassica Oleracea might also be due to its antioxidant activity.

It has been documented that Brassica Oleracae also have hypoglycemic effect in animals.³⁵The weight reducing ability of Brassica Oleracae might be due to its hypoglycemic effect .However, present study has not evaluated this effect.

Increasing awareness and consumption of plant derived products justify evaluation of their safety. We have found no gross toxic effect like loss of hairs, change in hair color, haematuria, aggressiveness in behavior, loss of activity and diarrhea in any animal during the whole experimental period. This indicates that Brassica Oleracea was completely safe at the administered dose and may be used as a nutritive alternative for weight management without any gross toxicity. Thus weight reducing effect of Brassica Oleracae can be attributed to combination of its phytochemical nature, hypolipidemic, antioxidant and hypoglycemic activities.

CONCLUSION:

Methanolic extract of Brassica Oleracea exhibited weight reducing effect in rabbits without any gross toxicity. Studies with large sample size using different doses in normal, obese and diseased animals should be conducted.

ACKNOWLEDGEMENTS:

The principal author is thankful to the junior staff of Pharmacology department, Karachi University especially Mr. Kashif, Mr. Anis and Mr. Ibrahim for their kind assistance in the completion of this work. I am also thankful to Madam Afroz for her guidance and encouragement during the study.

REFERENCES:

- Dahanukar, S. A., Kulkami, R. A. Rege, N. N. Pharmacology of Medicinal Plants and Natural Products. Indian J. Pharm 2000; 32: 5 18-81.
- 2. Auddy, B., Ferreiro, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F et al. Screening

- of Antioxidant Activity of Three Indian Medicinal Plants Traditionally used for the Management of Neurodegenerative Diseases. J. Ethnopharm 2003; 84:131-8.
- Rasonavivo P. Petitjean A. Ratsimamanga P. Urverg S. Pakoto R A. Medicinal plants used to treat malaria in Madagascar. J Ethnopharacol 1992; 37:117-27.
- Jimenez- Arellanes A, Meckes M, Ramireez R, Torres J, Luna - Herrera J. Activity against multidrug-resistant mycobacterium tuberculosis in Mexican plants used to treat respiratory diseases. J Phytother Res 2003; 17: 903-08.
- Marini-Bettolo, GB. Present aspects of the use of plants in traditional medicine. JE thnopharmacol 1980; 2:183-8.
- Croom, EM Jr.: Documenting and evaluating herbal remedies. Economic Botany, 1983; 37: 13-27.
- Verpoorte, R., Ven der Heij den, R.: Memelink. Engineering secondary metabolite production in plants. J. Transgenic Res, 2000; 9:323-43.
- Gross D. Indole Phytoalexins from Brassica Oleracea var. gongylodes. Planta Med 1994; 59: A-618.
- Byers T, Nestle M, McTieman A. American Cancer Society 2001 Nutrition and Physical Activity Guidelines Advisory Committee. American cancer society guidelines on nutrition and physical activity for cancer prevention: Reducing the risk of cancer with healthy food choices and physical activity. CA. Cancer. J. Clin 2002; 52: 92-119.
- Kris-Etherton PM, Hecker KD, Bonanome A. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med 2002; 113: 71S-88S.
- Ang-Lee MK, Moss J, Yuan CS. Herbal medicines and perioperative care. JAMA 2001;286: 208-16.
- Waqar MA, Mahmood Y. Antiplatelet, antihypercholesterolemic and antioxidant activities of Brassica Oleracea in high fat diet provided rats. World. Appl. Sci. J 2010; 8:107-12.
- 13. Ambrosone CB, McCann SE, Freudenheim

- JL. Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. J. Nutr 2004;134:1134-8.
- Jeffery EH, Araya M. Physiological effects of broccoli consumption. Phytochem Rev2009;8:283-98.
- Kane MP, Abu-Baker A, Busch RS. The utility of oral diabetes medications in type 2 diabetes of the young. Cuff. Diabetes Rev 2005; 1: 83-92.
- Hasani-Ranjbar S, Jouyandeh Z, Abdollahi M. A systematic review of anti-obesity medicinal plants - an update. J Diabetes Metab Disord 2013;12 (1):28. doi: 10.1186/225 1-6581-12-28.
- 17. Dulloo AG, Jacquet J. Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. Am J Clin Nutr 1998;68: 599-606.
- 18. Dickel ML, Rates SM, Ritter MR. Plants popularly used for losing weight purposes in Porto Alegre, South Brazil. J Ethno Pharmacol 2007; 109(1):60-71.
- 19. Appleby PN, Thorogood M, Mann JI, Key TJ. Low body mass index in non-meat eaters: the possible roles of animal fat, dietary fibre and alcohol. mt J Obes Relat Metab Disord 1998; 22:454-60.
- Nicholson AS, Sklar M, Barnard ND, Gore S, Sullivan R, Browning S. Toward improved management of NIDDM: a randomized, controlled, pilot intervention using a low fat, vegetarian diet. Prey Med1999; 29: 87-91.
- 21. Hossain MS, Ahmed M, Islam A. Hypolipidemic and hepatoprotective effects of different fractions of ethanolic extract of immature leaves of Mangiferaindica (Linn.) in alloxan induced diabetic rats. IJPSR 2010: 1: 132-8.
- National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press, 1996: 1-7.
- Hisham M. Osman, Mohamed E. Shayoub, Elsiddig M. Babiker. The effect of Moringaoleifera leaves on Blood parameters

- and Body weights of Albino rats and Rabbits. Jordan journal of Biological Scinces 2012; 5 (3):147-50
- 24. World Health Organization "Obesity, preventing and managing the global epidemic, report of a WHO consultation (Who Technical Report Series 894)"who, 2000.
- 25. Centre for public health excellence at NICE(UK), National collaborating centre for primary care (UK), "Obesity, the prevention, identification, assessment and management of overweight and obesity in adults and children" National institute for health and clinical Ecellence (UK) (NICE) Clinical guidelines, No. (43), 2006. http://www.ncbi.nlm.nilo.gov/books/NBK 63696/
- Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. Am J ClinNutr 2006; 84:289-98.
- 27. Connor WE, Duell PB, Connor SL: Benefits and hazards of dietary carbohydrate. Cuff Atheroscier Rep 2005; 7:428-34.
- 28. Gardner CD, Coulston A, Chatterjee L: The effect of a plant-based diet on plasma lipids in hypercholesterolemic adults: a randomized trial. Ann Intern Med 2005; 142:725-33.
- 29. Jenkins DJ, Kendall CW, Faulkner DA.

- Assessment of the longer-term effects of a dietary portfolio of cholesterollowering foods in hypercholesterolemia. Am J Clin Nutr 2006; 83:582—91.
- 30. Chandrasekaram, Vijayalakshin, Prakesh, Banasal, Meenakshi. Review article: Herbal approach for Obesity management. American Journal of Plant Sciences 2012; 3:1003-14.
- 31. Benort, P. Herve, P, Petitpretz, F. Parent, P duroux, G simonneav, "Primary pulmonary hypertension and Fenfluramine Use" British inert Journal 1993; 6:537-41.
- 32. Amin, Nagy. "Effect of carnitine and herbal mixture extract on obesity induced by high fat diet in rats" Dibetology and Metabolic syndrome, 2009;1(17):1-14
- 33. Garcia-Lafuente, a.; Guillemin, e, E; Villars, a.; Rostagno, M.A.; Martinez, J.A. Flavonoids as anti-inflammatory agents; Implications in cancer and cardiovascular disease. Inflamm res 2009; 58:537-52.
- 34. He alth Food and Nutrition.http;//vladimiriablogspot.com/20 10/03/cabbage-benefits-for-health.html.
- 35 Hazem A.H, Kataya and AlaaEldin A, Hamza. Red cabbage ameliorates diabetic nephropathy in rats. Evidence based complimentary medicine 2008; 5(3):281-7.