Biomedical & Pharmacology Journal Vol. **8**(Spl. Edn.), 715-719 (Oct. 2015)

Effects of the Alcoholic Extract of White Mulberry Leaves on Behavioral Performance of Rats

BAHRAMI ABBAS[1], JONEIDI-JAFARI AHMAD[2], ASILIAN HASAN[1], TAGHIZADEH MOHSEN[3], ARDJMAND ABOLFAZL[4] and AKBARI HOSEIN[5]

[1]Occupational Health Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
[2]Environmental Health Engineering Department, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[3]Nutrition Department, Faculty of Health, Kashan Medical Sciences and Health Services University, Kashan, Iran.
[4]Physiology Department, Faculty of Health, Kashan Medical Sciences and Health Services University, Kashan, Iran.
[5]Public Health Department, Faculty of Health, Kashan Medical Sciences and Health Services University, Kashan, Iran.

DOI: http://dx.doi.org/10.13005/bpj/774

(Received: August 15, 2015; accepted: September 20, 2015)

ABSTRACT

White mulberry tree is a genus of Morus in the family Moraceae. The leaves and root of this tree have been traditionally medical applications as a laxative, analgesic, diuretic, antitussive, and sedative agent as well as for reducing blood pressure. The present study aimed to investigate the effects of the alcoholic extract of the leaves of white mulberry tree on the behavioral performance of rats. In this study, 24 male Wistar rats were kept under the condition of normal 12-12 h light-dark cycle for adaptation with the animal house environment and experiments location (23- 27C^0). All animals have access to food and water ad libitum and were weighed weekly to control their natural growth. These rats were divided into four groups of six rats. First, the open field behavioral test was performed on all rats and after 35 days the gavage feeding of the alcoholic extract of white mulberry leaves was performed. Group one received distilled water and groups two to four respectively received concentrations of 150, 300, and 600 mg per unit body weight of the alcoholic extract white mulberry leaves. Then, open field behavioral test was performed again. Results showed that regarding the number of visited zones there is a significant difference between the control group and the groups receiving the extract. Up to the sixth week that the condition of all rats was identical the rate of weight increase was the same and from the seventh week on that the gavage of the hydro-alcoholic extract of white mulberry leaves began, the trend of the weight increase of the control group was different than that of the other groups. due to the effect of the alcoholic extract of white mulberry leaves on the weight of the rats at concentrations above, it can be concluded that the extract is effective for weight loss. Findings of open field test showed that this extract has anti-anxiety effects while imposing no toxic effects.

Key words: White mulberry leaf, Hydro-alcoholic extract, Open field test, Rat

INTRODUCTION

White mulberry tree is from family Moraceae and genus Mours that is cultivated for the purpose of feeding silk worms. Leaves and root of this tree have traditionally had medical applications and were used as laxative, analgesic, diuretic, antitussive, and sedative and to reduce blood pressure[1]. In addition, it has anti-diabetes, fat reducing, anti-microbial, and anti-oxidant properties[2]. White mulberry contains significant amounts of protein, carbohydrates, fat, fibre,

minerals, and some vitamins or provitamin substances, which based on dry weight of the leaves it contains 15.31 - 30.9 percent protein, 2.09 - 7.92 percent fat, 9.9 - 13.85 percent fiber, 27.6 - 43.6 non-dietary fibre, and 11.3 - 17.24 percent minerals[3]. In different studies performed on hydro-alcoholic extract of white mulberry leaves, varying results have been presented. Nade V. S. *et al.,*. (2015) in their studies with the goal of evaluating the anti amnesia properties of the ethyl acetate solution of the white mulberry leaves alcoholic extract determined that this part of the extract significantly improves learning and memory in rats and concluded that white mulberry is useful in the treatment of dementia and other cognitive disorders[4]. Hamzaa R. G. *et al.,* (2012) studied the antioxidant properties of white mulberry on the created oxidative stress in rats that were affected by gamma radiation, they concluded that white mulberry can potentially have protective effects against oxidative stress[5]. In Girish P. Laddha and G. Vidyadagar (2012) study to evaluate the anti psychotic properties of aqueous extract of white mulberry concluded that oral aqueous extract of white mulberry up to 2000 mg/kg has no toxicity and has the capability of the inhibition of serotonin and can be used as an antipsychotic agent[6]. The objective of this study is to evaluate the effect of the alcoholic extract of white mulberry on behavioral performance of rats.

MATERIALS AND METHODS

Twenty-four male Wistar rats were obtained from the Center for Laboratory Animal Production of the Physiology Research Center of Kashan University of Medical Sciences. To adapt with animal house environment and test location, they were kept in 12 hours light and 12 hours darkness condition for one week. All rats had access to water and food freely and were inspected and evaluated on a daily basis and were also weighed weekly to control their normal growth. These rats were divided into four groups of six rats. Group number one received distilled water and groups two to four respectively received the concentrations of 150, 300, and 600 milligrams per each kilograms of their body weight of the alcoholic extract of whit mulberry leaves. At first open field behavioral test was performed on all rats and after 35 days the oral gavage of the alcoholic extract of white mulberry leaves began and at the end of the thirteenth week of the beginning of the study the open field behavioral test was performed again.

The preparation of the alcoholic extract of white mulberry leaves

One kilogram of the leaves of white mulberry (Morus alba) was obtained from domestic trees and was dried in the shade at a temperature of 5 degrees Celsius and in a calm wind. After grinding, it was placed in the percolator and 70% ethylic alcohol was slowly added to it until alcohol covered its surface completely, after two hours alcohol was poured again in order to compensate for the receded alcohol surface. This operation was repeated a few times until at least three centimetres above leaves powder was covered in alcohol. Then it was placed at room temperatures for 72 hours. Then the valve of percolator was opened and the extract was separated. It was then placed inside the incubator with 50 degrees centigrade temperature in order to be concentrated. After determining the dry weight, 150, 300, and 600 milligrams per each kilogram of the body weight of the rat was prepared.

Open field behavioral test

To perform this test, Maze Route software that is an easy to use visual tracking system for behavioral analysis of laboratory animals was used. To do this, first the open field test section was opened and the intended protocol was selected so that each rat was placed in a square box with 60 by 60 by 30 dimensions for five minutes. The software divides the bottom of this box into 25 regions and each region is considered a zone. The software determined the time spent in each region in seconds, the number of visits into each region, and the distance traveled in each region in centimetres relative to the total distance traveled during this five minute period. The acquired data were analyzed using SPSS software version 16.

RESULTS

In this study 24 two month old male rats in the weight range of 170-180 grams were placed in four groups in a 13 weeks period. The results showed that in all intervention groups the mean of

Table 1: Mean and standard deviation of the weight the rats in four intervention groups from the start of the study to week thirteen

Groups Week	Control	150 mg/kg	300 mg/kg	600mg/kg
1	179 (14.7)	177.7(12.5)	184(9.5)	185.3(6.9)
2	195(13)	194.7(8.5)	204(8.6)	202(8.6)
3	220(16.5)	188.7(5.3)	192.3(10.8)	191.5(9.7)
4	216.7(17.9)	197.5(7.4)	200.7(11.6)	200.8(12.2)
5	223.5(17.7)	227.3(8.1)	218.5(13.7)	224.7(12)
6	236.7(18.3)	222.8(6.5)	220.5(14)	214.3(16.3)
7	206(19.1)	219.7(9.1)	222.5(15.2)	231.2(15.1)
8	246(19)	241.2(8.7)	243.5(14.8)	249.2(14.4)
9	273.3(2.2)	247.5(10.1)	254(16.5)	256.2(19.3)
10	282.8(23.8)	254.5(9.6)	260.5(15.7)	256.2(19.4)
11	287.7(20.9)	262.8(9.3)	266(17.5)	270(20.6)
12	296.7(24.2)	260.7(9.2)	270(17.4)	266.2(19.6)
13	296.5(19.6)	271.3(10.7)	270(18.2)	278(19.8)
P-value	<0.001	<0.001	<0.001	<0.001

Table 2. Mean and variance of the traveled distance before and after receiving alcoholic extract of the white mulberry leaves in the studied groups

Study period Groups	Before gavageX±S.D	After gavageX±S.D	P.value
Control	6.83 ± 16.5	5.24±16.32	0.942
Low dosage	1.41± 18	.174± 10.67	0.002
Average dosage	2.8± 21.67	4.04± 15.5	0.001
High dosage	.464± 19.5	1.75±10.33	0.001

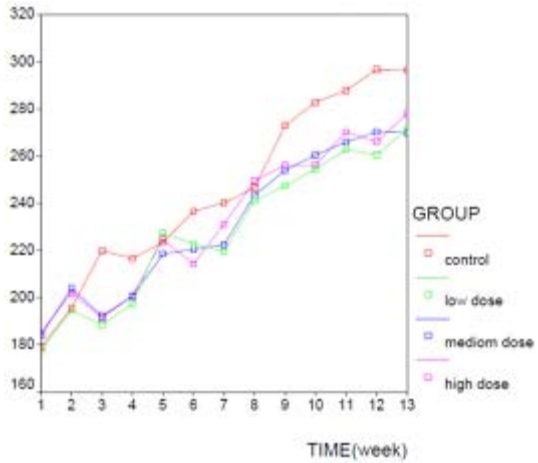

Fig. 1: The rate of the weight increase of the rats under study from the first to the thirteenth week

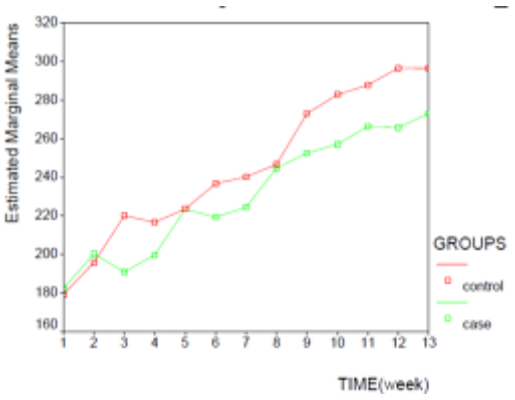

Fig. 2: The weight increase rate of the averaged studied groups compared with the control group

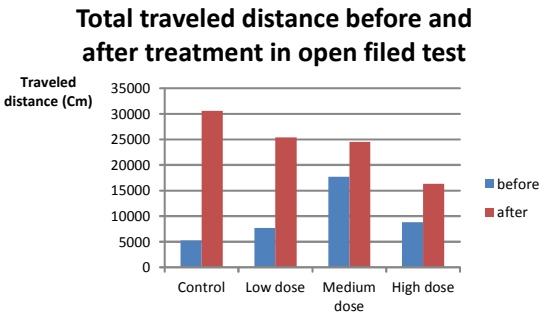

Fig. 3: The sum of the traveled distance in the studies groups of rats before the start of the study and at the end of the study

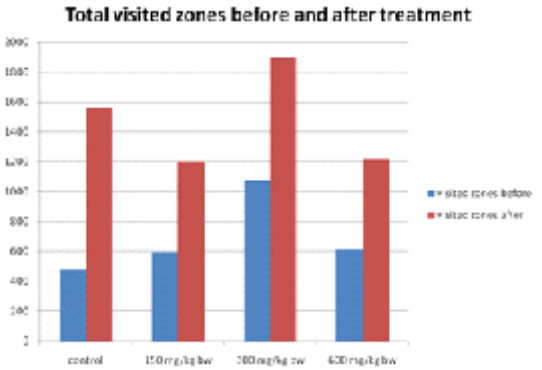

Fig. 4: The sum of the visited zones in the open field test before the beginning of the studay and at the end of the study

the weight of the rats during the study period had significant increase (P<0.001). The highest mean of the weight increase of rats was in the control group such that it reached from 179.1 grams at the beginning of the study to 296.5 grams at the end of the thirteenth week (P<0.0001), and the lowest amount of the weight increase was in the intervention group with 300 mg/kg bw dosage of white mulberry leaves alcoholic extract. However, no significant differences in the changes in the weight of the rats between the three intervention groups was observed (P=0.804). The trend of averaged weights of the studied rats under study during 13 week period of the experiments in the four groups is presented in Table 1.

The trend of the weight change of the rats is presented in Fig. 1. The results show that until the sixth week the conditions of all rats were identical and the weight increase trend has been identical. From the seventh week when the white mulberry leaves hydro-alcoholic extract gavage has been started, the increasing rate of the weight of the control group is different compared to the other groups.

The data of all treatment groups were average and compared with the control group all extract receiving groups in comparison with the control group, the difference in the growth rate from the beginning of giving the hydro-alcoholic extract of white mulberry leaves to them compared with the control group that received distilled water (Fig.2).

The results of the open field behavioral test are presented in Table 2 and Figs 3 and 4.

DISCUSSION

The results of the weekly weighing of the studied rats showed there is a significant difference between the groups receiving the hydro-alcoholic extract of white mulberry leaves and the control group (P<0.001). The results showed that up until the sixth week that the condition of all rats was identical the rate of the weight increase has been the same and from the seventh week that the gavage of the hydro-alcoholic extract of white mulberry leaves started the weight increase of the control group is different from that of other groups but there is no significant difference between extract receiving groups with regard to weight.

Wang *et al.,* (2010) reported that weight changes in different studied groups were not significant compared with the control group[7]. In this study the powdered fruit of the white mulberry was used as the food supplement. Similarly, Singab *et al.,* (2005) found no significant difference in the weight between the control group and the treatment group receiving the extract of the root of the mulberry tree[8]. This inconsistency can be attributed to the differences in the methods of extraction from roots and leaves. Laddha *et al.,* (2012) investigated the effects of aqueous extract of the leaves of white mulberry on the weights of Swiss and Wistar rats and observed no significant effects[6]. In contrast,

Nagalakshmamma *et al.,* (2012) demonstrated that one of the white mulberry properties is anti-obesity[9]. In Oh *et al.,* (2012) it is stated that the hydro-alcoholic extract of white mulberry leaves causes body weight loss[10]. Contrary, Hamdy (2012) investigated the effect of the hydro-alcoholic extract of white mulberry leaves on the enzymatic activities of rats and reported a significant weight increase in the animals[11].

The results of the open field test showed that only the number of visited zones was significantly different between control group and the treatment group which received hydro-alcoholic extract of white mulberry leaves ($P<0.05$). Gupta *et al.,* (2013) showed that groups receiving the hydro-alcoholic extract of white mulberry leaves had a significant difference with regard to the number of visited zones, compared with the control group[2]. It should be noted here that in this study white Swiss rats where used. Yadav *et al.,* (2008) injected the alcoholic extract of white mulberry leaves intraperitoneally with concentrations 50, 100, and 200 mg/kg of body weight to Swiss rats. The results of the open field test showed that there was a significant difference between all groups that received the extract and the control group regarding the visited zones ($P<0.05$)[3]. Gupta *et al.,* (2013) showed that the extract of the white mulberry leaves has therapeutic effects on anxiety Comparing our findings with other similar studies on weight reducing effects as well as anti-anxiety, with no toxic effects of this extract, we suggest conducting further studies on the weight loss and anti-anxiety effects of this extract on human while observing ethical aspects.

ACKNOWLEDGEMENTS

We hereby would like to show our gratitude and appreciation to the research deputy of the Kashan University of Medical Sciences and the Physiology Research Center of the Kashan University for their full support in conducting this study.

REFERENCES

1. Yadav A, Kawale L, Nade V. Effect of Morus alba L.(mulberry) leaves on anxiety in mice. *Indian journal of pharmacology*. **40**(1):32 (2008).
2. Gupta G, Kazmi I, Anwar F. Anxiolytic activity of Moralbosteroid, a steroidal glycoside isolated from Morus alba. 2013.
3. Butt MS, Nazir A, Sultan MT, Schroën K. Morus alba L. nature's functional tonic. *Trends in food science & technology.* **19**(10): 505-12 (2008).
4. Nade V, Kawale L. International Journal of Pharmaceutical Sciences and Drug Research.
5. Hamzaa R, El Shahat A, Mekawey H. The antioxidant role of mulberry (Morus alba L.) fruits in ameliorating the oxidative stress induced in γ-irradiated male rats. *Biochem Anal Biochem.* **1**(122): 2161-1009 (2012). 1000122.
6. Vidyasagar GPLaG. anti-psychotic effect of aqueous leaves extract of morus alba in animal models. *International Journal of Pharmacy.* **2**(3): 513-9 (2012).
7. Yang X, Yang L, Zheng H. Hypolipidemic and antioxidant effects of mulberry (Morus alba L.) fruit in hyperlipidaemia rats. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. **48**(8-9): 2374-9 (2010).
8. Singab AN, El-Beshbishy HA, Yonekawa M, Nomura T, Fukai T. Hypoglycemic effect of Egyptian Morus alba root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. *Journal of ethnopharmacology*. **100**(3):333-8 (2005).
9. Nagalakshmamma K. History and active pharmacokinetic principles of mulberry.
10. Oh K-S, Ryu SY, Lee S, Seo HW, Oh BK, Kim YS, *et al.,* Melanin-concentrating hormone-1 receptor antagonism and anti-obesity effects of ethanolic extract from Morus alba leaves in diet-induced obese mice. Journal of ethnopharmacology. **122**(2):216-20 (2009).
11. Hamdy SM. Effect of Morus Alba Linn extract on enzymatic activities in diabetic rats. *Journal of Applied Sciences Research.* **8**(1):10-6 (2012).