Ekoloji 28(107): 175-181 (2019)

# Cotoneaster Manna Effects on Animal Model of Aggression and Depression like Behaviors in the Environment

**Shirali Kharamin [1], Heibatollah Sadeghi Mansoorkhani [2], Roggayeh Kharamin [3], Rezvan Babamir [3], Hashemi Mohammad Abad Nazir [4*]**

[1] PhD. in Clinical Psychology, Assistant professor, Yasuj University of Medical Sciences Medicinal Plants Research Center, Yasuj, IRAN

[2] Professor at School of Medicine, Medicinal Plants Research Center, Yasuj, IRAN

[3] Student of Yasuj Smart High School, Yasuj, IRAN

[4] Department of Psychiatry, Faculty of Medicine, Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, IRAN

* Corresponding author: nazir.hashemi@gmail.com

**Abstract**

**Objectiv**e: complementary medicine (CAM), especially herbal medicine, is widely used in worldwide. Although use of herbal medicine among patients with mental disorders seems common, limited scientific data exist about the efficacy, side effects and safety of these herbs. In other hands, there are also concerns about the possible negative psychiatric effects. Cotoneaster manna, as an herb, has been used pervasively in traditional and modern medicine for treatment of physical disorders as therapeutic and protective medicine. This study was established to assess domestic common believes and concerns (especially psychiatric problem side effects) about this herb and also assess its effects on animal model of aggression and depression **Methods and materials:** in this experiment 24 male Albumin Wistar rats were divided to three groups, control, treatments (500 and 1000 mg/kg) and Bilineaster herbal oral drop (Cotoneaster manna drop) was gastrogavaged for five days. Resident intruder paradigm, chronic mild stress, and the forced swim test were administrated and data were calculated according to protocols. **Results:** ANOVA revealed a significant difference between three group in both of swimming ($p=.002$) and mobility ($p=.004$) with higher scores for treatment groups. There was no significant difference between three groups in offensive aggressive behavior and social exploration behaviors in the environment ($p>.05$). **Conclusion:** The result showed that Cotoneaster manna not only increased anti-depressive like behaviors, but also does not induce any aggressive behavior in the environment.

**Keywords:** animal model, aggression, depression, cotoneaster

Kharamin S, Mansoorkhani HS, Kharamin R, Babamir R, Abad Nazir HM (2019) Cotoneaster Manna Effects on Animal Model of Aggression and Depression like Behaviors in the Environment. Ekoloji 28(107): 175-181.

## INTRODUCTION

Today, complementary medicine (CAM) alone or alongside conventional medicine is widely used to treat and restore health to the sick and prevention of disease in healthy individuals. One important example of CAM is herbal medicine which is defined as plant-derived preparations claimed to have therapeutic benefits and is the most commonly used CAM among people. It is known that between 65 and 80 % of the world's population use herbal medicines as their primary form of health care. This tendency and preference is also demonstrated in patients with psychiatric disorders. It seems that herbs have various useful chemical constituents which is useful for treatment of various psychological disorder like schizophrenia, depression, anxiety &panic disorders and other symptoms and syndromes. The use of CAM especially herbal medicine among patients with psychiatric disorders seems common. Some studies reported that among adults with a 12-month mood, anxiety, or substance use disorder, 34% reported using CAM in the past 12 months (Ahmed 2010). A data from a nationally representative sample of 2055 people interviewed during 1977–1988 revealed that 57% of those suffering anxiety attacks, and 54% of those with severe depression reported using herbal medicine during the previous 12 months to treat their disorder. In a recent survey questionnaire 22.2% of patients with mental disorders

 Received: 27 Feb 2018 / Accepted: 11 Oct 2018

were using some form of CAM in the Turkish community which the most common type of CAM (n = 146, 64%) used was herbal therapy.

In addition to using as a medicine, the herbal remedies may be encountered in psychiatric practice when they are used to produce changes in mood, thinking, or behavior as a side effect; or interact with psychiatric medications in the environment. Although use of CAM among patients with psychiatric disorders seems common, limited data exist about their safety, efficacy (compared to conventional) and other pharmacognostic, and pharmacological properties. In other side, studies showed that attitudes and beliefs regarding CAM explained much more variance in CAM use than clinical and demographic variables alone. Cotoneaster manna is one of the most pervasively used herbal medicine, especially in traditional treatment. It, known in Iran as Shir-Khesht, is found as dew drops falling on Cotoneaster species plants. The manna is white to yellow, round or shapeless pieces with a very sweet taste and cooling properties. Its effects on infant jaundice, and radio protective effect Ameli et al. (2017) have been investigated in a number of studies. In spite of these pervasive studies, there is still a number of question about dosage, side effects and its psychological effects. In addition, there is a very restricted domestic believe (according to researcher experiences) in south of Iran about aggression and depression side effects after using this medicine in infants. Shirkhesht (Cotoneaster) personality is very famous word in these areas and means a personality with aggressive behaviors and depressive mood in the environment. They believe that as consequence of Cotoneaster taking in infancy period, we will be confronted with this personality in the later age. In other hand, hereunto, there is no study about the psychiatric and behavioral effects of Cotoneaster manna in the environment. Therefore, this research was established to investigate two aims; the first assessment the traditional common believes about this herb and the second to assess its psychiatric effects in animal model of aggression and depression for first time (Awad et al. 2012).

## MATERIALS AND METHODS

### Animals

All animals used in this experiment were obtained from Center of Experimental Animal (Yasuj University of Medical Sciences). For main groups 24 male Albumin Wistar rats (180-220 gm) were divided in control and treated (500mg and 1000mg) groups of 8 rats in each group. For mate group 24 female Albumin Wistar rats (180-220 gm) and for intruder group in The Resident-intruder Paradigm, 8 male Albumin Wistar rats (160-200gm) also were allocated (Azadbakht 2005, Salakhova et al. 2018, Bedel et al. 2018). The animals were housed individually under controlled conditions (23.5 _ 1.5_C, under a 12-hour light/dark cycle with lights on at 8:00 AM, food and water provided ad libitum). These conditions were varied in stage of mild chronic stress for main groups rats (control and treated groups) as will be described in procedures. The animals were allowed to adapt to laboratory conditions for at least 1 week before the experiments started. All experiments, except RIP, were performed during the light phase of the light/dark cycle. They were housed individually and paired as will described in procedures. The study was performed with the consent of and under surveillance by the Committee of Ethics in Animal Research (Yasuj University of Medical Sciences) (Bahceci 2013).

### Drug Preparation and Administration

Bilineaster herbal oral drop was purchased from Sobhan Darou (Rasht, Iran). Each ml of drop contains 300 mg Cotoneaster manna. At the first, 24 male rats were randomly divided into three groups (8rats per each). Bilineaster were orally administrated for treated groups (500 and 1000 mg per kg) for 5 days. The control group received only distillated water during this time. All drugs and water were given at a single dose at 24 hours. These doses and duration were chosen based on tradition hakim reports and earlier studies (Fallah et al. 2014).

### Behavioral Testing

### Resident intruder paradigm (RIP)

The resident intruder test was performed according to previously described methods Farhat et al. (2006) after termination of treatment stage (5days). In this stage of experiment, the animals were housed individually in observation cages (80 3 55 3 50 cm), each with a sterilized female to avoid social isolation and to facilitate territorial behavior in the environment. After 10 days, the aggression test was performed using a dark red light (<2 lux, during the dark period). Approximately 1 h before the start of the confrontation, the female of the experimental rat was removed from the observation cage. The cage was moved to an observation table and after approximately 15 min of adaptation, a male intruder (of smaller size than the resident rat) was transferred from his home cage and introduced into the resident's cage for a period of 10 min. The intruder was typically attacked and defeated by the resident, as exemplified by his exhibition of freezing behavior and

assumption of a sub-missive posture in the environment (Pryce et al. 2005). The rats were considered to be aggressive when they displayed behavioral parameters mentioned in protocol. Subsequently, intruders were returned to their home cages (Haddad 2010). The rats were subjected to daily resident intruder tests for 3 consequent days and the means of three days were calculated as data. Two most important profiles in this paradigm are used as indicators of offensive aggressive behaviors; total offense score (sum of lateral threat, upright, clinch, keep down and chase) and social exploration score (sum of social explore, ano-genital sniffing and social groom). To avoid individual differences in defeat intensity, residents were confronted each day with a different intruder in a Latin square design (Islahudin et al. 2017).

### Chronic Mild Stress (CMS)

After the termination of the resident intruder test, three groups were subjected to a 10-day chronic mild stress paradigm. The test was performed using the method described by the literature. The following stressors were used: (i) 24 h of food deprivation; (ii) 24 h of water deprivation; (iii) changing cages, (iv) 3 hours of 45° cage tilt; (v) forced swimming in cold water 10 or 15 min; (vi) flashing light during 120-210 min; (vii) removing mates; (Viii) wet cages (200mL water in sawdust bedding, 12hours); (Viiii) confronting with foot shock (24 MA). Stress was applied at distinct periods every day, in order to minimize its predictability (James and Bah 2014).

### The Forced Swim Test (FST)

Ten days after CMS starting, the forced swim test (FST) protocol was utilized conform the literature. This model is a well-known screening tool for potential antidepressant drugs. Ten days after beginning of CMS model, the rats were placed inside cylinders with tap water at 23 ± 1 °C and adjust the water depth according to the rat's size, so that it cannot touch the bottom of the container with its hind legs. According to protocol times spent (5 min test stage) in three parameters, Struggling/climbing, immobility, and swimming, were calculated as depressive-like Behavior index (Kessler 2001).

### Procedure

One week after housing the animal, treatment in two control groups (500 and 1000 mg/kg) was administrated. The control group received only distillated water during this time. Then the RIP test performed for three times and data was collected by two blinded persons. After that CMS procedure was administrated for ten days. Eventually, depressive like behaviors were assessed by animal model of FST. In this stage last four minutes of 5minutes testing data was recorded and calculated by blinded person (Koolhaas et al. 1980).

### Statistical Analysis

The results are reported as means ± SE. Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. Values were considered to be statistically significant when $P < 0.05$ compared to the control. All calculations and analyses were performed using SPSS v.23 statistical software. Using prism software, the graphs were prepared.

## RESULTS

### Effect of Cotoneaster manna on Offensive Aggressive Behavior

**Fig. 1** revealed no significant difference between three groups in offensive aggression index in the environment. The ANOVA revealed no significant main effects for treatment in lateral threat ($F=1.41$, $p=.266$), upright ($F=1.34$, $p=.283$), clinch ($F=.43$, $p=.65$), keep down ($F= .6$, $p=.56$) and chase ($F= .96$, $p=.40$). There is no also a considerable variation between three groups in the level of social exploration behavior in the environment (as demonstrated in **Fig. 2**). Animals treated with Cotoneaster manna (500 and 1000) did not show any significant difference in social exploration ($F= .23$, $p=.79$) and ano-genital sniffing ($F= .04$, $p=.96$), compared to the control group. It is citable that degree of freedom was equal to 2 and 21 for all mentioned ANOVAs. It should be mentioned that there was no any reported biting behavior in all of the three groups (Koolhaas 2013).

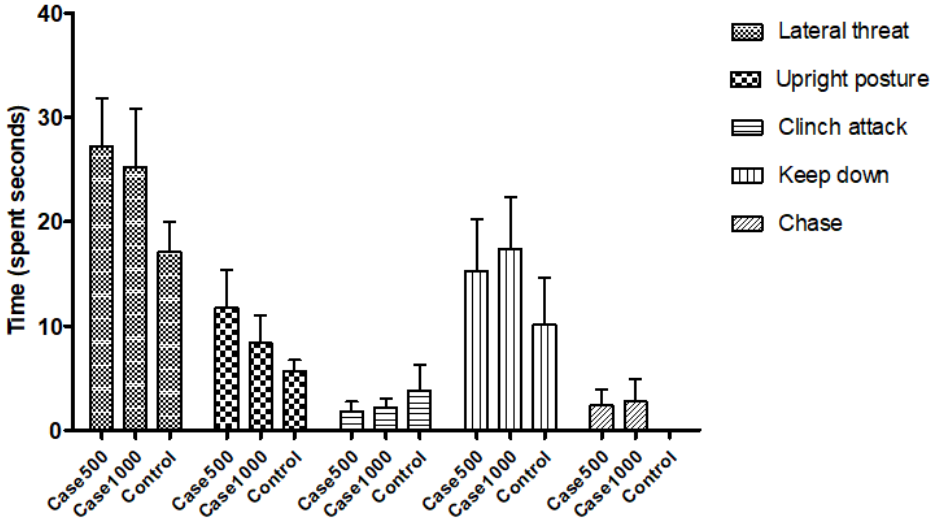


**Fig. 1.** Representative results of offensive aggression Indexes in two cases and control groups

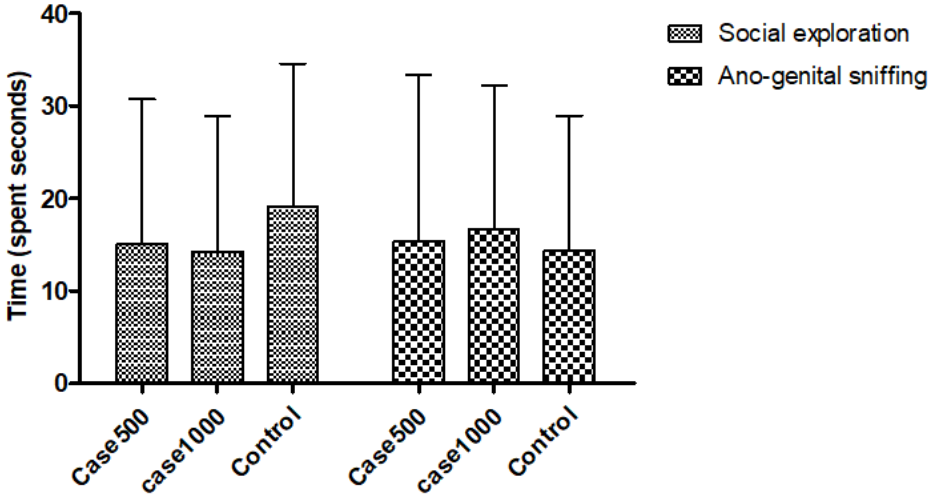


**Fig. 2.** Comparing indexes of Social exploration scores in three groups

### Effect of Cotoneaster Manna on Sepression like Behaviors in the FST

**Fig. 3** shows the results of three groups in the FST. This figure shows that swimming and total mobility time in the forced swimming test was increased in a significant manner in both treatment groups. ANOVA revealed a significant difference between three group in both of swimming (F= 8.28, p=.002) and immobility (F= 7.18, p=.004). Post- hoc analysis revealed no significant difference between two treatment groups. As demonstrated in mentioned figure, immobility was reduced significantly in both treatment groups (F= 6.84, p=.005) with compare to control group. In addition, there was no significant effect of Cotoneaster manna on time spent for climbing (F= 2.31, p=.12). The degree of freedom for all ANOVA on FST was equal to 2 and 21 (Rafieian-Kopaei 2016).

## DISCUSSION

The present study was established to respond these questions; whether traditional ideas, especially aggressive and depressive behaviors side effects, about Cotoneaster manna are right or not in the environment. The second, how is the Cotoneaster manna effects on animal model of aggression and depression. In this experiment 24 male Albumin Wistar rats were divided to three groups; control, treatment with 500mg/kg, and treatment with 1000 mg/kg. Using RIP, MCS, and FST procedures, rat's behaviors in aggression and depression were investigated in the environment. The major findings of this study were that Cotoneaster manna did not change aggressive behaviors in rat and that it could relive depressive – like behaviors in animal model of depression after administrating MCS model. To our knowledge, this is the first study that investigated the psychological and behavioral effect of cotoneaster. The

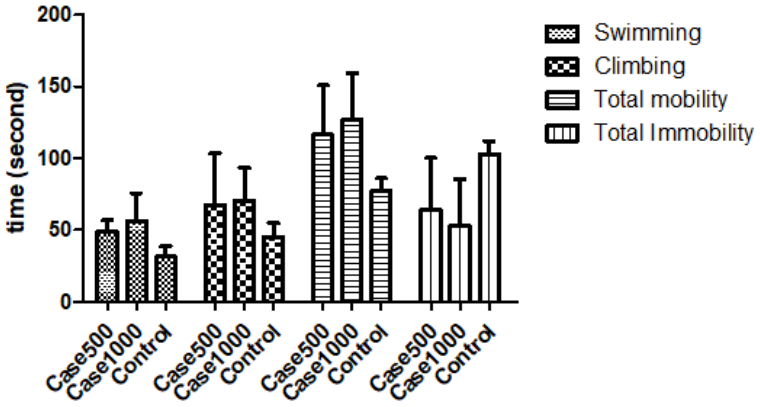


**Fig. 3.** Swimming, climbing, total mobility and total Immobility spent times in the FST of three groups

resident intruder paradigm is considered to be highly specific for the detection of anti-aggressive effects of drugs. The present findings clearly demonstrate that there was no significant difference between three groups in behavioral profile of offensive aggression (**Figs. 1** and **2**). The data from this study did not support the notion that Cotoneaster increases offensive aggression in rats. In other words, the domestic common believes about long term effect of this herb (especially aggression) were rejected by these findings. Several justifications could be proposed to explain this inconsistency. First, these domestic common believes may be irrational and scientifically have not been grounded during the past history. Second, because majority of people with history of Cotoneaster manna used, are people with a history of neonatal jaundice (hyperbilirubinemia). The observed aggressive behavior may be related to neurological abnormality (especially aggression) induced by hyper bilirubin Shanti (2013) that attributed erroneously to this herbal by people.

The FST paradigm was used to assess the likely Cotoneaster effects on depressive like behaviors in animal in the environment. This test remains one of the most common animal models used for screening potential antidepressant agents and is sensitive, believable, and useful measurement in the evaluation of antidepressant drugs. The present findings clearly demonstrate that Cotoneaster mamma increased or protected anti-depressant like behaviors in rats (compared to control group) after a period of mild chronic stress paradigm. It increased significantly time spent in mobility and swimming (as indicators of anti-depressive likes behaviors) in treatment groups (Suleiman 2014). There was no difference between treatment groups with 500 and 1000 mg/kg. These findings are not only inconsistent with previous domestic common believes, but also they showed an anti-depressant effect for this herbal medicine. Several biological and behavioral mechanisms, especially Cotoneaster biochemical compositions, could affect results in the present test. This may be related to phytochemical compositions of Cotoneaster; for example, phenolic, flavonoid, ferulic acid, and chlorogenic acid. A number of studies have supported the anti-depressant effect of these phytochemical compositions. In other hand, this results not only do not support people opinions about depressive behaviors consequences of Cotoneaster using, but also is completely in contrary to them. This inconsistency may be, to some extent, related to misattributing of neurological and psychological consequences of neonatal jaundice to Cotoneaster using (as mentioned previously). In addition, these significant differences were not dose dependent. In other words, there was no significant difference between treatment groups in these findings.

This study contains some limitations that are important to acknowledge. To confirm the anti-depressant effects, we need to compare this drug with a golden standard which was absent in this study. Therefore, it is recommended to establish a new experiment with an anti- depressant treatment such as fluoxetine.

To conclude, the current findings suggest that treatment with Cotoneaster manna not only does not increased depressive like behaviors, but also it protects and increases anti-depressive behaviors indicators in the environment (Caspi and Moffitt 2006). In other hand, the findings did not support any increasing aggressive behaviors after treatment with this herb in rats (Wong et al. 1998, Woodward 2009).

**ACKNOWLEDGEMENT**

At all stages of this study (design, data management, analysis and manuscript writing) researchers Shirali Kheramin, Heibatollah Sadeghi Mansoorkhani, Roghayeh Kharamin and Rezvan Babamir played a significant role.

**ETHICAL APPROVAL**

All procedures performed in studies involving human participants were in accordance with the Yasuj University of Medical Sciences Research Ethics Committee and in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**REFERENCES**

Ahmed A (2010) Herb's Used in Psychological Disorders, Inventi Impact: Ethnopharmacology.

Ameli Z, Assarroudi A, Akrami R (2017) Effect of Bilineaster Drop on Neonatal Hyperbilirubinemia. Evidence Based Care, 6(4): 66-73.

Awad AI, AlAjmi S, Waheedi M (2012) Knowledge perceptions and attitudes toward complementary and alternative therapies among Kuwaiti medical and pharmacy students. Medical principles and Practice, 21(4): 350-354.

Azadbakht M (2005) The effect of purgative manna on the infant jaundice. Iranian Journal of Pharmaceutical Sciences, 1(2): 95-100.

Bahceci B (2013) Complementary and alternative medicine use in patients with mental disorders in Turkey. Complementary therapies in clinical practice, 19(4): 221-226.

Bedel C, Tomruk Ö, Yolcu S, Albayrak L (2018) Duodenal Perforation by an Ingested Nail. J Clin Exp Invest, 9(3):135-6. https://doi.org/10.5799/jcei.458763

Caspi A, Moffitt TE (2006) Gene–environment interactions in psychiatry: joining forces with neuroscience. Nature Reviews Neuroscience, 7(7): 583.

Fallah R, Ali Fallahzadeh M, Noori-Shadkam M (2014) Evaluation of safety and efficacy of purgative manna (billinaster drop) and glycerin suppository in icterus of healthy term newborns. Current drug safety, 9(1): 29-33.

Farhat A, Mohammadzadeh A, Amir M (2006) Effect of cotoneaster tricolor pojark manna on serum bilirubin levels in neonates. International Journal of Pharmacology, 2(4): 455-8.

Haddad F (2010) Analysing the radioprotective effect of Cotoneaster nummularia in mouse bone marrow cells using micronucleus assay. Journal of Cell and Molecular Research, 1(2): 77-82.

Islahudin F, Shahdan IA, Mohamad-Samuri S (2017) Association between belief and attitude toward preference of complementary alternative medicine use. Patient preference and adherence, 11: 913.

James PB, Bah AJ (2014) Awareness, use, attitude and perceived need for Complementary and Alternative Medicine (CAM) education among undergraduate pharmacy students in Sierra Leone: a descriptive cross-sectional survey. BMC complementary and alternative medicine, 14(1): 438.

Kessler RC (2001) The use of complementary and alternative therapies to treat anxiety and depression in the United States. American Journal of Psychiatry, 158(2): 289-294.

Koolhaas J, Schuurman T, Wiepkema P (1980) The organization of intraspecific agonistic behaviour in the rat. Progress in Neurobiology, 15(3): 247-268.

Koolhaas JM (2013) The resident-intruder paradigm: a standardized test for aggression, violence and social stress. Journal of visualized experiments: JoVE, 77.

Pryce CR, Rüedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J (2005) Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. Neuroscience & Bio behavioral Reviews, 29(4-5): 649-674.

Rafieian-Kopaei, M (2016) Cotoneaster: A safe and easy way to reduce neonatal jaundice. Journal of clinical and diagnostic research: JCDR, 10(4): SC01.

Salakhova, V. B., Belinskaya, D. B., Erofeeva, M. A., Ulyanova, I. V., Zotova, L. E., Khammatova, R. S., Mizonova, O. V (2018) Modern methods of diagnosing addiction to psychoactive substances: neurophysiological aspects. Electronic Journal of General Medicine, 15(6), em94. https://doi.org/10.29333/ejgm/100633

Shanti P (2013) Herbal medicines for depression and anxiety: a comprehensive state of the art review. Global Journal of Research on Medicinal Plants & Indigenous Medicine, 2(5): 317.

Suleiman A (2014) Attitudes and beliefs of consumers of herbal medicines in Riyadh, Saudi Arabia. J Community Med Health Educ, 4(2): 269.

Wong AH, Smith M, Boon HS (1998) Herbal remedies in psychiatric practice. Archives of general psychiatry, 55(11): 1033-1044.

Woodward AT (2009) Complementary and alternative medicine for mental disorders among African Americans, black Caribbeans and whites. Psychiatric Services, 60(10): 1342-1349.