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# Effect of bee pollen hydro-alcoholic extract on cognitive function in mice

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## ABSTRACT

*Background : Bee pollen is believed to be important sources of new chemical substances with potential Therapeutic efficacy.The aim of this studay was rol of bee pollen on the cognitive function of mice. Material and methods: Bee pollen hydro-alcoholic extract (200, 400, 800, 1600mg/kg ,po) normalsalin (10ml/kg,po) were adminestred for30days.passive avoidance method was used for the assessment of cognitive function in animal. The results indicated a significant improvement in the cognitive function with dose of 800mg/kg.*

***Keywords:*** *Bee pollen , Cognitive ,Passive avoidance ,Mice*

## INTRODUCTION

Today, solution providing for the management and treatment of cognitive disorders and age-related diseases is highly regarded (1). In addition, allocating of huge funds for researches related to cognitive impairment shows the importance of this issue (1, 2). According to epidemiological studies conducted in Europe (Sweden, Netherlands, UK and Spain) are rising the prevalence of cognitive dysfunction and dementia, so it can be a potential threat to public health (3). Although superoxide and hydroxyl radicals are produced as part of the body normal activity (4, 5). Nevertheless, stable and long-term oxidative stress is a major cause of cognitive impairment (6). As bee pollen is a nutrient, it is highly regarded in the industry food (7).
Nowadays, the bee pollen is used as antimicrobial and antifungal compound (8). In addition, it considered to be as a product with antioxidant property (9). Capcarova and colleagues in 2013 reported that treatment with bee pollen improved and increased the activity of superoxide dismutase and antioxidant levels in mice (10). Polyphenols especially flavonoids were obtained from bee pollen and a lot of attention is to this compounds for diseases treatment due to their antioxidant property (11-13). In previous studies reported that long-term administration of flavonoids may be helpful for cognitive dysfunction improvement in humans (14, 15). The aim of study was to evaluate the effect of bee pollen on cognitive function in mice.

## Material and method

### Preparation of bee pollen hydro-alcoholic extract

Bee pollen was purchased from ardabil state . For preparation of bee pollen hydro-alcoholic extract, we used maceration method. Briefly, 300 g of bee pollen was macerated by 70% ethanol for 72h. Then mixture was filtered . The obtained extract was concentrated by rotary vacuum and dried with to put in the oven (35°C) (16).

## Animals

In this study, 36 male N_MARI mice (body weight 20-25 g) were purchased from the Experimental Animal Center of Jundishapur University of Medical Sciences, Ahvaz, Iran. The animal were kept in an air-conditioned room at a control tempreture (23+_2°C) and humidity (50°C ) under a light and dark cycle. Standard plate food and tap water were available adlibitum

## Experimental design

The step-down apparatus used to perform passive avoidance consisted of a box measuring 25cm×25cm×20 cm with an electric fied grid floor.There was around plastic platform 1cm high and 9cm in diameter , which could be enclosed by a 20cm long hollow plastic cylinder with and inner diameter of 10cm.Mice was subsequently divided into six groups (n=8). Bee pollen hydroalcohlic extract four groups was adminestred once orally for 30 days. The control groups normalsalin(10ml/kg) but blank group did not recived anything.

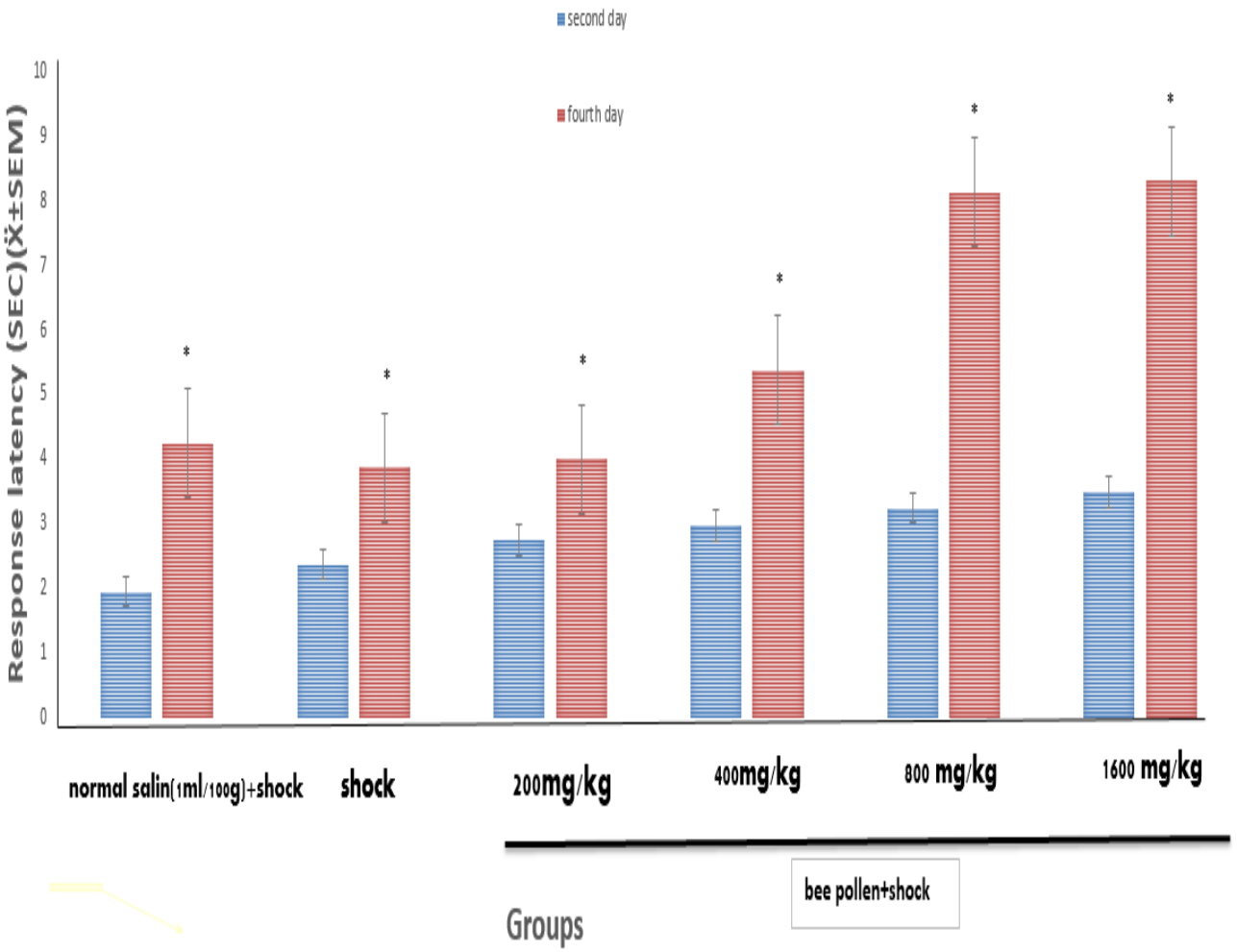
Recording of memory indices began 24h after administration of the last dose of drugs. On the first day, five animals from each group were given access to the learning apparatus for 3min to familiarize with the new environment. On the second day, each mouse was individually placed on the platform inside the cylinder.after 10s ,the cylinder was removed and the step-down latency was measured. On the third day, the same procedure was performed as on day 2, and 1s foot shock (1mA) was applied as soon as the animal left the platform with all four legs. On the fourth day, the step-down latency of mice was again recorded and both young and aged group were compared.(18)

## Statistical analysis

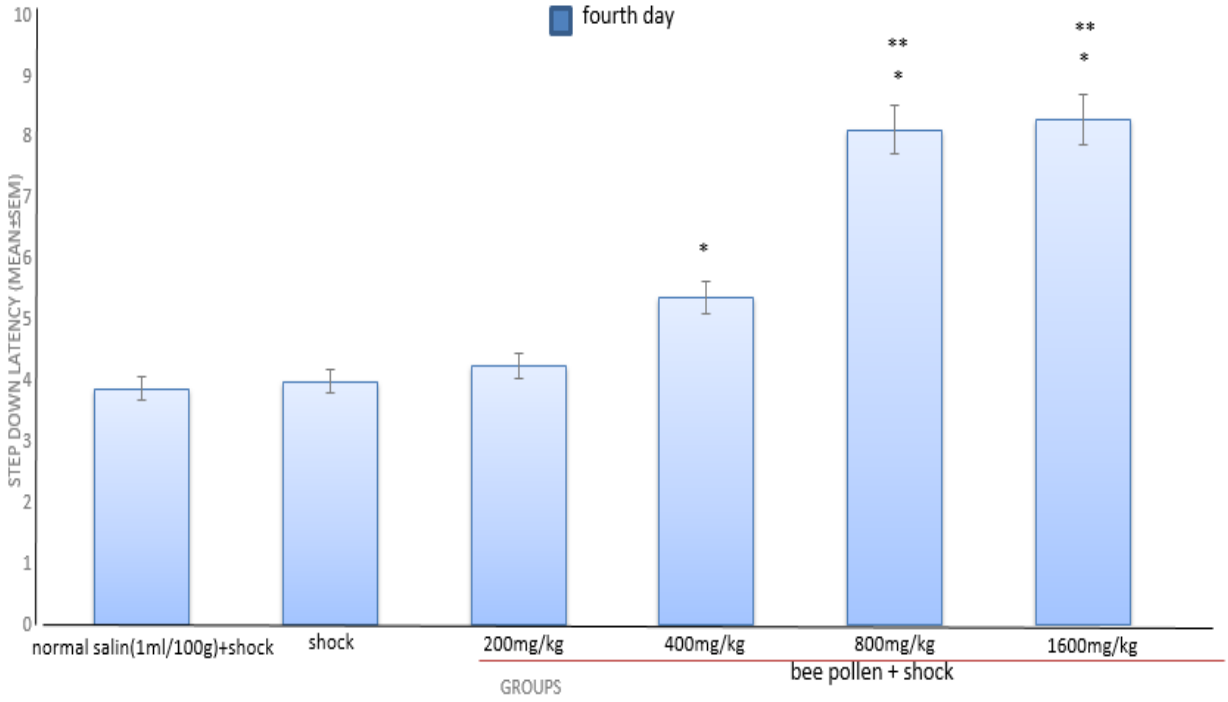
All data are presented as Mean ± SEM. Paired sample T- Test was performed for different determination of step-down latency between 2nd and 4th day in each group. In addition, One-way ANOVA analysis followed by LSD test was used to compare the step-down latency in 4th day in all groups. $p \leq 0.05$ was set as significant different.

## Results

The results of this study indicated that the mean of step-down latency on day 4 in comparison with day 2 became longer($p \leq 0.05$) in all of groups of mice. All groups in comparison to control and shock group show significant difference ($p \leq 0.05$) except 200mg/kg-treated groups in step-down latency on day 4(fig2).The mean of step-down latency on day4 ,The groups which recived( 400,800,1600mg/kg,po) extract respectively, incomparison with the groups which received 200mg/kg extract showed significant difference ($p \leq 0.05$),(fig2).also,the group received 400mg/kg extract in comparison with 800,1600mg/kg extract showed signifcant difference($p \leq 0.05$) ,while this difference was not between 800,1600mg/kg extract.(fig2)




Graph 1- comprasion of step-down latency between 2nd and 4th day in control and exprimental groups. The groups ,which recieved normal saline (1ml/100g) (control group), only induced electric shock (1 mA), and treated by 200, 400, 800, 1600 mg/kg bee pollen hydro-alcoholic extract respectively. Step-down latency was showed as Mean ± SEM. Paired sample T- Test was used for analysis of groups ($p \leq 0.05$) (n=8). * Significant different between 2nd and 4th day




Graph 2- comprasion of step-down latency in all groups at 4th day. The groups, which recieved normal saline (1ml/100g) (control group), only induced electric shock (1 mA), and treated by 200, 400, 800, 1600 mg/kg bee pollen hydro-alcoholic extract respectively. Step-down latency was showed as Mean ± SEM. One-way ANOVA analysis followed by LSD test was used for analysis of groups ($p \leq 0.05$) (n=8). * Significant different with groups of control, only shock and treated by 200 mg/kg bee pollen hydro-alcoholic extract. ** Significant different with the group treated by 400 mg/kg bee pollen hydro-alcoholic extract.

## Discussion

Many chemical drugs are used for enhancement of memory and prevention of dementia that may have severe side effects and toxicity. Therefore, in today's world return to the use of medicinal plants is much attention for research in the case of applicable from the standpoint of their choice and how drug consumption due to the low and negligible side effects (17). As oxidative stress and free radicals is one of the most important factors in the incidence of dementia and memory disorders (18). Given that bee pollen has antioxidant property based on many conducted studies (19). In the present study was investigated its effect on memory retention in young mice.

The production of free radicals associated with normal cellular processes such as cell metabolism, mitochondrial respiration, lipoxygenase and cyclooxygenase activity (20). However, its amount increase accompanied by age increasing (21). During aging the oxidative stress consider as a risk factor in increase of oxidized lipids and proteins at CNS that eventually lead to cell damage (22). Our results showed that bee pollen could improve memory and behavior in small white mice so that dose of 800 mg/kg had most effect. The findings were

achieved based on the increase of step-down latency time in the group treated by bee pollen compared to control group. Our findings are consistent with obtained results by other researchers for example Chen jue and colleagues in 1989 examined anti-aging effects of bee pollen in old mice (10 g as daily administration). At the end of study the function evaluation of liver and heart and measurement of lipids were determined that bee pollen led to reducing of aging and tissue damage (23). In addition, it has well been demonstrated that bee pollen has high potential for protection of tissue due to rich source of flavonoids with anti-oxidant and anti-estrogenic properties (24). In a study was investigated 12 types of bee pollen and determined that all types have high level of phenolic compounds with antioxidant property. It was also confirmed that they have protective effect on vital organ after daily administration to mice (25). The evaluation of effect of bee pollen and its isolated-acetone fraction on memory was indicated that they led to diminution of amnesia induced by carbonic acid and scopolamine (26). Eraslan et al, 2009 showed that treatment by 100 mg/kg bee pollen disrated the adverse effect of propoxur (a carbamate insecticide) (27).

According to conducted studies bee pollen as antioxidant source protects cell from oxidative stress damages and leads to amplification of total antioxidant capacity, thus can be useful for management of memory loss and forgetfulness. Bee pollen has high amount of lecithin that is affect in reduction of LDL and increment of HDL and ultimately result in rapid evacuation of fat from body (28). The high level precense of polyphenols content (a super bioflavonoids) lead to bee pollen consider as prominent antioxidant (8). In addition, the phenolic compounds of bee pollen inhibit from oxidation of molecules and diminish the risk of oxidation-related disease (29, 30).

Given the role of pollen in the removal of free radicals and oxidative stress, it seems that pollen can be use as a therapeutic strategy for reduction of oxidative stress in CNS. It should be noted that for ensure the beneficial effect of bee pollen is need to study on various animal models and eventually human models according to available laws and criteria.

## Competing Interest

The authors have no financial conflicts of interest for this paper.

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