

# *The effects of hydro alcoholic-extracts garlic on depression induced by reserpine in rats*

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## **Abstract**

**Introduction:** Previous studies showed that besides Garlic therapeutic effects on cardiovascular diseases, it is also effective on Central Nervous System (CNS) disorders like depression, Alzheimer and Parkinson. This study was done for evaluating Garlic effects on depression.

**Methods and materials:** In an experimental study for evaluation of anti-depression effects of Garlic, its hydro-alcoholic extract was prepared, concentrated and different dilution was made. Forty two Sprague-Dawley rats with weight average of 200-250 grams were selected and randomly divided into seven groups each with six members. In six groups of them, depression was induced by treating with 10mg/kg of reserpine. Four groups of depressed animals received 50, 100, 150 and 200mg/kg of weight of garlic extracts intra-peritoneally (IP) daily. 150 and 200mg doses in early days led to death of majority of animals and so, these concentrations omitted from study. Treatment of depression with 50 and 100mg doses continued and one group of animals treated by Amytriptylline IP daily.

**Results:** Garlic treated group with 100mg/kg, cured after 9 days and reduced signs of depression like diarrhea, weight loss, eye hemorrhage, sedation and righting reflex was observed in comparison with untreated reserpine group significantly ( $P < 0.01$ ,  $P < 0.001$ ). In reserpine treated group with 50mg/kg of Garlic, reduction of depression signs was observed but it was not significant. 100mg/kg of Garlic extract produced better function in foot and hand hemorrhage, sedation and righting reflex than amytriptylline ( $P < 0.05$ ,  $P < 0.001$ ) while hanging from tail and feet, and eye hemorrhage in amytriptylline treated group was better than Garlic extract group ( $P < 0.05$ ). In weight loss and reduction of diarrhea, Garlic extract was better than amytriptylline ( $P < 0.05$ ,  $P < 0.001$ ).

**Conclusion:** Garlic extract in reasonable doses can reduce many signs of depression.

**Keywords:** Garlic, Depression, Reserpine, Rat, Amytriptylline.

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## Introduction

Nowadays, plant products or total extract have found routine usage in all around the world. It is clear that modern medicine takes its roots in ancient medicine and plant therapy but the diversity of medicinal plants are so much that we can't acclaim that we know all of their effects. Necessity of attention to treatment and side effects of medicinal plants and scientific use of these plants, preparation of effective substances of medicinal plants and understanding major pharmacological mechanisms of them, calls for evanescence of all of the researchers in medical sciences. World Health Organization (WHO) suggests to all governments to take necessary attention to use their own medicinal plants and ancient medicinal systems and with suitable organization of them, works toward optimization of national health systems.<sup>(1)</sup> It must be taken in mind that medicinal plants, like chemical drugs have special usage and side effects and we have to know them quietly for using them correctly. In many cases, mis-nomenclature, misidentification, contamination of tissue plants with pesticide and heavy metals and even combination of medicinal plants with highly strengths chemical drugs (e.g. Diazepam, phenylbutazon, testosterone, ...) may lead to severe and dangerous side effects like bone marrow suppression, hypertension and cardiac arrhythmia. Garlic belongs to Liliaceous and this tribe has 11 geneses. *Allium* is one of these genuses and has 250 species.<sup>(2)</sup>

Previous studies showed that Garlic has not only anti Alzheimer effects but also it has anti-aging properties.<sup>(4-7)</sup> Also, it has been reported that Garlic increases secretion of serotonin by inhibiting mono amine oxides (MAO).<sup>(8,9)</sup> It has been mentioned in ancient Egyptian medicine that Garlic has anti tumor and anti headache effects.<sup>(10)</sup> Different studies in 1983 showed that Garlic has anti accumulative effect on blood platelet.<sup>(11)</sup> Also, in recent years, many studies have been done on Garlic's effects on cardiovascular diseases including hypertension, chest pain and heart failure.<sup>(12)</sup> It has been suggested that Garlic has important role in decreasing blood triglyceride, cholesterol and glucose.<sup>(13)</sup> Garlic effects on *Salmonella typhi* has been extensively studied in Shahid Beheshti University in Tehran.<sup>(3,14)</sup> Reserpine is a plant alkaloid which is extracted from Indian plant *Rauwolfia Serpentina*. Reserpine inhabits biogenic amine reservoir. The mechanism of action is probably involved in up taking of  $Mg^{2+}$  and ATP. This process took place in whole body and leads to discharging of norepinephrine, dopamine and serotonin in brain and peripheral neurons. Chromaffin granules in centre port of adrenal also discharged from catecholamine but relatively lower than neurons. As the little amount of drug attached to vehicle's membrane for days, it seems that the reserpin effects on adrenergic vesicles are irreversible. Although enough amount of reserpine decreases catecholamine reservoir to zero level, but little amount inhibit

nerve transfer and this inhibition is relative to amine discharge. Reserpine enter the brain easily and with discharging brain amines, causes depression and Parkinson's signs. Large amount of reserpine can cause sleepness, though weakness, nightmare and severe depression.<sup>(15,16)</sup>

### **Methods and materials**

This is an experimental study. In this study Sprauge-Dawley laboratory mice were purchased from Razi Research Institute (Hesarak-Karaj). Mean weight of tested mice was 200-250 grams. Rat food was prepared by Pars Co. Animals had water piping availability and were under 12 hours light and 12 hours darkness. Forty two test mice divided randomly to seven groups each has six mice. First group: positive control, used for comparing with depressed and treated mice. Members of this group didn't receive any drugs and extract. Members of the second group (negative control) received 10mg/kg reserpine I.P. Third, fourth, fifth and sixth groups received 50, 100, 150 and 200mg/kg of Garlic extract IP after depression with reserpine. Seventh group received one IP dose of 0.2mg/kg amytriptylline after depression with reserpine. Symptoms (signs) of depression after taking drugs in second and seventh group in Garlic extracts taking groups were studied and recorded in special tables and entered into computer and were analyzed in next steps.

**A) Material and drugs:** Reserpine was purchased from Sigma by Kimia Pajouh as pure powder. Amytriptylline was purchased from Pars Daru Co.

Glacial acetic acid was used as solvent of reserpine and 96% ethyl alcohol was used for preparation of Garlic extract. Hamadan Garlic was grinded, dried, powdered and it's extract was prepared.

### **B) Equipment and tools:**

1. Electric grinder, Model 830, 220V, Hong Kong.
2. Special alcohol
3. Soxhlet with electric oven, Iran.
4. Vacuum distillation, Harward.
5. Deionizer apparatus, Xilon, Iran
6. Glass cage for observation of depression signs

### **C) Data collection and analysis**

Some signs of depression were selected for study. Weight loss was a quantitative sign and other studied signs belong to qualitative ones. For quantitative signs and on the basis of their severity, different score was used.<sup>(18)</sup> These studied signs were included: diarrhea, foot and hand bleeding, eye bleeding, eye closeness. We gave four scores to severe depressed animals with severe diarrhea. The same score was given to an animal with foot and hand bleeding, severe eye bleeding and completely closed eyes. For sedation signs like hanging from tail, hanging from feet, hanging from hands, neck tightening, landing on the back of hand, the same score was given. If animal hang from tail, foot and hand and had not any reflexes and hadn't contraction of whole body, took 2, 6 and 8 score respectively. If animal landed on the back of hand and had not any reflexes, took 4 scores. For righting reflex like animal twist one, two or three times and dropping it to earth

some scores was given. For example, if animal falls in it's side after one twist took 1 score, if animal falls in it's back took four scores, if animal falls after three times twist in it's side took 4 scores and if animal falls on it's back took six scores. If animal didn't receive drug, it took zero score in the table. With respect to obtained scores, effectiveness of Garlic extract and drug evaluated and compared.<sup>(18)</sup> In statistical analysis, Kruskal-Wallis, ANOVA, Mann-Whitney and t-test were used.

### Results

Due to high mortality in 150 and 200mg/kg of Garlic, actually these groups omitted from study. In 50mg/kg of Garlic, in comparison with control group, no significant decrease in depression signs was observed. So, reasonable results obtained from 100mg/kg of Garlic group. Finally four groups studied which were as follow:

Group 1: This group received one IP dose of 10mg/kg reserpine.

Group 2: This group first received one IP dose of 10mg/kg of reserpine and from second day received Garlic hydro-alcoholic extract (100mg/kg) daily.

Group 3: This group firstly received one IP dose of 10 mg/kg of reserpine and from second day received Garlic hydro-alcoholic extract (50mg/kg) daily.

Group 4: This group first received one IP dose of 10 mg/kg of reserpine and from second day received amytriptyline (0.2mg/kg) daily.

In all above mentioned groups, depression signs evaluated as follow:

V1) hanging of animal from tail

V2) landing on the back of hand

V3) hanging of animal from feet and reflex

V4) hanging of animal from hand and reflex

V5) foot and hand bleeding

V6) eyes bleeding

V7) eye closeness

V8) diarrhea

VR1) two times twisting of animal and dropping on laboratory table, if animal falls to it's side took 1 score and if falls on it's back took 4 score.

VR2) four times twisting of animal and dropping on laboratory table, if animal falls to it's side took 2 score and if falls on it's back took 5 score.

Each animal from each group for above mentioned signs took score. Third group (50mg/kg of Garlic), in comparison with control group (group 1) has no significant difference and so daily analysis of first, second and fourth group showed following results:

In first day: V4, V5 P=0.008, P=0.023, significant.

In second day: V4, V5, V6, V7, VR1 with P-value of 0.034, 0.006, 0.015, 0.002 respectively, significant.

In third day: V2, V3, V5, V6, V7, V8, VR1, VR2 with P-value of 0.033, 0.004, 0.011, 0.007, 0.008, 0.015, <0.001, 0.033 respectively, significant.

In fourth day: V3, V4, V5, V8, VR1 with P-value of 0.001, 0.029, 0.048, 0.004, <0.001 respectively significant.

As more than 50% of first group rat died from fifth days on, statistical

analysis for three groups done only in first four days. Statistical analysis for first to fourth days was as follow: V3 (P=0.005), V5 (P=0.002), V7 (P=0.026), V8 (P=0.002), VR1 (P<0.001), VR2 (P=0.038). In all above

mentioned results, differences were resulted from second group's function. Minimum weight loss has occurred in group 2 (100mg/kg) with P<0.001, (table 1).

**Table 1: Mean and standard deviation of weight loss in the first four days (in three groups)**

GROUP	GROUP 1	GROUP 2	GROUP 4
Mean ± SD	2.23 ± 2.52	1.756 ± 1.96	1.998 ± 4.92

#### **Comparison of second and fourth group in first to fourth days:**

Second group in comparison with fourth group had done better, V2 (P = 0.019), V3 (P = 0.03), V5 (P = 0.001).

#### **Comparison of second and fourth group between fifth and eight days:**

In some objects fourth group done better than second group significantly,

V1 (P=0.012), V3 (P=0.032), V6 (P=0.049).

In decrease of diarrhea (V8) second group (P=0.018) had better response. In weight loss, results have been showed in table 2. It is important to know that we had not only weight loss but also weight increase.

**Table 2: Mean and standard deviation of weight loss (in two groups)**

DAY GROUP	DAY 1 UP TO DAY 4 MEAN ± SD	DAY 5 UP TO DAY 8 MEAN ± SD	P-VALUE
second	2.899 ± 0.02	2.501 ± 1.92	0.000
fourth	2.509 ± 3.07	1.239 ± 1.17	0.000

#### **conclusion**

Depression was made in rats by reserpine. First on the basis of previous experiments<sup>(18)</sup> 5mg/kg of this drug was used. With this dose rats become depressed but this depression hadn't continuation and in the next day they returned to the natural state. So, for making depression in such a way that depression appears and lasts for next

few days, different doses of reserpine were used in primary experiments and the best dose was 10mg/kg for this Sprague-Dawley race. To study hydro-alcoholic effects of Garlic extracts on depressed animals, 50, 100, 150 and 200mg/kg of animal weight was used in four different groups. Using these doses caused high incidence of mortality in tested animals as a result

of brady arrhythmia which may be in turn was produced as a consequence of interaction of Garlic and reserpine in tested animals. Jafari et al<sup>(20)</sup> reported that Garlic may decrease contraction strength and heart rate in Sprague-Dawley mice. On the other hand, reserpine makes parasympathetic system dominance in such a way that vague nave has been exited and heart rate has been decreased and produced contraction of smooth muscles of gastric system has been lead to diarrhea. Also, this drug causes myosis, decrease of blood pressure and vein dilatation.<sup>(15,16)</sup> Thus, taking Garlic simultaneously or in a little time after taking reserpine may produce dangerous interactions which may lead to death unless the Garlic used in low dosage. Garlic effect on depression is dose dependent and among four tested doses, only 100mg/kg had a significant decrease in depression signs. So, in using medicinal plants or a drug and a medicinal plant, special attention must be made in the case of interaction. Jahani Hashemi et al<sup>(19)</sup> findings in a study on rats suggested that anesthesia with Halothane and Pentobarbital in Garlic diets make a significant decrease in heart rates, prolonged anesthetic periods and induction of anesthesia and recovery of the animal. Optimum dose of Garlic decreased symptoms significantly. Severe diarrhea of reserpine taking animals after daily treatment with Garlic extract from third days on significantly decreased while in the previous study with Valerian, significant decrease of diarrhea was not

observed.<sup>(18)</sup> It was interesting that amytriptyline which is a three cycle anti-depression drug must more effectively decrease diarrhea than Garlic but actually we didn't observe such a result and in decreasing foot and hand bleeding, Garlic was significantly more effective than amytriptyline and so was observed in treatment with Valerian.<sup>(18)</sup> In righting reflex, Garlic extract was excellent ( $P < 0.001$ ). Also in some of the sedation signs, Garlic extract made suitable response while Valerian extract decreased all of the sedation signs.<sup>(18)</sup> All of the sedation signs resulted from reserpine with amytriptyline in the fourth to eighth days of treatment decreased better than Garlic extract. These signs were including:

- 1- Hanging of animal from tail and no reflex.
- 2- Hanging of animal from feet and no reflex.

With respect to these points that: increase of above mentioned signs and in bleeding from eyes among the fourth and eight days, amytriptyline treated group had better responses than Garlic extract ones, one may conclude that amytriptyline in early days is not very effective and it can be effective from fourth day on. In depressed patients also, response to treatment did not appear rapidly and the first response revealed at least after 24-25 days.<sup>(16,17)</sup> While Garlic extract in it's favorite dose (100mg/kg) could decrease depression signs in early days. Weight loss was little in Garlic treated mice and they showed even weight increase

from seventh day on and meanwhile depression signs decreased to zero level. In final days of study amytriptyline was unable to make favorite response for weight loss of tested animals. On the whole, Garlic extract in reasonable dose can significantly decrease the depression signs

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# تأثیر عصاره هیدروالکلی سیر بر افسردگی القاء شده با رزپین در رت

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## چکیده

**سابقه و هدف:** شواهد و گزارش ها حاکی از آنست که سیر علاوه بر دارا بودن اثرات درمانی در اختلالات قلبی و عروقی، در درمان اختلالات مربوط به سیستم عصبی مرکزی (C.N.S) نظیر افسردگی، آلزایمر و پارکینسون نیز مؤثر است. هدف از این مطالعه بررسی تاثیر سیر بر افسردگی بوده است.

**روش بررسی:** در یک مطالعه تجربی برای بررسی اثرات ضدافسردگی سیر، عصاره هیدروالکلی سیر همدان تهیه و تغلیظ گردید و غلظتهایی از آن تهیه شد. تعداد ۴۲ رأس موش صحرایی با میانگین وزنی (۲۵۰-۲۰۰) گرم از نژاد اسپیراگ داوولی به طور تصادفی به هفت گروه شش تایی (n=۶) تقسیم گردیدند، در شش گروه از حیوانات به کمک رزپین ۱۰ mg/kg افسردگی ایجاد گردید. به چهار گروه از حیوانات بعد از افسردگی روزانه یکمرتبه عصاره سیر با دوزهای ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی گرم بر کیلوگرم به صورت تزریق داخل پریتون (I.P.) تجویز گردید. دوزهای ۲۰۰ و ۱۵۰ میلی گرم در روزهای اولیه باعث مرگ و میر اکثریت حیوانات گردید و لذا این دو گروه از مطالعه حذف گردیدند. درمان افسردگی با دوزهای ۵۰ و ۱۰۰ میلی گرم تداوم یافت و یک گروه از حیوانات نیز تحت درمان آمی تریپتیلین ۰/۲ mg/kg روزانه به صورت I.P. قرار گرفتند.

**یافته ها:** گروه تحت درمان سیر با دوز ۱۰۰ mg/kg بعد از ۹ روز بهبودی کامل یافتند و کاهش علائم افسردگی نظیر اسهال، کاهش وزن، خونریزی چشمها، نحوه آویزان ماندن از سر و دم و دست و پا (Sedation) و حرکات Righting Reflex نسبت به گروه رزپین بدون درمان، با اختلاف بسیار معنی دار مشاهده گردید ( $P<0.001$  ,  $P<0.01$ ). در گروه رزپین تحت درمان با سیر ۵۰ mg/kg، کاهش علائم افسردگی رویت شد اما این کاهش معنی دار نبود. دوز ۱۰۰ mg/kg از عصاره سیر در خونریزی از دست و پا و در Sedation و در حرکات Righting Reflex عملکرد بهتری از آمی تریپتیلین داشت ( $P<0.001$  و  $P<0.05$ ) در حالیکه در نحوه آویزان ماندن از ناحیه دم و پا و در خونریزی از چشمها، آمی تریپتیلین بهتر از سیر بود ( $P<0.05$ ). در میزان کاهش وزن و کاهش اسهال، عصاره سیر بهتر از آمی تریپتیلین بود ( $P<0.001$  و  $P<0.05$ )

**نتیجه گیری:** عصاره سیر به شرط آن که در دوزهای معقول استفاده شود می تواند علائم زیادی از افسردگی را کاهش دهد.

**واژگان کلیدی:** سیر، افسردگی، موش، رزپین، آمی تریپتیلین