STUDY OF THE EFFECT OF *Allium porrum* ON HYPERTENSION INDUCED IN RATS

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ABSTRACT

The present study was designed to investigate the effect of alcoholic extract of *Allium porrum* (250 and 500mg/kg) on hypertension induced experimentally in male rats by oral administration of L-NG-Nitroarginine methyl Ester (L-NAME) (50mg/kg/day) for four successive weeks. Alcoholic extract of *Allium porrum* (250 and 500mg/kg) was administered by oral route daily for 8 weeks before hypertension induction and 4 week simultaneously with L-NAME. Systolic blood pressure (SBP) was measured every week by tail-cuff method; blood samples were collected at the end of the experiments for determination of serum cholesterol, nitric oxide, malondialdehyde and total antioxidant capacity.

Oral administration of alcoholic extract of *Allium porrum* (250 and 500mg/kg) resulted in a significant reduction of the elevated SBP induced by L-NAME (50mg/kg) compared with hypertensive control group. It was also observed that alcoholic extract of *Allium porrum* showed a significant antioxidant activity and hypcholesterolemic effect, in addition to increase significantly serum nitric oxide. It is concluded that alcoholic extract of *Allium porrum* have promising effect in experimentally induced hypertension in rats, which may be attributed to its antioxidant capacity. [www.relaquim.com](http://www.relaquim.com)

Keywords: *Allium porrum*, hypertension, antioxidant, rats.

RESUMEN

El presente estudio fue diseñado para investigar el efecto del extracto alcohólico de *Allium porrum* (250 y 500mg/kg) en la hipertensión inducida experimentalmente en ratas machos por la administración oral del L-NG-nitroarginina metil ester (L-NAME) (50mg/kg / día) durante cuatro semanas sucesivas. El extracto alcohólico de *Allium porrum* (250 y 500 mg/kg) se administró por vía oral diariamente 8 se-

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importance of hypertension studies arises from the fact that the prevalence of HT in Egypt was estimated by 26.3% of Egyptian population and increased progressively with age, from 7.8% in 25- to 34-year-olds to 56.6% in those 75 years or older (Ibrahim et al., 1995). High blood pressure is defined as chronic elevations of systemic arterial blood pressure \(\geq 140/90\) mm Hg (Giles et al., 2005). Persistent hypertension is one of the risk factors for stroke, myocardial infarction, heart failure and arterial aneurysm, and is a leading cause of chronic kidney failure (Pierdomenico et al., 2009). Mild to moderate essential hypertension is usually asymptomatic and called silent killer (Pitt and Adams, 1998). In patients with hypertension, endothelial activation and damage lead to changes in vascular tone, vascular reactivity, and coagulation and fibrinolytic pathways (Padwal et al., 2007). Multiple evidences suggest that oxidative stress alters many functions of the endothelium, including modulation of vasomotor tone. Inactivation of nitric oxide (NO) by superoxide and other reactive oxygen species (ROS) occur in conditions such as hypertension (Hemmelgarn et al., 2006). Normally nitric oxide is an important regulator and mediator of numerous processes in the nervous, immune and cardiovascular systems, including smooth muscle relaxation thus resulting in vasodilation of the artery and increasing blood flow, it also acts as a suppressor of migration and proliferation of vascular smooth-muscle cells (Chiong, 2008).

The main classes of drugs that treat hypertension has its side effect which ranges from dizziness to heart block passing by hypercalcemia, hypokalemia, hyponatremia, hyperglycemia, hyperlipidemia, hyperuricemia, glucose intolerance, impotence, bradycardia and depression (Wright et al, 2005; Kragten and Dunsleman, 2007; Wright et al., 2008). Herbal remedies can effectively be used to treat high blood pressure instead of the pharmaceutical drugs to avoid adverse reactions caused by antihypertensive medications. Herbs like ginkgo (Ginkgo biloba), garlic (Allium sativum), and onion (Allium cepa) has the ability to relax blood vessels muscles and reduce the levels of cholesterol and used in the treatment and prevention of cardiovascular diseases including hypertension (Brankovic et al., 2011). The leek, Allium ampeloprasum var. porrum (L.), also known as Allium porrum. It which is a plant belonging to fa-
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Family Alliacea (Liliaceae), commonly named leek, is a biennial herb, closely related to garlic and onion (Peumans et al., 1997). *Allium* genus such as garlic (*A. sativum*), onion (*A. cepa*) and leek (*A. porrum*) are widely known vegetables, cultivated and consumed as flavors and foods throughout the world. In fact, garlic, onions and leek were likely cultivated in ancient Egyptian times; they are the oldest cultivated plants and are still used both as a food and for medical purposes. They are a rich source of a number of phytonutrients which make them important elements of the diet. They are also useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, type II diabetes, hypertension, cataract and disturbances in the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia) (Griffiths et al., 2002).

**MATERIALS AND METHODS**

**Animals**

Adult albino male Wister strain rats, weighing 200-250 g, were used in all experiments of this study. They were obtained from the animal house colony of the National Research Center (Dokki, Giza, Egypt) and were housed under conventional laboratory conditions throughout the period of experiments. The animal were fed a standard rat pellet diet and allowed free access to water. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985)

**Drugs and herbs**

Garlic tablets (Sekem Company, Egypt) and alcoholic extract of *Allium porrum* bulbs, and leaves (cultivated in Egypt) were used in this study. All drugs were freshly dissolved in distilled water and were given orally. The concentration was adjusted so that each 100 g animal body weight received 0.5 ml, containing the required dose of each drug.

**Chemicals and test reagent kits**

Chemicals:-
- L-NAME from Sigma Co., Egypt
- Ethyl alcohol absolute form El Nasr Pharmaceutical Co., Egypt
- Diethyl ether from Chemicals from El Nasr Pharmaceutical

Reagent kits:-
- Nitric oxide colorimetric according to Montgomery and Dymock, (1961).
- Total antioxidant according to Kora-vcic et al., (2001).
- Cholesterol according to Allain et al., (1974)

All kits are colorimetric method and were obtained from Biodiagnostic Co., Egypt.

**Allium porrum identification**

*Allium porrum* were collected from Banha city, Egypt in the months of May and June; 2006. The plant was botanically identified and authenticated by Dr. Ahmed Abd Al-Aziz Abd Al-Fattah, Ass. Prof. of Aromatic and Medicinal Plant Department, Agricultural Research Centre. Then leaves and bulbs were dried and ground to powder using clean mill and mortar then soaked in 1L of 70% ethanol and left to stand 24 hours with shaking, the solution was filtered and alcohol removed from the filtrates using rotary evaporator under 60-70°C (Movahedian et al., 2006).

**Acute toxicity study and the effect of long administration of *Allium porrum* on liver and kidney functions were studied as following**

- *Allium porrum* extract was suspended in distilled water then given orally in graded
doses to rat up to 5g/kg and the control group received the same volume of the distilled water. The percentage mortality was recorded 24 hours later.

-Tow groups of rats (n=8). First group rats were weighed and given the extract of *Allium porrum* (250, 500mg/kg) as single daily oral dose for 8 successive weeks. Second group rats were given distilled water orally and served as control group. Blood samples were collected at zero time and after 4 and 8 weeks for assessment of liver function tests (serum ALT, AST, ALP) and kidney function tests (BUN, serum creatinine).

The results of acute toxicity study showed that the extract used were safe up to 5 g/kg and the experimental dose were used in the present study were 1/20 and 1/10 of (5g/kg) of the extract (250, 500 mg/kg). Also the long oral administration proved that there were no significant difference between control and treated groups at basal, 4 and 8 weeks after treatment with extract.

**Experimental design**

Hypertension was induced according to Majithiya *et al.*, (2005), rats were weighed and given L-NAME (50 mg/kg) in distilled water (D.W.) daily oral for 4 successive weeks. The blood pressure was measured by a tail-cuff method then rats were divided into five groups, of 8 rats /each as follows:

- **Group 1**: rats given orally distilled water (0.5ml/100g) throughout the experiment and served as negative control group,
- **Group 2**: rats given L-NAME (50 mg/kg/day) as single daily oral dose for 4 successive weeks and served as positive control group,
- **Groups 3, 4**: rats given orally *Allium porrum* extract (250,500 mg/kg) respectively for 8 weeks before induction of hypertension and 4 week simultaneously with L-NAME (50 mg/kg/day),
- **Group 5**: rats were orally given garlic solution (53 mg/kg/day) for 8 weeks before induction of hypertension and 4 weeks simultaneous with L-NAME (50 mg/kg/day).

Systolic blood pressure and heart rate of animals were measured each week by non invasive blood pressure monitor (ML 125NIBP, AD Instruments, Australia). Rats were restrained in the tubes for 10-20min/day for 5 days prior to recording blood pressure in the tail-cuff technique, rats were warmed for 30min at 28°C in a thermostatically controlled heating cabinet (Ugo Basille, Italy) for better detection of tail artery pulse, the tail was passed through a cuff and a tail-cuff sensor that was connected to an amplifier (ML 125 NIBP, AD Instruments, Australia). The amplified pulse was recorded during automatic inflation and deflation of the cuff.

At the end of the experiment blood samples were withdrawn from retro orbital venous plexus of rats after light ether anesthesia and the serum was separated by centrifugation at 3000 rpm for 10 min. All parameters were measured in serum using colorimetric kits.

**RESULTS**

**Statistical analysis**

Data was expressed as mean ± SE where (n=8). Statistical comparisons between the control group and the treated groups were carried out using two-way ANOVA in systolic blood pressure measurement while rest of the experiments were carried out using one-way ANOVA, followed by Duncan’s multiple range test. Significance difference between groups were determined at the corresponding time at p<0.05.

The effects of oral administration of *Allium porrum* alcoholic extract (250,500 mg/kg) and garlic (53 mg/kg) daily for 12 successive weeks, 8 weeks before induction of hypertension and 4 successive weeks simultaneous with L-NAME (50mg/kg/day, orally) resulted in the following:

**Pulse rate**

The mean value of pulse rate of the normal
control group (given distilled water) was 327.04±19.43 beats/min that didn’t change during the experimental period. No significant effects on the pulse rate were observed by administration of L-NAME (50mg/kg/day, orally), *Allium porrum* alcoholic extract (250 and 500mg/kg), nor garlic (53mg/kg) during the 4 weeks of the experimental period. (Table 1 and Figure 6).

**Blood pressure**

Systolic blood pressure (SBP) was recorded and mean value of the normal control group was 98.14 ±5.29 mmHg which didn’t change significantly during the experimental period. Administration of L-NAME increased the systolic blood pressure significantly starting from the first week till the end of the experiment by 34, 44, 46, and 73% respectively as compared to that of the normal control group. Administration of *Allium porrum* extract (250 and 500 mg/kg) and garlic daily resulted in significant attenuation of the elevated systolic blood pressure in the L-NAME-treated rats respectively by 10, 13, and 14% in the 1<sup>st</sup> week, 14, 16 and 17% in the 2<sup>nd</sup> week, 17, 23, and 23% in the 3<sup>rd</sup> week and 25, 27 and 28% in the 4<sup>th</sup> week, compared with positive control group but still significantly higher than the normal group (Figure 1).

**Serum nitric oxide (NO) level**

The mean value of NO metabolite of the normal control group (given distilled water) was 52.236±3.423 (µmol/L). Significant decrease in serum NO by 31% in comparison with normal control rats was resulted due to treatment with L-NAME (50mg/kg/day, orally). Administration of *Allium porrum* alcoholic extract (250 and 500mg/kg), and garlic (53mg/kg) significantly re-

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**Table 1.** Effect of oral administration of *Allium porrum* alcoholic extract [A.p.ext.] (250, 500mg/kg) and garlic (53mg/kg) on pulse rate of L-NAME treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pulse rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
</tr>
<tr>
<td>Normal control</td>
<td></td>
</tr>
<tr>
<td>Given distilled water</td>
<td>327.04</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
</tr>
<tr>
<td>L-NAME (50mg/kg)</td>
<td>323.8</td>
</tr>
<tr>
<td>L-NAM (50mg/kg) + A.p.ext.(250mg/kg)</td>
<td>315.63</td>
</tr>
<tr>
<td>L-NAM (50mg/kg) + A.p.ext. (500mg/kg)</td>
<td>319.75</td>
</tr>
<tr>
<td>L-NAME(50mg/kg) + garlic (53mg/kg)</td>
<td>330.9</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n=8).
Statistical comparison of difference between the control group and the treated groups was carried out using two-way ANOVA followed by Duncan’s multiple range test.
No significant difference between groups at the corresponding time at p<0.05.
Fig (1): Effect of oral administration *Allium porrum* alcoholic extract [A.p.ext.] (250, 500mg/kg) and garlic (53mg/kg) on systolic blood pressure in L-NAME treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using two-way ANOVA followed by Duncan’s multiple range tests.

@ Statistically significant from L-NAME-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.

Fig (2): Effect of oral administration of *Allium porrum* alcoholic extract [A.p.ext.] (250, 500mg/kg) and garlic (53mg/kg) on serum nitric oxide (NO) level in L-NAME treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range tests.

@ Statistically significant from L-NAME-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.

Fig (3): Effect of oral administration of *Allium porrum* alcoholic extract [A.p.ext.] (250, 500mg/kg) and garlic (53mg/kg) on serum malondialdehyde (MDA) level in L-NAME treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range tests.

@ Statistically significant from L-NAME-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.

Fig (4): Effect of oral administration of *Allium porrum* alcoholic extract [A.p.ext.] (250, 500mg/kg) and garlic (53mg/kg) on serum total antioxidant capacity (TAC) level of L-NAME treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range tests.

@ Statistically significant from L-NAME-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.
versed the decreased release of NO level to reach approximately normal control value (Figure 2).

**Serum malondialdehyde (MDA) level**

The mean value of MDA of the normal control group (given distilled water) was 2.56±0.43 nmol/ml. Significant elevation in serum MDA by 103% were observed in L-NAME (50mg/kg) treated group. Administration of *Allium porrum* alcoholic extract (250 and 500mg/kg), and garlic (53mg/kg) significantly reversed the elevation of MDA levels to reach approximately normal control values (Figure 3).

**Serum total antioxidant capacity (TAC)**

The mean value of TAC of the normal control group (given distilled water) was 1.754±0.135 Mm/L. Significant elevation in serum TAC were observed by administration *Allium porrum* alcoholic extract (250, 500mg/kg) and garlic (53mg/kg) by 69, 101 and 82% respectively (Figure 4).

**Serum total cholesterol (TC) level**

The mean value of TC of the normal control group (given distilled water) was 75.683±5.78 mg/ dl. Significant increase in serum total cholesterol by 37% compared with normal control group of rats resulted due to treatment with L-NAME. Administration of *Allium porrum* alcoholic extract (250 and 500mg/kg), or garlic (53mg/kg) significantly reversed the increase of TC levels to reach approximately normal control values (Figure 5).

**DISCUSSION**

In this study HT is induced by L-NAME at a dose of 50 mg/kg daily for 4 weeks [2 (Majithiya et al., 2005). Treatment with L-NAME cause significant decrease in serum NO metabolite. This finding is agreed with result produced in this study where L-NAME (50mg/kg/day, orally) resulting in
significant decrease in serum NO. L-NAME is a nitric oxide synthase (NOS) inhibitor, thus it inhibit nitric oxide (NO) synthesis from its precursor L-arginine which has been shown to be the active principle of the endothelium derived relaxing factor leading to vasoconstriction and hypertension (Nakamura et al., 1997). In current study the induced time-dependent significant elevation of systolic blood pressure from normal rats was attenuated by oral administration of *Allium porrum* (250,500 mg/kg) and garlic (53 mg/kg) daily for 12 successive weeks, 8 weeks before induction of hypertension and 4 successive weeks simultaneous with L-ANME (50mg/kg/day, orally) and this result is agreed with Pedraza-Chaverrí et al., (1998) which concluded that the L-NAME-induced hypertension is blocked in garlic fed rats by antagonizing in vivo the inhibitory effect of L-NAME on NO production. This result well explained, after studying the mode of action of L-NAME which cause endothelium-dependent contractions in isolated arteries and inhibit endothelium-dependent relaxations to a variety of agonists. Furthermore L-NAME-induced decrease in glomular filtration rate (GFR) is enhanced according to Van der Linde et al., (2003).

Oral administration of *Allium porrum* (250, 500 mg/kg) and garlic (53 mg/kg) in L-NAME induced hypertensive rats resulted in significant increase in serum nitric oxide and this could describe the attenuating effect of *Allium porrum* on elevated blood pressure may be due to increase serum nitric oxide by stimulating nitric oxide synthase. These results were in agreement with Das et al., (1995) who reported that alcoholic garlic extracts increased nitric oxide synthase activity in a dose-dependent manner by activation of calcium-dependent nitric oxide synthase and the subsequent production of nitric oxide in in-vitro study on platelets. The g-Glutamylcysteines are compounds found in *Allium sativum* and *Allium porrum* may lower blood pressu-
isoalliin, respectively) (Kubec et al., 2000), make *Allium porrum* may be act by the same mechanism of *Allium sativum* (garlic). It is also reported in that rabbits treated with 250, 500, or 1000 mg/kg of body weight of a hydroalcoholic extract of leek showed a significant decrease of total cholesterol and LDL cholesterol levels without a significant change in HDL cholesterol and TG content with respect to the hyper-cholesterolemic group (Movahedian et al., 2006). Animal data show that garlic significantly decreases hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase activity, and may have some effects on cholesterol α hydroxylase, fatty acid synthetase, and pentosephosphate pathway enzyme activity (Bathaie and Akhondzadeh, 2008).

In the current study oral administration of alcoholic extract of *Allium porrum* and garlic resulted in restoration of serum malonaldehyde (MDA) concentration in L-NAME-treated group and this elevation in serum MDA occur by L-NAME administration agreed with Miguel-Carrasco et al., (2008). Where MDA is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress mediated by lipid peroxidation in renal tissue (Ö ktem et al., 2006). Jain and Wise, (1995) stated that accumulation of lipid peroxidation products can induce vasoconstriction.

Ö ktem et al., (2011) reported that chronic L-NAME (75 mg/kg/day) administration resulted in a significant depletion of serum nitric oxide (NO), significant increase in renal tissue MDA level activities and decrease in renal tissue super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. Also, long-term NO inhibition has been associated with increased vascular superoxide anion (Nabha et al., 2005).

So the effect of alcoholic extract of *Allium porrum* in decreasing the serum MDA and significant increase in total antioxidant capacity in current study could be explained by the presence of its phenolic content, this result is agreed with (Tsai et al., 2005) who reported that aqueous extracts of *Allium porrum* appeared to contain more phenolic compounds than those of garlic and green onion and thus the antioxidant activities of *Allium porrum* is bigger than green onion and garlic. Also Rose et al., (2005) demonstrated that several organosulfur compounds identified in *Allium* species have antioxidant properties, the organosulfur compounds found in *Allium porrum* and garlic extract, can reduce lipid peroxidation and hydrogen peroxide formation.

Borek (2001) reported that organosulfur compounds, such as S-allylcysteine and S-allylmercaptocysteine which extracted from *Allium porrum* and garlic exert an antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells. They also inhibit lipid peroxidation, reducing ischemic/ reperfusion damage and inhibiting oxidative modification of LDL. Yin et al., (2002) demonstrated that the antioxidant protection of organosulfur agents was concentration dependent.

**CONCLUSIONS**

We can concluded that oral administration of *Allium porrum* has protective effect against hypertension induced by L-NAME in rats and this effect may be achieved due to the presence of sulfur amino acid compounds in it which antagonize the inhibitory effect of L-NAME on nitric oxide NO release leading to smooth muscle relaxation resulting in vasodilatation. Thus safety and efficacy of *Allium porrum* make the isolation of its active constituents and testify their therapeutic effect on different biological diseases is recommended.
REFERENCES


