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Acute and Sub-acute Toxicity of *Allium sativum* Linn. (Garlic) Bulbs in Animal Model

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Allium sativum Linn. (Garlic) (Family-Liliaceae) is one of the commonly used medicinal plants in Myanmar traditional medicine for the treatment of hypertension. The present study was carried out to determine the phyto-chemical constituents and to find out the acute and sub-acute toxicity of dried bulb powder of garlic on animal model. The acute toxicity test of the dried bulb powder was carried out in albino mice. In sub-acute toxicity study, the dried bulb powder in the doses of 300 mg/kg and 600 mg/kg were administered orally to the albino rats daily for 90 days. At the end of 90 days, all the rats were sacrificed. Blood samples of the rats were collected and tested for haematological and biochemical parameters. The internal organs were removed and collected for histopathological studies. It was found that the dried bulb powder contained alkaloids, flavonoids, glycosides, polyphenol, amino acid, steroid/terpene, glycoside, tannin, saponin, carbohydrate and resin. In the acute toxicity study, it was found that LD_{50} value of the bulb powder was more than 2 g/kg. In the sub-acute toxicity study, the dried bulb powder at the dose of 300 mg/kg and 600 mg/kg showed no significant changes in body weight, hematological (blood complete picture) and biochemical (serum creatinine and blood urea, liver function test) parameters when compared with those of the control group. Histopathological studies of the internal organs of the rats showed no pathological changes. Therefore, it can be concluded that the dried bulb powder of garlic had no acute toxic effect in the mice and sub-acute toxic effect in the rats.

Key words: Toxicity, Garlic bulbs, Animal model

INTRODUCTION

Allium sativum Linn. (Family-Liliaceae) is a medicinal plant which is widely cultivated in Shan State in Myanmar. It is commonly known as garlic. The bulb of *Allium sativum* Linn. has been used for culinary and medicinal purposes in many countries since ancient times. The bulb of *Allium sativum* Linn. is traditionally used for the treatment of fever, cough, asthma, flatulance, arthritis, skin disease, hypertension, diabetes mellitus, etc.[1, 2]

The bulbs of the garlic has been reported to have many medicinal activities such as hypolipidemic, hypocholesterolaemic, anti-microbial, antihypertensive, hypoglycaemic, hepatoprotective, antioxidant and anticoagulant activities.[3, 4]

Oral administration of petroleum ether extract of garlic bulb to rats significantly prevented the rise in serum cholesterol and triglyceride levels caused by arthrogenic diet and it was found to have significant protective effect against arthrogenic diet induced arthrosclerosis.[5] One study has reported that ethanol extract of garlic supple-

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mentation to the hypercholesterolemic men caused lowering plasma concentration of total cholesterol and LDL cholesterol. Garlic extract supplementation in animal diets similarly reduced plasma concentration of total cholesterol and triglyceride.[6] Garlic extract was found to have anti-bacterial activity (against *E. coli, Samonella typhosa*, *Shigella paradysentriae*, *Staphylococcus* species, *Proteus vulgaris*) antifungal and antiviral activities.[7, 8]

Garlic bulbs yield essential oil containing sulphur compound, allicin which possesses various medicinal activities.[3] It was found that oral administration of garlic extract decreased the blood pressure in spontaneously hypertensive rats.[9] It was reported that garlic capsule administration on hypertensive patients decreased blood pressure level.[10]

Bulbs of the garlic have been used traditionally by Myanmar people as a remedy for hypertension. It is also obtained in some Myanmar Traditional Medicine Formulations for hypertension. In chronic toxicity study, some degrees of liver and lung toxicities have been observed in rats with high oral dose of garlic juice.[11] Despite the wide spread use of garlic, a few scientific studies have been undertaken to investigate the safety of garlic. Moreover, there is no scientific report of toxicity studies of the dried bulb powder of garlic in Myanmar.

Therefore, this study was done to investigate the acute and sub-acute toxicity of dried bulb powder of *Allium sativum* Linn. in animal model as well as its phytochemical constituents.

MATERIALS AND METHODS

Study design was an experimental animal study. Control parallel study design for toxicity studies was used. Site of study was in Pharmacology Research Division, DMR-LM.

Plant materials

The bulbs of *Allium sativum* Linn. (garlic) from Shan State were collected from market in Yangon. The bulbs were identified by the Botanist from Department of Botany, Yangon University. The bulbs were air-dried under the shade. Then, the air-dried bulbs were made into powder by using grinding machine.

Phytochemical and physicochemical studies

Phytochemical studies of the dried bulb powder of garlic were done qualitatively for the presence of alkaloids, flavonoids, glycolsides, steroids, saponins, tannins, resin, amino acid, phenol and cyanogenic glycoside by using the method of Physio-chemical Standard of Unani Formulations.[12] The physicochemical tests of dried bulb powder were done by the method of WHO.[13]

Acute toxicity study

The acute toxicity study of the dried bulb powder of garlic was done to determine the degree of toxicity to administration of the drug and to find out the median lethal dose (LD_{50}) by using the method of Litchfield and Wilcoxon.[14] Forty albino mice (ddy strain of both sexes weighing 25-35 gm) from Laboratory Animal Services Division, DMR-LM were used in this study. The mice were randomly divided into 4 groups with 10 mice each.

Each group of mice was placed separately in each mouse cage. They were fasted for 18 hours but allowed free access to water before administration of the dried bulb powder of garlic. The mice in group I were given distilled water orally and served as the control group. Group II, III and IV were given 0.5 g/kg, 1 g/kg and 2 g/kg body weight of the dried bulb powder of garlic, respectively through oral route. After that, the mice were allowed free access to water and standard pellet diet *ad libitum*. Then, they were observed for general condition, behavioral changes, motor activities and mortality daily up to 2 weeks.

Sub-acute toxicity study

Sub-acute toxicity study was done by using the method described in WHO.[15] Thirty albino rats (Wistar strain) of both sexes

weighing 150-200 g were used in this study. They were randomly divided into 3 groups and each group contained 10 rats. Each group of rats were placed separately in each rat cage. Two dose levels (300 mg/kg body weight and 600 mg/kg body weight) of the dried bulb powder of garlic (suspended in distilled water) were used in this study.

The rats in group I were given distilled water through oral route and served as the control group. Group II and group III were given 300 mg/kg body wt (bwt) (ie., 15 times that of human dose) and 600 mg/kg bwt (ie., 30 times that of human dose) of the garlic bulb powder, respectively through oral route daily for 90 days. The rats were allowed free access to water and standard pellet diet *ad libitum*. They were observed for general conditions, behavioral changes, motor activities as well as mortality daily for 90 days. Body weights were measured weekly.

At the end of the experimental period, all rats were anesthetized with chloroform and blood samples were immediately collected from their hearts by cardiac puncture for haematological (Blood for complete picture and platelet count) and biochemical analysis such as Liver Function Tests [serum bilirubin, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)] and renal function test (serum creatinine, blood urea). Autopsy examination was performed to examine gross pathological lesions of the internal organs.

Then, the internal organs such as brain, heart, lungs, liver, stomach, small intestine, pancreas, spleen and kidneys were removed, weighed and preserved in 10% formalin solution. Tissues slides were prepared and stained with hematoxylin and eosin. Histopathological examinations were performed by the pathologist.

Data analysis

The data were expressed as mean±SD. The statistical difference between the means of treatment group and control group was tested by unpaired 't' test. A difference at $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical and physicochemical studies

The phytochemical study of the dried bulb powder of garlic showed the presence of alkaloids, flavonoids, glycosides, steroids/ terpenes, saponin, tannins, amino acids, polyphenol, resin, carbohydrate and amino acid. Cyanogenic glycoside was not detected.

Physicochemical tests of the crude powder of the dried bulb powder of garlic showed water and volatile matter content (9%), swelling index (1.5 cm), foaming index (<100), total ash (5.2%), ethanolic extract (6.3%), chloroform extract (0.6%), petroleum ether extract (0.3%) and watery extract (58.3%).

Acute toxicity study

There were no lethality or any signs of toxicity observed in the mice after oral administration of the dried bulb powder of garlic at the doses of 0.5 g/kg, 1 g/kg and 2 g/kg, respectively.

Sub-acute toxicity study

Effect of the dried bulb powder of garlic on body weights and organ weights

The results were shown in mean±SD. The average initial body weights of the control rats and the rats treated with the dried bulb powder of garlic at the dose of 300 mg/kg and 600 mg/kg were 217±28.87 g, 208.9±8.77 g and 219±20.11 g, respectively. At the end of 90 days, the average final body weights of the control and tested rats, were 277.5±44.54 g, 250.8±37.7 g and 275.5±58.28 g, respectively.

It was found that there were no significant differences in the average body weights between control and two test groups throughout the experimental period ($p>0.05$). Lethality and signs of toxicity were not also observed. There was no significant difference in the average organ weights among

the rats of control and test groups ($p>0.05$). The results were shown in Table 1.

Table 1. Changes in the organ weights of rats in control group and groups treated with dried bulb powder of garlic

Internal organs (gm)	Group I (Control)	Group II garlic bulb powder (300 mg/kg)	Group III garlic bulb powder (600 mg/kg)
Brain	1.58±0.18	1.49±0.2	1.6±0.16
Lungs	1.28±0.28	1.2±0.13	1.16±0.18
Heart	1.01±0.22	0.89±0.13	0.9±0.17
Liver	12.24±3.89	10.54±2.5	10.37±3.35
Stomach	2.43±.59	2.23±0.34	2.17±0.37
Intestine	0.6±.036	0.46±0.18	0.56±0.2
Pancreas	0.75±0.31	0.78±0.42	0.76±0.42
Spleen	0.88±0.4	1.23±0.84	0.64±0.24
Kidney	2.6±0.74	2.22±0.48	2.18±0.49

Sample size for each group (n=10)
The data were expressed in mean±SD. Statistical comparisons were made between the values of the control and the groups receiving the dried bulb powder of garlic.

Table 2. Effect of the dried bulb powder of garlic haematological parameters of rats

Parameters	Group I (Control)	Dried bulb of garlic powder		Normal range
		Group II (300 mg/kg)	Group III (600 mg/kg)	
Haemoglobin level (g/dl)	14.6 ±1.65	13.71 ±0.65	12.77 ±0.42	11.4-19.2
Total WBC count ($x10^3$ /µl)	8.6 ±3.56	8.68 ±1.27	9.02 ±3.99	5-25
Neutrophil (%)	23.7 ±9.25	15.5 ±5.74	22.2 ±18.65	9-34
Lymphocyte (%)	69.5 ±9.3	77.4 ±8.09	69.1 ±17.98	65-84
Monocyte (%)	6.5 ±5.29	7.1 ±5.09	8.7 ±5.06	0-5
Eosinophil (%)	0.3 ±0.48	0	0	0-6
Platelet count ($x10^3$/µl)	776.8 ±301.47	815 ± 240.43	800 ±216.02	500-1000

Sample size for each group (n=10)
The data were expressed in mean±SD. Statistical comparisons were made between the values of the control and the groups receiving the dried bulb powder of garlic.

Effect of the dried bulb powder of garlic on haematological parameters

There were no significant differences of haemoglobin level, white blood cell count and platelets count between the rats treated with 300 mg/kg and 600 mg/kg dose of the bulb powder of garlic and the control group ($p>0.05$) (Table 2). These results were also within the reference normal ranges.[16]

Effect of the dried bulb powder of garlic on biochemical parameters

There were no significant differences of serum bilirubin, serum creatinine and blood urea among three groups. The results of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of the rats in control group and treated group were in the reference normal range (Table 3).[17]

Table 3. Effect of dried bulb powder of garlic on biochemical parameters of rats

Biochemical parameters	Group I (Control)	Dried bulb powder of garlic	
		Group II (300 mg/kg)	Group III (600 mg/kg)
Serum bilirubin (µmol/l)	11.4 ±1.43	10 ±2.45	15.7 ±11.98
Serum aspartate amino transferase (AST) (IU/L)	179.1 ±42.69	185.6 ±79.37	130.2 ±18.71
Serum alanine aminotransferase (ALT) (IU/L)	163.5 ±58.95	198.8 ±58.62	111.4 ±30.97
Serum alkaline phosphatase (ALP) (µmol/l)	201 ±12	175.7 ±28.22	193.1 ±18.39
Serum creatinine (mg/dl)	1.18 ±0.14	1.18 ±0.21	1.22 ±0.2
Blood urea (mg/dl)	49.7 ±11.61	53.9 ±8.76	44.2 ±9.3

Sample size for each group (n=10)
The data were expressed in mean±SD. Statistical comparisons were made between values of control and groups receiving the dried bulb powder of garlic.

Effect of the dried bulb powder of garlic on histology of internal organs

The gross examinations of the internal organs of the control and two groups of rats treated with the bulb powder of garlic were found to be normal. In histopathological examinations of the rats, no pathological lesions were found in the tissues of brain, lungs, heart, liver, stomach, intestines, pancreas, spleen and kidneys of the control group and the treated groups of rats.

DISCUSSION

Alliun sativum Linn. (Garlic) is traditionally used as a remedy for hypertension in Myanmar. Garlic is widely used around the world as the food condiment and herbal medicine for many years.

The present study has been carried out to assess the safety of dried bulb powder of garlic in animal model. In the phyto-chemical study, it was found that dried bulb powder of garlic did not contain toxic plant compound like cyanogenic glycoside. One study reported that the acute oral toxicity test of garlic extract was studied in rats and mice. The group of mice receiving 30 ml/kg bwt of garlic extract showed no toxic effect.[18]

In this study, the oral route of administration was used because it is the intended route for use in human. In acute toxicity study, it was observed that the oral administration of the dried garlic bulb powder was not toxic in mice up to the maximum feasible dose of 2 g/kg. This result showed that median lethal dose (LD_{50}) of the garlic bulb powder was above 2 g/kg.

In chronic toxicity study, it was reported that some degrees of liver and lung toxicities have been observed in rats with high oral dose of pure garlic juice (ie., 5 ml/kg).[11] Some people suffer from allergies to garlic. It can also cause indigestion, nausea and vomiting when taking large amount of garlic.[3] Garlic is generally recognized as safe food and most people enjoy garlic.

In sub-acute toxicity study, garlic bulb powder did not produce any toxic effect or lethality among tested animals received two doses of garlic bulb powder (300 mg/kg and 600 mg/kg) orally daily for 90 days. There was no significant difference in body weights and organ weights due to two doses of garlic bulb power when compared with those of control group.

In haematological studies, there were no significant difference in haemoglobin level, white blood cell counts and platelet counts between the groups of the rats treated with bulb powder of garlic and the control. Regarding the biochemical parameters, there was no significant difference in serum creatinine and blood urea levels between two groups of the rats treated with 300 mg/kg and 600 mg/kg of the garlic bulb powder and the control group.

Regarding the liver function test, there was no significant difference in serum bilirubin level of the rats given with different doses of the garlic bulb powder and the control rats. Liver enzymes (serum AST, ALT, ALP) were found to be in reference normal range.[17]

The most widely used criteria of the toxic action of the drugs and chemicals are reduction of body weight gain, detection of gross and histologic abnormalities in the organs, change in organ weights and the increase in mortality rate. Liver enzymes are good indicators of liver function.[16]

Serum creatinine and blood urea are used as an indicator of kidney function. It was observed that the biochemical results of rats treated with dried bulb powder of garlic produced no damage to liver and kidneys. The tissue samples taken from brain, heart, lung, liver, stomach, small intestine, pancreas, spleen and kidney of both rats treated with garlic bulb powder and the rats of control group showed no pathological lesions on gross examination and histopthological examination. The results indicated that oral administration of garlic bulb powder daily for 90 days showed no toxic effects on rats. Therefore, it can be concluded that the garlic bulb powder possess no acute toxic effect in mice and no sub-acute toxic effect in rats.

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