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28-Day Repeated Dose Oral Toxicity of a Herbal Mixture Dia-2, Containing Standardized Extracts of *Allium Sativum* and *Lagerstroemia Speciosa* In Sprague Dawley Rats.

KS. Kesavanarayanan[1], P. Kalaivani [1], G.Ramakrishnan[1], S.Sathiya[1], V.Ranju[1], R.Jyothipriya[1], Chinnasamy Selvakkumar[1], C.Saravanababu[1], J.venkhatesh[1], R.Ilavarasan[2], S.Kavimani[3], D Prathiba[4]

***Corresponding author:**

D.Prathiba

[1]Centre for Toxicology and Developmental Research, Sri Ramachandra University, Chennai 600 116, Tamilnadu, India.
[2]Captain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha, Arumbakkam, Chennai 600 106, Tamilnadu, India.
[3]Department of Pharmacology, Division of Pharmacy, Mother Therasa Institute of Health Sciences, Pondicherry, India.
[4]Department of Pathology, Sri Ramachandra University, Chennai 600 116, Tamilnadu, India.

Abstract

Allium sativum [ASE] and *Lagerstroemia speciosa* [LSE] are widely used in folk medicine as a medication for diabetes. DIA-2 is a polyherbal antidiabetic formulation containing fixed combination [1:1 w/w] of standardized aqueous extracts of *Allium sativum* bulbs containing 1.1 % alliin w/w and 40 % hydroalcholic extract of *Lagerstroemia speciosa* leaves containing 1.28% w/w corosolic acid. Earlier studies in our laboratories have demonstrated the oral safety of DIA-2 on acute oral exposure to female Sprague Dawley [SD] rats and the antidiabetic activity of DIA-2 in high-fat diet fed/streptozotocin-induced diabetic rats. The ingredients of DIA-2 have long history safety but however, there is little toxicological information regarding the oral safety on repeated exposure of ASE and LSE when given as a combined mixture. The present study evaluated the repeated oral toxicity of DIA-2 in both the sexes of SD rats. Rats were treated orally once with 62.5, 125, 250 mg/kg body weight, and animals were observed till the 28 days of study. On repeated oral administration, DIA-2 showed did not exhibit any clinical signs of toxicity, mortality, significant change in food, water consumption, body weight, mortality, clinical chemistry, hematology, organ weight, gross pathology and histopathology when varying doses of the DIA-2 were administered orally once daily for a period of 28 days. The NOAEL [No Observed Adverse Effect Level] of DIA-2 in this study was identified to be greater than 250 mg/kg/day. The results from the study suggest that there are no toxicologically significant effects on 28 day repeated oral administration of DIA-2 and the data also provide satisfactory preclinical evidence on its oral safety to support its use as a therapeutic agent in the treatment of diabetes mellitus.

Keywords: Allium sativum, Lagerstroemia speciosa, diabetes mellitus, repeated oral toxicity, herbal formulation.

Introduction

Obesity and type 2 diabetes mellitus are two major metabolic disorders that are prevalent all over the world [1], in which obesity is strongly associated with incidence of type 2 diabetes mellitus [2].Insulin resistance syndrome appears to be the common contributing factors for most of the metabolic disorders [3]. Obesity is characterized by an increase in the adipose deposits, a condition that promotes the development of insulin resistance [4]. Controlling obesity represents an approach to decrease the degree of insulin resistance and delay the incidence of type 2 diabetes followed by its associated complications [5].

Oral hypoglycemic agents (OHA)/insulin or both are prescribed in the therapeutic management of diabetes mellitus, but each class of drugs has its own side effects. Hypoglycemia and adiposity are the most common side effects shared by most classes of anti-hyperglycemic agents. Hence, these agents treat the key symptom of diabetes [i.e. hyperglycemia] but aggravate the condition of adiposity and thus beneficial for short term use and not optimal for long term use [5]. Despite of their role in effective treatment of diabetes, most of the currently available OHA are still to be optimized. So there arises a need for better candidates [6].

Several medicinal plants were being used as anti-diabetic therapies as part of our diet since prehistoric time [7]. *Allium sativum* [commonly known as garlic] and *Lagerstroemia speciosa* [also known as banaba] are one such dietary medicinal food used all over the world in the treatment of several disorders. Garlic and Banaba were shown to posses either antidiabetic effects [8; 5] or have direct effects on adipose tissue [9; 10]. Multiple therapeutic effects in the absence of toxicity can be achieved with plant drug combinations due to their diverse pharmacological actions [11]. Hence, we hypothesized that a combination of these two herbs

may show a great therapeutic potential in the management of obesity associated diabetes devoid of any side effects. Though garlic and banaba were used as dietary medicinal food traditionally, the general perception that these herbal drugs may be safe and free from toxicity may not be true, when given as a combination. Few studies on the toxicological profile of *Allium sativum* [12; 13; 14; 15] were reported indicating the need for identification a safe dose range for garlic. In case of *Lagerstroemia speciosa*, there was no toxicity related information except few animal studies and clinical studies [16; 5]. The present study investigates the toxicological effects of DIA2, a polyherbal formulation consisting fixed combination [1:1 w/w] of standardized ingredients [ASE and LSE] after 28-day repeated oral administration in both the sexes of Sprague-Dawley rats.

Materials and Methods

Chemicals and reagents

The diagnostic biochemical kits were purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India. All other chemicals and reagents used in the study were of analytically grade.

Plants extracts and preparation of DIA-2

Authentic samples of ASE and LSE were obtained commercially from M/s. Amsar Pvt. Ltd, Indore, India and M/s. K.Patel, PhytoExtractions Pvt Ltd, Mumbai, India respectively. Both ASE and LSE has been standardized and optimized and claimed to contain 1.1 % alliin w/w and 1.28 % w/w corosolic acid respectively. In addition, the heavy metal content and microbial contaminants were within the permissible limits. Both the extracts were triturated individually to a fine powder using mortar and pestle, weighed and mixed uniformly in the ratio 1:1 w/w. The resulting powder mixture is called as DIA-2. DIA-2 suspension was prepared freshly daily using reverse osmosis water before dosing.

Animals and husbandry

Healthy young male and female Sprague dawley (SD) rats of body weight range 150 ± 20% mean were selected for the study. The animals were housed individually in sterile bedded polypropylene cages and provided with food (Nutrilab Rodent, Tetragon Chemie Pvt Ltd, India) and purified water *ad libitum*. They were kept in a well ventilated room (air exchange: 70:30; air cycles: 15/min) under a standard temperature of 23±2°C and 40–65% relative humidity, with a 12-h light/dark artificial cycle. All the animals were acclimatized for 7 days prior to the study. Guidelines of "Guide for the care and use of laboratory animals" (Institute of laboratory animal resources, National academic press 1996; NIH publication number #85-23, revised 1996) were strictly followed throughout the study. The study was approved by Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University; Chennai, India (IAEC/XI/ SRMC & RI /61/2006).

Repeated dose 28-day oral toxicity study

The study was performed as per the Organisation for Economic Co-operation and Development (OECD) test guideline 407 (Adopted: 3 October 2008). Both sexes of young healthy adult SD rats ranging from 140-160g were acclimatized individually in sterile polypropylene cages for 7 days. Our earlier study on acute oral toxicity studies indicated that acute oral LD_{50} of DIA-2 was greater than 2000 mg/kg in female SD rats and in another study using high fat (HFD)/streptozotocin (STZ) induced diabetic rat, DIA-2 was found to exhibit anti-diabetic activity without exhibiting any toxic signs as evidenced from the histopathological finding till the dose range of 125 mg/kg body weight. DIA-2 at 250 and 500 mg/kg body weight did not show any protection after 14 days of treatment in HFD/STZ induced diabetic rats [17]. On the basis of these results, three therapeutic doses of 62.5 mg/kg (Low dose), 125 mg/kg (Mid dose) and 250 mg/kg (High dose) were selected for the study. All the animals were randomized accordingly with their body weights into 4 groups of 10 animals each (5 males, 5 females/each group). Group I served as control which received vehicle at 10ml/kg body weight orally. Group II, III and IV received DIA-2 at 62.5 mg/kg, 125 mg/kg and 250 mg/kg, per oral, respectively, for a period of 28 days. The experimental animals received their respective treatments orally twice daily.

Study design

Clinical observations which include general behaviour, respiratory pattern, cardiovascular signs, motor activities, reflexes, changes in skin, fur and mortality signs were observed for all the experimental animals continuously at 30 min, 1h, 2h and 4h after the administration of the doses and thereafter once a day for 28 days preferably at the same time (1 hour after the dose administration). Body weights were taken on 0^{th}, 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days of dose administration. Individual doses were calculated based on the most recent weekly body weights and were adjusted each week to maintain the targeted dose level. Doses were administered to all groups at a constant dose volume of 10 ml/kg. Feed and water consumption was monitored and recorded throughout the study and was expressed as 7 day cumulative data.

On the last day of dose administration all the animals were kept for overnight fasting (water *ad libitum*). The overnight fasted animals were anaesthetized under general anaesthesia using isoflourane, blood samples were collected using heparinised microhematocrit tubes by retro-orbital puncture into a potassium EDTA containing blood collection tubes (for haematological) and 11% w/v tri-sodium citrate (TSC) containing tubes (for biochemical fanalysis). Blood smear was prepared from the EDTA containing blood sample, air dried and stained (Hemacolor rapid staining of blood smear, E.Merck, Mumbai, India) for differential leukocyte count (DLC). Haematological analysis were performed using automated haematology analyzer (Model PE 6000 Rapid Diagnostics Pvt Ltd, New Delhi, India), which includes analysis of haemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC], platelet count and hematocrit (HCT).

The 11% TSC containing blood tubes were centrifuged immediately in refrigerated centrifuge (Model 5810 R, Eppendorf AG, Germany) at 1500 x g for 15 min. The plasma thus collected was analysed for glucose, triglyceride, cholesterol, alkaline phosphatase (ALP) aspartate transaminase (AST) alanine transaminase (ALT) lactate dehydrogenase (LDH), total bilirubin creatinine, urea, protein and albumin levels by using biochemical kits (Accurex Biomedical Pvt. Ltd, Thane, India) in semi automated biochemical analyzer (Model: Star 21 Plus, Rapid Diagnostics Pvt Ltd, New Delhi, India).

Histopathology

After blood collection, end terminal body weight measurements were made and all the experimental animals were sacrificed by cervical dislocation under general anaesthesia for gross pathological examination of all major internal organs. Organs like liver, kidneys, adrenals, brain, spleen, heart, lungs and testes/ovaries were dissected immediately after euthanasia, blotted free from blood and weighed. The major organs including liver, kidney, brain, heart testes, epididymis, prostate, seminal vesicle, uterus, ovary, thyroid and parathyroid were collected from vehicle control and high dose groups (250 mg/kg) and when appropriate from the lower dose groups (62.5 and 125 mg/kg). All the specimens were fixed in 10% neutral buffered formalin for 48 h. Thereafter the fixed tissues were processed for paraffin embedment, sectioned to 4 μm thick using the microtome (Leica RM2125RT, Germany). The sections were stained with haematoxylin and eosin (H&E) stain for general histopathological evaluation.

Statistical analysis

Data were expressed as mean ± standard error mean. Data obtained from repeated dose studies were analysed by Student's t-test using GraphPad prism 5.0 to determine significant difference between the means of control and test groups. p value ≤0.05 was considered significant.

Results and Discussion

Herbal medicines are consumed by humans either as food or medicine [18]. *Allium sativum* and *Lagerstroemia speciosa* are one such herbal medicine consumed either as food or medicine by humans for treating various disorders. Though the therapeutic effect of these medicinal plants in metabolic disorder management are established [16;19] but still there is limited or no scientific data regarding their safety aspects when given as a herbal mixture.

High dose (500 mg/kg body weight/day) of *Allium sativum* has been reported for its hepatotoxic potential and low doses of garlic (100 or 250 mg/kg body weight/day) are considered to safe [20; 21; 22]. High concentrations of triterpene acids, like corosolic acid have also been know for its toxic effects [23]. DIA-2 is a herbal mixture containing standardized extracts of *Allium sativum* and *Lagerstroemia speciosa*. The present study was undertaken to access the possible effects of DIA-2 after repeated oral administration for 28 days to either sex of SD rats.

The determination of repeated oral toxicity of DIA-2 could be evaluated only after obtaining initial toxic information on its acute oral exposure. Our earlier study demonstrated the oral safety in female SD rats to ascertain the toxicity related information after single administration of DIA-2 at a dose (2000 mg/kg) recommended by OECD-423 test guideline. The absence of clinical signs of toxicity, lack of changes in body weight and gross pathology of vital organs on necropsy suggested that DIA-2 is orally safe up to 2000 mg/kg body weight [17].

The selection of dose for the study is based on the therapeutic dose of DIA-2. The low dose (62.5 mg/kg), high dose (250 mg/kg) is selected based on its anti-hyperglycemic effect as investigated in our earlier study [17] and the intermediate dose (125 mg/kg) is a geometric mean between the high and the low dose. Administration of DIA-2 at these selected dose levels for 28 days to either sex of SD rats showed no treatment related changes regarding clinical signs, mortality, body weights, feed and water consumption, hematology, clinical chemistry, organ weight, gross and histopathology in rats of either sex.

Clinical signs are used as a measure of humane endpoints in safety evaluation studies; administration of DIA-2 did not produce any toxic clinical signs or mortality during the experimental period. Weekly changes in body weights are shown in fig-1, which revealed that administration of DIA-2 had no significant effect on the body weight in either sex when compared to vehicle control group. No significant difference was also observed on weekly feed [fig-2] and water consumption [fig-3] between the DIA-2 and vehicle treated groups.

Few studies on garlic use have reported to cause allergic reactions, alteration of platelet functions [24], however the platelet function is not impaired by single and repeated oral consumption at dietary dose [25]. The effect of DIA-2 on haematological test performed at the end of the study did not differ between groups [table-1]. DIA-2 treatment also showed no significant changes in plasma biochemistry compared to the control group [table-2].

Organ weight data's obtained from toxicology studies is an integral component in the safety assessment of pharmaceuticals [26] and food/nutritional products [27]. The organ weight is considered to be an important endpoint for identification of toxic effects of chemicals on target organs [28]. The relative organ weight (organ weight expressed as a percentage of body weight) was calculated from the terminal body weight and shown in table-3. There was no significant difference in relative organ weight in either sexes of rats in both DIA-2 and vehicle treated animal groups.

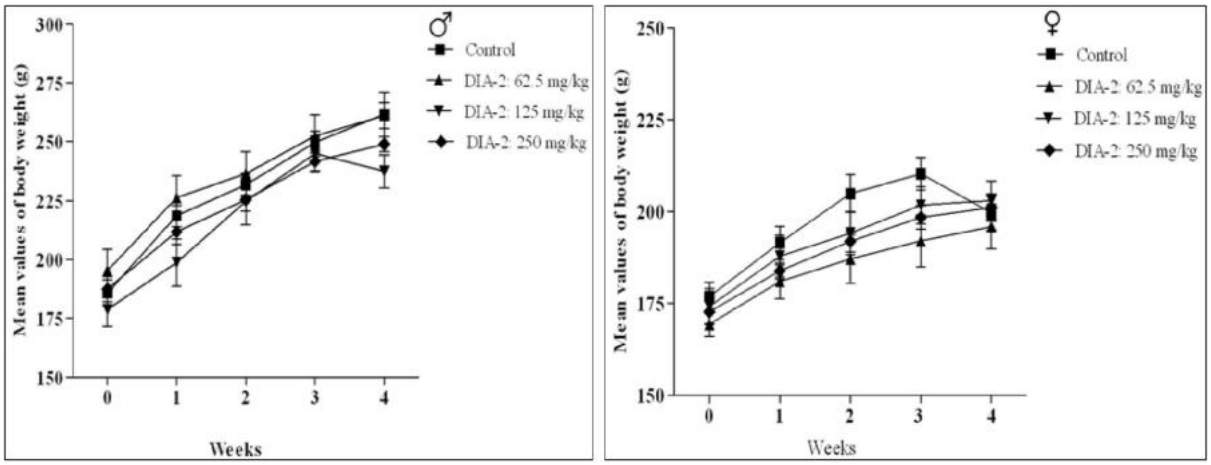

Fig-1: Effect of repeated administration of DIA-2 on body weight of male (left) and female (right) Sprague Dawley rats. Values are expressed in mean ± SEM (n=5); Statistical analysis was performed with Student't' test following one way ANOVA using GraphPad prism 5.0

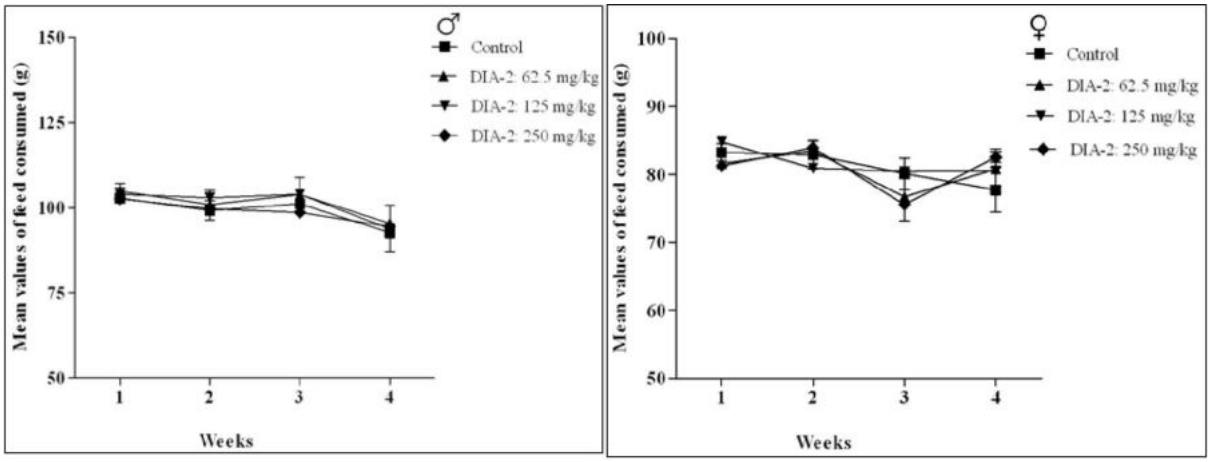

Fig-2: Effect of repeated administration of DIA-2 on feed consumption of male (left) and female (right) Sprague Dawley rats. Values are expressed in mean ± SEM (n=5); Statistical analysis was performed with Student't' test following one way ANOVA using GraphPad prism 5.0

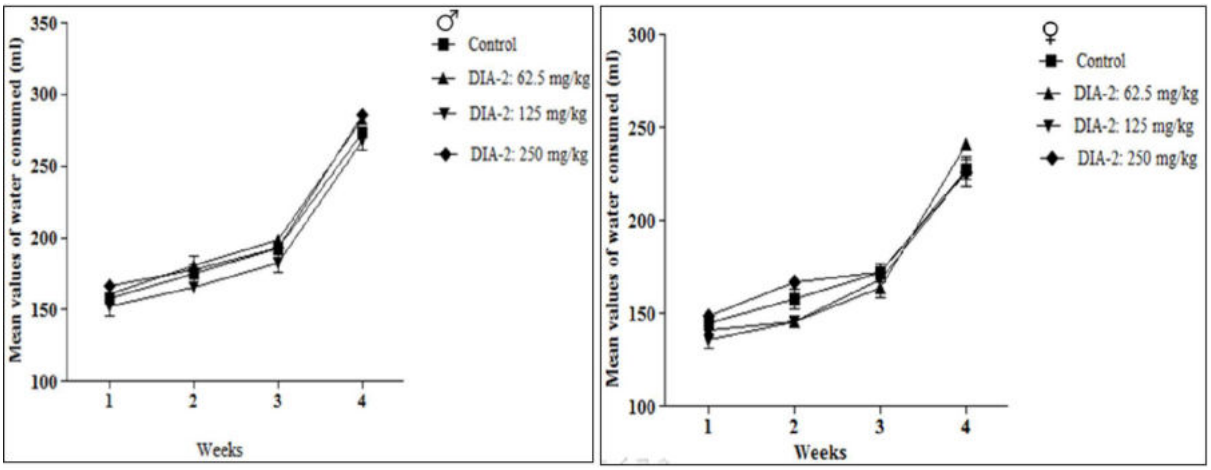

Fig-3: Effect of repeated administration of DIA-2 on water consumption of male (left) and female (right) Sprague Dawley rats. Values are expressed in mean ± SEM (n=5); Statistical analysis was performed with Student't' test following one way ANOVA using GraphPad prism 5.0

Histopathological examinations of the major organs [liver, kidney, brain, heart] were shown in fig-4. The histological changes of sexual organs of male and female rats and endocrine gland, thyroid were shown in fig-5-6. On histological examination of all the

Table.1: Effect of repeated administration of DIA-2 on haematology of male and female Sprague Dawley rats

Parameters (Units)	Sex	Control (Water 10 ml/kg)	DIA-2 62.5 mg/kg	DIA-2 125 mg/kg	DIA-2 250 mg/kg
White Blood Cells ($10^3\mu L$)	M	11.40±1.83	7.86 ± 0.41	9.75 ± 1.85	9.96 ± 1.31
	F	7.30 ± 1.07	7.22 ± 1.11	6.76 ± 0.61	9.16 ± 0.61
Red Blood Cell (106μL)	M	7.57 ± 0.63	6.14 ± 0.59	6.82 ± 0.74	5.30±0.16
	F	6.49 ± 0.73	5.42 ± 0.63	5.92 ± 0.60	5.86 ± 0.69
Hemoglobin (g/dL)	M	14.20 ± 0.28	14.2 ± 0.18	14.10 ± 0.36	13.80 ± 0.23
	F	14.00 ± 0.27	13.56 ± 0.28	13.80 ± 0.21	13.84 ± 0.24
Hematocrit (%)	M	41.08 ± 3.52	31.86 ± 3.02	35.40 ± 3.66	30.84±2.26
	F	34.70 ± 3.88	29.62 ± 3.84	31.96 ± 3.01	30.70 ± 3.31
Platelet (%)	M	215.00 ± 55.37	246.8 ± 56.08	121.00 ± 46.95	166.60 ± 42.79
	F	270.60 ± 66.81	182.40 ± 35.50	254.80 ± 51.82	163.20 ± 55.74
Neutrophil (%)	M	16.20±2.48	17.60±0.93	17.23±1.28	20.80±2.08
	F	17.80±3.68	17.40±1.36	17.40±0.60	18.80±2.35
Eosinophil (%)	M	1.60±0.51	2.20±0.66	2.00±0.71	2.08±0.28
	F	1.40±0.51	2.10±0.64	1.84±0.30	2.12±0.31
Basophil (%)	M	0.40±0.24	1.00±0.32	0.62±0.15	0.62±0.08
	F	0.80±0.37	0.26±0.08	0.46±0.10	0.70±0.11
Monocytes (%)	M	2.20±0.37	2.40±0.68	1.80±0.37	1.80±0.58
	F	1.60±0.51	2.20±0.58	2.20±0.58	2.00±0.45

Values are expressed in mean ± SEM (n=5); Statistical analysis was performed with Student't' test following one way ANOVA using GraphPad prism 5.0

organs in high dose of DIA-2 (250 mg/kg body weight) treated animals, exhibited no apparent pathological alterations when compared to vehicle treated animals.

In the safety assessment of chemicals to humans, it is essential to determine no-observed-adverse-effect-levels (NOAEL) or lowest-observed-adverse-effect-levels (LOAEL) from animal experiments [29]. The lowest-observed adverse effect level (LOAEL) of DIA-2 was ≥ 250 mg/kg and the no-observed adverse effect level (NOAEL) was ≤125 mg/kg.

In conclusion, our results showed that there was no treatment-related toxicity in rats of either sex following 28-days oral administration of 62.5, 125 and 250 mg/kg body weight of DIA-2. DIA-2 is found to be orally safe under the tested experimental condition. The data's obtained from the study supports the potential use of DIA-2 as a medicinal food or dietary supplement in the management of diabetes..

Table.2: Effect of repeated administration of DIA-2 on plasma biochemistry of male and female Sprague Dawley rats

Dose	Sex	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	ALP (U/L)	SGOT (U/L)	SGPT (U/L)	LDH (U/L)	Total Bilirubin (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Protein (g/dl)	Albumin (g/dl)
Control	M	97.72±13.02	51.62±8.83	48.28±7.96	178.54±25.62	109.15±16.64	39.27±12.87	371.17±14.29	0.03±0.01	1.70±0.07	10.51±2.95	4.91±0.09	1.96±0.11
Water (10 ml/kg)	F	115.06±6.53	39.43±3.56	98.43±14.57	184.40±49.10	80.75±4.07	34.12±2.55	270.60±28.39	0.05±0.01	1.60±0.03	15.46±0.90	5.42±0.45	2.21±0.16
DIA-2	M	76.61±6.38	56.50±5.41	72.51±7.22	261.12±27.36	91.11±4.61	47.94±5.31	272.68±38.35	0.03±0.01	1.61±0.04	14.09±1.03	5.22±0.27	2.05±0.15
(62.5 mg/kg)	F	116.72±3.84	36.24±2.46	81.35±9.81	270.52±46.62	110.93±10.82	44.79±3.97	360.18±31.77	0.06±0.02	1.63±0.03	18.41±1.03	5.75±0.30	2.13±0.14
DIA-2	M	94.63±3.05	44.84±8.88	57.50±3.75	257.52±11.60	101.42±3.51	46.69±6.01	274.84±25.60	0.02±0.01	1.62±0.04	12.78±0.79	4.97±0.18	2.06±0.17
(125 mg/kg)	F	107.26±3.43	45.17±5.16	75.79±5.16	313.40±45.23	111.44±15.98	41.99±2.26	370.70±48.20	0.03±0.01	1.70±0.07	18.82±1.16	5.75±0.36	2.36±0.20
DIA-2	M	103.40±2.56	45.57±3.13	56.56±2.21	264.46±39.16	101.75±9.23	59.15±16.84	290.40±37.44	0.03±0.01	1.60±0.04	16.31±1.64	5.20±0.38	1.94±0.15
(250 mg/kg)	F	109.92±6.02	39.21±2.99	71.20±4.95	155.87±12.40	118.90±10.47	43.93±6.16	345.08±48.82	0.05±0.00	1.65±0.09	20.10±1.14	5.97±0.15	2.42±0.10

Values are expressed in mean ± SEM (n=5); Statistical analysis was performed with Student't' test following one way ANOVA using GraphPad prism 5.0

Table.3: Effect of repeated administration of DIA-2 on relative organ weight of male and female Sprague Dawley rats

Dose	Sex	Organ Weight (gm)								
		Body Weight	Liver	Kidneys	Adrenal	Brain	Spleen	Heart	Lung	Testes/Ovary
Control	Male	242.2 ± 9.02	8.10±0.20	1.94 ± 0.06	0.06 ± 0.00	1.34 ± 0.06	1.10 ± 0.11	0.99 ± 0.08	1.49 ± 0.20	4.33±0.06
	Female	193.80 ± 3.07	6.90 ± 0.29	1.43 ± 0.02	0.04 ± 0.00	1.25 ± 0.02	0.95 ± 0.10	0.78 ± 0.04	1.43 ± 0.03	0.08 ± 0.01
DIA-2 (62.5mg/kg)	Male	245.40±5.80	7.84±0.10	2.00±0.09	0.06±0.01	1.36±0.01	0.96±0.08	0.89±0.03	1.52±0.09	4.11±0.02
	Female	180.60 ±6.47	6.70 ±0.41	1.49 ±0.06	0.05 ±0.01	1.29 ±0.02	0.81±0.08	0.77 ±0.02	1.65 ±0.15	0.06 ±0.01
DIA-2 (125mg/kg)	Male	236.75 ± 6.80	7.81 ± 0.30	1.75 ± 0.05	0.05 ± 0.00	1.41 ± 0.07	0.99 ± 0.09	0.88 ± 0.03	1.74 ± 0.06	4.15 ± 0.14
	Female	186.60 ± 5.94	6.99 ± 0.23	1.43 ± 0.03	0.06 ± 0.01	1.28 ± 0.02	0.91 ± 0.06	0.70 ± 0.03	1.46 ± 0.08	0.11 ± 0.02
DIA-2 (250mg/kg)	Male	233.25 ± 3.00	7.80 ± 0.19	1.73 ± 0.02	0.05 ± 0.01	1.35 ± 0.04	1.17 ± 0.05	0.89 ± 0.03	1.49 ± 0.04	4.14 ± 0.14
	Female	190.20±2.60	7.22±0.20	1.48±0.03	0.05±0.00	1.28±0.01	1.01±0.02	0.70±0.04	1.49±0.03	0.10±0.01

Values are expressed in mean ± SEM (n=5); Statistical analysis was performed with Student't' test following one way ANOVA using GraphPad prism 5.0

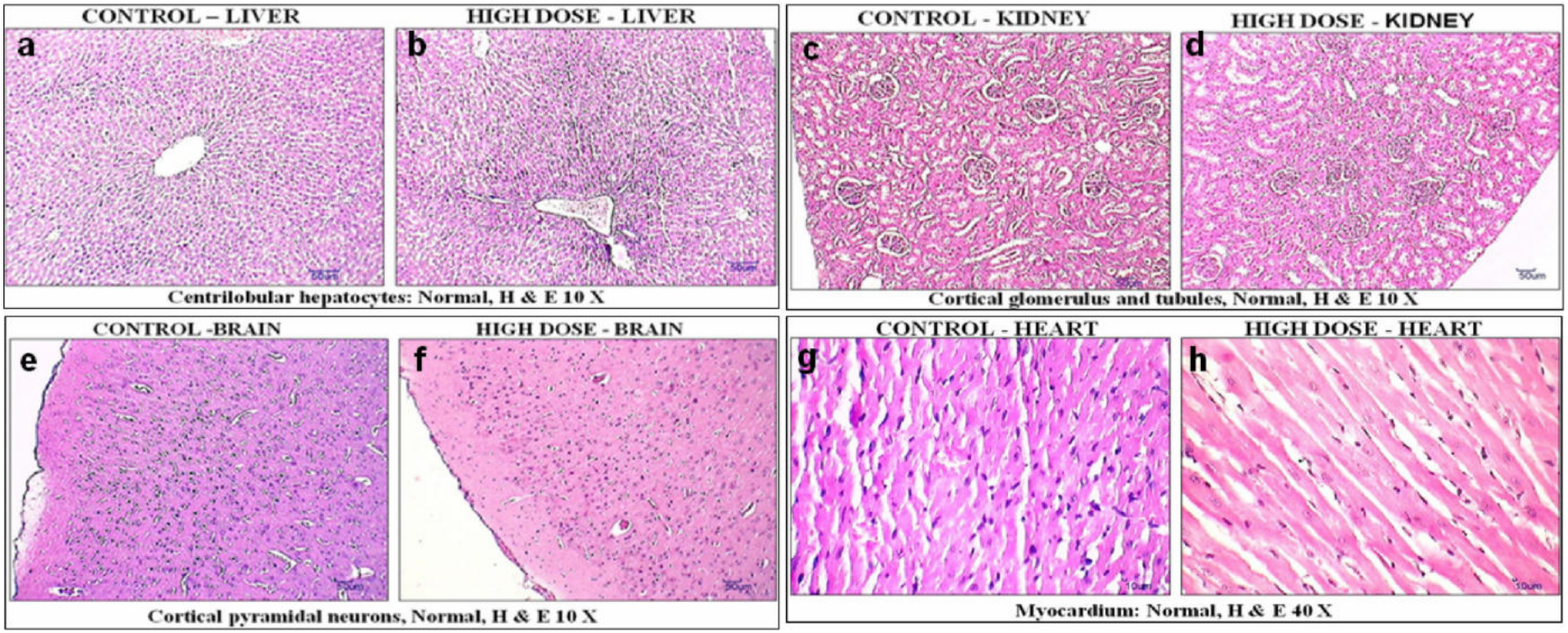

Fig-4: Color plate of liver (a, b) kidney (c, d) brain (e, f) and heart (g, h) histopathology from representative animals of vehicle treated control group and high dose of DIA-2 (250 mg/kg/day) group (hematoxylin–eosin stain).

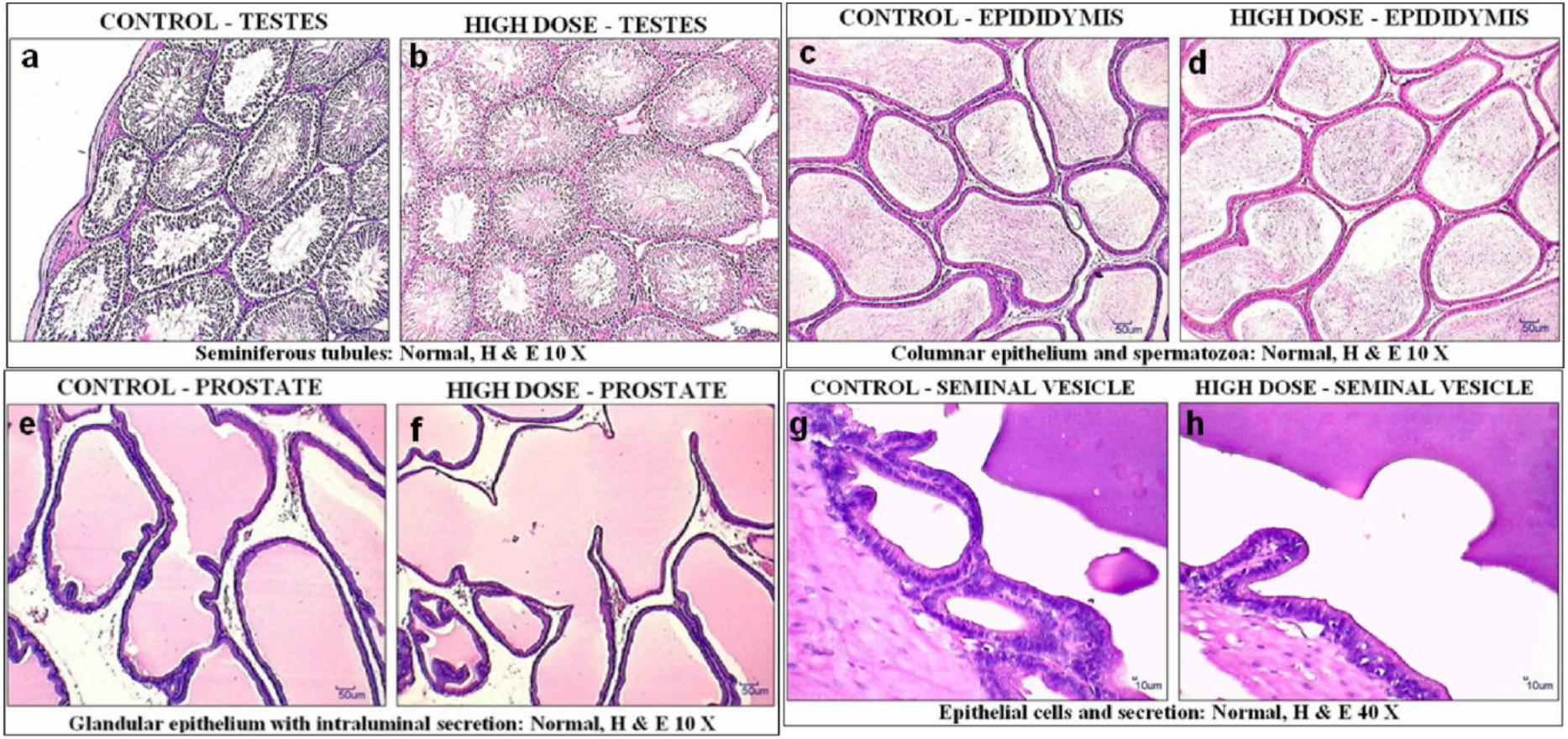

Fig-5: Color plate of testes (a, b), Epididymis (c, d), prostate (e, f) and seminal vesicle (g, h) histopathology from representative animals of vehicle treated control group and high dose of DIA-2 (250 mg/kg/day) group (hematoxylin–eosin stain).

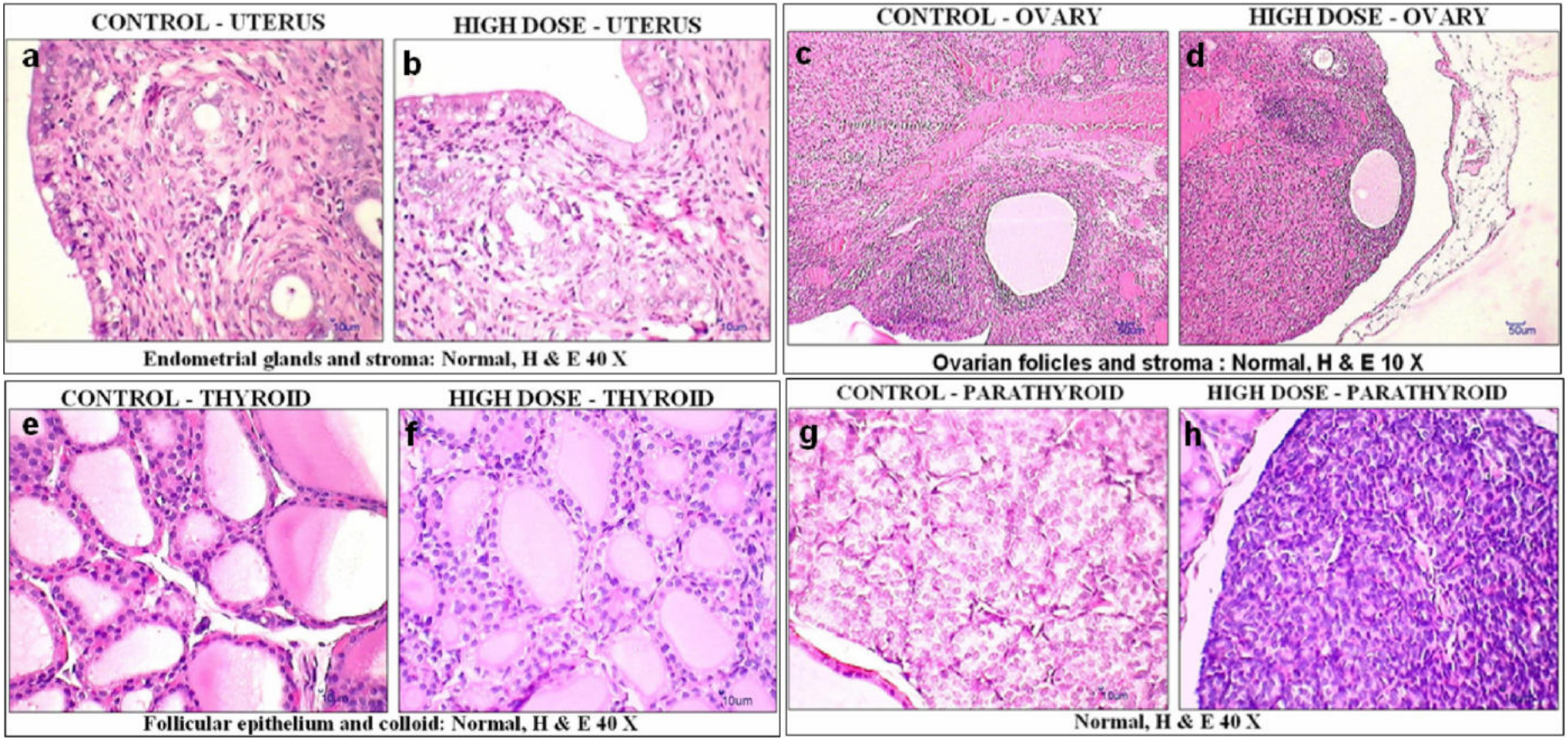

Fig-6: Color plate of uterus (a, d), ovary (c, d), thyroid (e,f) and parathyroid (g,h) histopathology from representative animals of vehicle treated control group and high dose of DIA-2 (250 mg/kg/day) group (hematoxylin–eosin stain).

References

[1]. Zimmet P, Boyko EJ, Collier GR, de Courten M. Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. *Ann N Y Acad Sci*. 1999; 892:25-44.

[2]. Amin KA, Nagy MA. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. *Diabetol Metab Syndr*. 2009; 1:17.

[3]. Kelly GS. Insulin resistance: lifestyle and nutritional interventions. *Altern Med Rev*. 2000; 5:109-132.

[4]. Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma [PPARgamma] and PPARgammacoactivator-1 [PGC1]. *Public Health Nutr*. 2007;10:1132-1137.

[5]. Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Antidiabetes and Anti-obesity Activity of *Lagerstroemia speciosa*. *Evid Based Complement Alternat Med*. 2007; 4:401-407.

[6]. Das SK, Chakrabarti R. Non-insulin dependent diabetes mellitus: present therapies and new drug targets. *Mini Rev Med Chem*. 2005; 5:1019-1034.

[7]. Gupta R, Bajpai KG, Johri S, Saxena AM. An overview of Indian novel traditional medicinal plants with anti-diabetic potentials. *Afr J Tradit Complement Altern Med*. 2007; 5:1-17.

[8]. Liu, C.T., Sheen, L.Y., Lii, C.K., 2007. Does garlic have a role as an antidiabetic agent? *Mol Nutr Food Res*. 2007; 51: 1353-1364.

[9]. Ambati S, Yang JY, Rayalam S, Park HJ, Della-Fera MA, Baile CA. Ajoene exerts potent effects in 3T3-L1 adipocytes by inhibiting adipogenesis and inducing apoptosis. *Phytother Res*. 2009; 23:513-518.

[10]. Bai, N., He, K., Roller, M., Zheng, B., Chen, X., Shao, Z., Peng, T., Zheng, Q. Active compounds from *Lagerstroemia speciosa*, insulin-like glucose uptake-stimulatory/inhibitory and adipocyte differentiation-inhibitory activities in 3T3-L1 cells. *J Agric Food Chem*. 2008; 56: 11668-11674.

[11]. Tiwari AK and Rao JM: Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Sci*, 2002; 83:30-37.

[12]. Alnaqeeb MA, Thomson M, Bordia T, Ali M. Histopathological effects of garlic on liver and lung of rats. *Toxicol Lett*. 1996; 85:157-164.

[13]. Morcos NC, Camilo K. Acute and chronic toxicity study of fish oil and garlic combination. *Int J Vitam Nutr Res*. 2001; 71:306-312.

[14]. Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK, Maulik SK. Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. *Food Chem Toxicol*. 2001; 39:793-797.

[15]. Mikail HG. Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. bulbs in experimental rabbits. *Journal of Medicinal Plants Research*. 2010; 4: 322-326.

[16]. Stohs SJ, Miller H, Kaats GR. A Review of the Efficacy and Safety of Banaba [Lagerstroemia speciosa L.] and Corosolic Acid. Phytother Res. 2012;26:317-324.

[17]. Kesavanarayanan KS, Sathiya S, Kalaivani P, Ranju V, Sunil AG, Saravana Babu C, Kavimani S, Prathiba D. DIA -2, a polyherbal formulation ameliorates hyperglycemia and protein-oxidation without increasing the body weight in Type II diabetic rats. *Eur Rev Med Pharmacol Sci*. 2012 [Article in press].

[18]. Perera PK, Li Y. Functional herbal food ingredients used in type 2 diabetes mellitus. Pharmacogn Rev. 2012;6:37-45.

[19]. Madkor HR, Mansour SW, Ramadan G. Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycaemia, dyslipidaemia and oxidative stress in streptozotocin-nicotinamide diabetic rats. Br J Nutr. 2011;105:1210-1217.

[20]. Rana SV, Pal R, Vaiphei K, Singh K. Garlic hepatotoxicity: safe dose of garlic. Trop Gastroenterol. 2006; 27:26-30.

[21]. Nakagawa S, Masamoto K, Sumiyoshi H, Harada H. Acute toxicity test of garlic extract. J Toxicol Sci. 1984; 9:57-60.

[22]. Sumiyoshi H, Kanezawa A, Masamoto K, Harada H, Nakagami S, Yokota A, Nishikawa M, Nakagawa S. Chronic toxicity test of garlic extract in rats. J Toxicol Sci. 1984; 9:61-75.

[23]. Akihisa T, Kamo S, Uchiyama T, Akazawa H, Banno N, Taguchi Y and Yasukawa K. Cytotoxic activity of Perilla frutescens var. japonica leaf extract is due to high concentractions of oleanolic and ursolic acids. J Nat Med. 2006; 60:331-333.

[24]. Borrelli F, Capasso R, Izzo AA. Garlic [Allium sativum L.]: adverse effects and drug interactions in humans. Mol Nutr Food Res. 2007; 51:1386-1397.

[25]. Scharbert G, Kalb ML, Duris M, Marschalek C, Kozek-Langenecker SA. Garlic at dietary doses does not impair platelet function. Anesth Analg. 2007; 105:1214-1218.

[26]. Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, Perry R, Schafer K. Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology

studies. Toxicol Pathol. 2007; 35:751-755.

[27]. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K, Pitsch S. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicol Pathol. 2007; 35:742-750.

[28]. Bailey SA, Zidell RH, Perry RW. Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicol Pathol. 2004; 32:448-466.

[29]. Aida Y, Kamata E, Nakadate M. A study of the relationships between exposure periods and no-effect doses in repeated dose toxicity tests. Eisei Shikenjo Hokoku. 1992;110:48-53.