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QUALITY STANDARDIZATION AND TOXICITY STUDY OF GARLICON A CARDIOVASCULAR FORMULATION

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Abstract: Herbal medicines have long therapeutic history and still serving the needy. However the quality standardization and toxicity study of herbal medicines still remains a challenge because of complex nature of phyto-constituents and therefore it is difficult to establish standard and quality control profile for raw material as well as finished product. Most of the medicines are effective but one major drawback is lack of standardization. So, there is a need to develop a standardization technique and toxicity study according to guidelines to come together this system of medicine in the main stream of health science. The main objective of this study was to perform standardization and toxicity study of a polyherbal formulation i.e. "Garlicon tablet" which contains Garlic (*Alium Sativum*), Arjuna Ghan (*Terminalia Arjuna*), Nagarmotha powder (*Cyperus Rotundus*), Shuddha guggul (*Commiphora Mukul*), Awala Ghan (*Emblica Officinalis*), Dalchini powder (*Cinnamomum Zeylanicum*) The formulation used to treat cardiovascular diseases. It was standardized on the basis of organoleptic characters, phytochemical screening, and physico-chemical, microscopical, analytical parameters. The set parameters were found to be sufficient for the quality control/quality assurance purposes. The Garlicon formulation was also subjected for acute and sub-acute study. Toxicity study for Garlicon indicated that it was a safe polyherbal formulation and free from any toxic effect. Thus it can be concluded that the Garlicon was found to be well-tolerated by rats. Acute and sub-acute oral toxicity studies in rats using the highest attainable dose of 2000 mg/kg did not cause any lethal effect.

Key Words: Herbal medicines, Garlicon, Standardization and Toxicity study

INTRODUCTION

In the last few decades, there has been an exponential growth in the field of ayurvedic medicine. There are great need of quality standardization and toxicity study of ayurvedic formulations. Standardization is a system to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect. WHO has also issued Guidelines for Quality Standardization methods for medicinal plant material in 1992 with a clear objective to provide general test methods for correct botanical evaluation and identification of medicinal plants widely used in traditional and home remedies. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs. Two kinds of side effects have been reported for herbal medicines. The first, considered to be intrinsic to herbal drugs themselves, is mainly related to predictable toxicity due to toxic constituents of the herbal ingredients and over dosage, and the second is allergy. Garlicon is an Ayurvedic polyherbal formulation present in tablet form and it contains six different ingredients of different category namely Garlic (*Alium Sativum*), Arjuna Ghan (*Terminalia Arjuna*), Nagarmotha powder (*Cyperus Rotundus*), Shuddha guggul (*Commiphora Mukul*), Awala Ghan (*Emblica Officinalis*), Dalchini powder (*Cinnamomum Zeylanicum*). All other ingredients have different therapeutic uses which support, is used to treat cardiovascular diseases.

However, one of the impediments in the acceptance of the Ayurvedic formulation is the lack of standard quality control profile. The quality of herbal medicine i.e. the profile of the constituents in the final product has implication in efficacy and safety. Due to complex nature and inherent variability of the constituents of plant based drugs, it is difficult to establish quality control parameter and modern analytical technique are expected to help in circumventing this problem. The safety problems emerging with herbal medicinal products are due to a largely unregulated growing market where there is a lack of effective quality control. Lack of strict guidelines on the assessment of safety and efficacy, quality control, safety monitoring and knowledge on traditional medicine/complementary and alternative medicine are the main aspects which are found in different regulatory systems. So, to perform quality standardization according to guidelines given by WHO and toxicity study according to OECD guidelines for this Garlicon tablet and for its raw materials are necessary. To determine identity, quality, quantity and safety assessment of herbal drug based on standardization and toxicity study this study is essential and so one can determine the quality, safety and efficacy of drugs.[1,2,3,4]

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MATERIALS AND METHODS

The Garlicon Tablet is marketed formulation and it contains six different ingredients with different activity. It is used to treat cardiovascular diseases.

Organoleptic characters (For Garlicon tablet and its Raw materials): Garlicon tablet and its raw materials were evaluated for its colour, odour, taste and texture, appearance.[5, 6] Result shown in Table 2.

Foreign Matters: The raw materials were analyzed for the presence of foreign materials[5,6]. Results obtained given in Table 4.

Table 1: Contents of Garlicon Tablet

Name of Ingredients	Quantity	Category
Garlic (*Alium Sativum*)	250mg	Anti-hyperlipedemic
Arjun Ghan (*Terminalia Arjuna*)	100mg	Anti-hyperlipidemic & Anti Atherosclerotic Activity
Nagarmotha powder (*Cyperus Rotundus*)	75mg	Cardio protective property
Amala Ghan (*Embillica officinalis*)	50mg	Cardio protective property
Shuddha Guggul (*Commiphora Mukul*)	50mg	Antihyperlipidemic
Dalchini Powder (*Cinnamomum Zylanicum*)	25mg	Vasodialator Properties

Table 2: Organoleptic Properties for Garlicon Tablet and Its Raw Materials

Name of Drugs	Organoleptic properties				
	Colour	Odour	Taste	Texture	Appearance
Garlic	Pale Yellow	Characteristic	Pungent	Smooth	Powder
Arjun Ghan	Pale Brown	Characteristic	Astringent	Smooth	Powder
Nagarmotha powder	Brownish Black	Fragrant	Starchy	Smooth	Powder
Amala Ghan	Light Grey	Characteristic	Astringent	Smooth	Powder
Shuddha Guggul	Light Yellow	Characteristic	Characteristic	Smooth	Powder
Dalchini Powder	Pale Brown	Characteristic, Sweet, Fragrant	Aromatic, Pungent, And Sweetish	Smooth	Powder
Garlicon Tablet	Dark Pink	Characteristic	Characteristic Bitter	Smooth	Tablet

Physical Parameters

Determination of Ash Value Physical constants such as the ash values and extractive values for the Garlicon Tablet raw materials were determined and the results obtained were recorded below.

Total ash: Total ash content of Garlicon Tablet and raw materials was determined, taking samples from the suppliers[7,8]. The values obtained and their acceptable limits defined were given in Table 4.

Acid Insoluble: Ash From the Total ash, the Acid insoluble ash content of the individual raw materials and Garlicon Tablet were determined[9,10]. Results were given in Table 4.

Table 4: Presence of Foreign Matter for Raw Materials of Garlicon Tablet

Raw materials of Garlicon tablet	Foreign Matter
Garlic	Absent
Arjun Ghan	Absent
Nagarmotha powder	Absent
Amala Ghan	Absent
Shuddha Guggul	Absent
Dalchini Powder	Absent

Determination of Extractive Value: Water soluble Extractive and Alcohol soluble Extractive values for the raw materials and Garlicon Tablet were determined in water and alcohol[11,12]. Results were given in Table 5.

Table 4: Total Ash Value and Acid Insoluble Ash Values for Raw Materials of Garlicon Tablet

S.No.	Raw materials of Garlicon tablet	Total Ash Values (%w/w)	Limits (%w/w)	Acid Insoluble Ash Values (%w/w)	Limits (%w/w)
1.	Garlic	0.8	NMT 1	0.17	NMT 1
2.	Arjun Ghan	19.9	NMT 25	0.26	NMT 1
3.	Nagarmotha powder	12.87	NMT 20	2.56	NMT 5
4.	Amala Ghan	6.3	NMT 7	0.41	NMT 2
5.	Shuddha Guggul	2.21	NMT 3	0.72	NMT 1
6.	Dalchini Powder	1.8	NMT 5	1.1	NMT 2

Table 5: Water Soluble Extractive Values for Raw Materials of Garlicon Tablet

Sr. No.	Raw materials of Garlicon tablet	Water soluble Extractive values (%w/w)	Limits (% w/w)	Alcohol soluble Extractive values (%w/w)	Limits (% w/w)
1.	Garlic	2.91	NLT 2.5	1.59	NLT 0.1
2.	Arjun Ghan	28.9	NLT 20	28	NLT 20
3.	Nagarmotha powder	21.27	NLT 15	15.15	NLT 10
4.	Amala Ghan	68.8	NLT 50	54.8	NLT 40
5.	Shuddha Guggul	64.4	NLT 53	5.2	NLT 2
6.	Dalchini Powder	2.8	NLT 1	7.7	NLT 2

Loss on Drying: Loss on drying for the raw materials and Garlicon Tablet were done with samples taken from the suppliers[12,13]. The results obtained and tabulated in Table 6.

Table 6: Loss on Drying For Raw Materials of Garlicon Tablet

Name of test	Garlic	Arjun Ghan	Nagarmotha powder	Amala Ghan	Shuddha Guggul	Dalchini Powder
Result (%)	4.8	2.7	2.1	4.5	4.5	5.1
Limits (%)	NMT 8	NA	NA	NMT 8	NA	NA

Flow Properties for raw materials: Flow properties for all raw materials were determined[12,13,14]. Results shown in Table 7.

pH of suspension of the drugs: pH of freshly prepared 1% w/v suspension of tablet (in powder form) and raw materials and 10% w/v suspension in distilled water was determined using simple glass electrode pH meter[15,16]. Result shown in Table 8.

Melting point: The melting point for tablet (in powder form) and raw materials were observed by capillary method[15,16]. Result shown in Table 8.

Table 7: Flow Properties of Raw Materials of Garlicon Tablet

Name of tests	Raw Materials of Garlicon Tablet					
	Garlic	Arjun Ghan	Nagarmotha powder	Amala Ghan	Shuddha Guggul	Dalchini Powder
Bulk Density (gm/ml)	0.45	0.62	0.45	0.45	0.45	0.68
Tap Density (gm/ml)	0.55	0.71	0.56	0.55	0.49	0.55
Carr's index (%)	18.18	11.97	19.64	15.94	8.16	19.11
Hausner's Ratio	1.2	1.1	1.03	1.2	1.08	1.2
Angle of repose (θ)	37°2'	35°4'	32°6'	36°8'	34°6'	32°4'

Table 8: pH and Melting Points of Raw Materials of Garlicon Tablet

Chemical parameters	Raw Materials of Garlicon tablet					
	Garlic	Arjun Ghan	Nagarmotha powder	Amala Ghan	Shuddha Guggul	Dalchini Powder
pH of suspension of the drugs	5.9-6	5.6-5.6	5-6.5	2.5 - 3.8	4.5-5	2-5.5
Melting point (°C)	113°C	163°C	161°C	36°C	171°C	223°C

Determination of Heavy Metal Contents for Garlicon tablet only: Heavy Metal Contents for Garlicon tablet were determined by Atomic Absorption Spectroscopy[17]. Result shown in Table 9.

Table 9: Heavy Metal Content in Garlicon Tablet

Name of metals	Result (ppm)	Limits (ppm)
Arsenic (Ar)	0.004	3
Mercury (Hg)	0.0088	1
Cadmium (Cd)	0.003	0.3
Lead (Pb)	0.0039	10

Total Phyto-constituents: All the raw materials were tested for Total Phyto-constituents[14,16,18]. Result shown in Table 10 and 11.

Table 10: Phytochemical Screening of Raw Materials of Garlicon Tablet in Aqueous Extract

Phytochemical parameters	Raw materials of Garlicon tablet Water extract					
	Garlic	Arjun Ghan	Nagarmotha powder	Amala Ghan	Shuddha Guggul	Dalchini Powder
Alkaloids	-	+	+	-	+	+
Tannins	-	+	+	+	+	-
Saponins	+	+	+	-	-	+
Flavonides	-	+	-	+	+	-
Glycosides	+	+	+	+	-	-
Steroids	-	-	-	-	-	+
Terpenoids	+	-	-	-	+	-
Phenols	-	-	+	+	-	-
Carbohydrates	-	+	-	+	-	+

Table 11: Phytochemical Screening of Raw Materials of Garlicon Tablet in Methanol Extract

Phytochemical parameters	Raw materials of Garlicon tablet Methanol extract					
	Garlic	Arjun Ghan	Nagarmotha powder	Amala Ghan	Shuddha Guggul	Dalchini Powder
Alkaloids	+	+	+	+	+	+
Tannins	-	+	+	+	-	+
Saponins	+	+	+	+	-	+
Flavonides	-	+	+	+	-	-
Glycosides	+	+	+	+	-	+
Steroids	-	-	-	+	-	-
Terpenoids	+	-	-	-	-	+
Phenols	-	-	+	+	+	-
Carbohydrates	-	+	-	+	+	+

(+) indicated present / (-) indicated absent

Microscopic Parameters: Microscopic characteristics of raw materials[14,16,18] (Fig. 1, 2, 3,4,5,6)

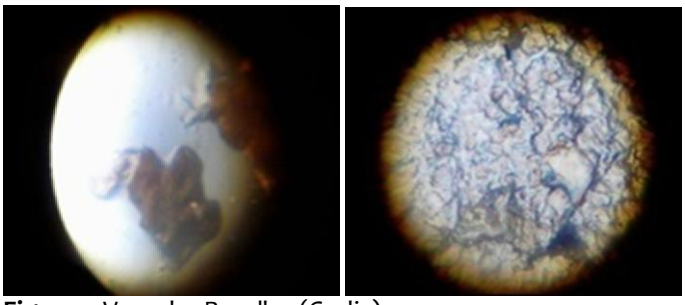

Figure 1: Vascular Bundles (Garlic)
Figure 2: Fragments of Cortex Cells (Dalchini)

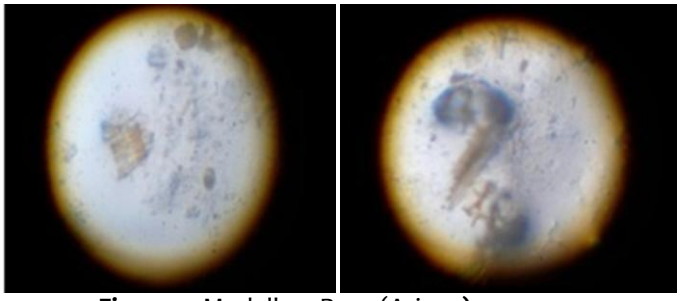

Figure 3: Medullary Rays (Arjuna)
Figure 4: Trichomes (Amala)

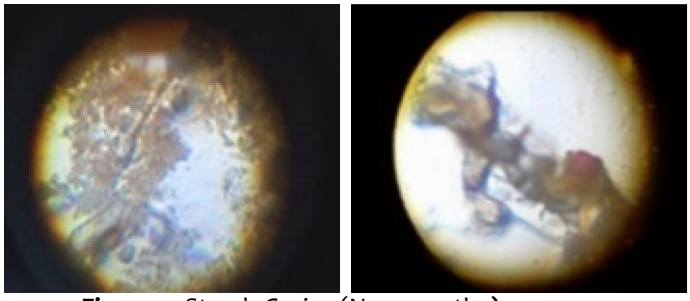

Figure 5: Starch Grains (Nagarmotha)
Figure 6: Xylem Vessels (Shuddha Guggul)

Chromatographic Parameters (For Garlicon Tablet)

TLC and HPLC: TLC and HPLC analysis were done for Garlicon tablet TLC reading shown in Table 12 and graph for Garlicon Tablet shown below[18, 19].

Table 12: TLC for Garlicon tablet

Name Of Mobile Phase	Rf value
n-Butanol: Water: Acetic acid: Formic acid	0.78

Evaluation Parameters (For Garlicon Tablet)

Garlicon Tablet were evaluated for following parameters[20,21] Weight variation test, Hardness, Disintegration, Friability, LOD, pH and Flow properties (Table 13 and Fig. 7)

Table 13: Evaluation Parameters (For Garlicon tablet)

Evaluation Parameters for Garlicon Tablet	Results
Shape	Round
Thickness (mm)	3
Weight variation (%)	729±5%
Moisture content (% w/w)	3.6
Solubility	In water and methanol
pH	6.9
Disintegration time(min)	27 (In water)
Hardness (kg/cm^2)	2.3
Friability (%)	0.6
Bulk density(gm/ml)	0.49
Tap density(gm/ml)	0.60
Carr's index (%)	18.33
Hausner's ratio	1.2

*Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API); NA -Not Available; NMT – Not More Than

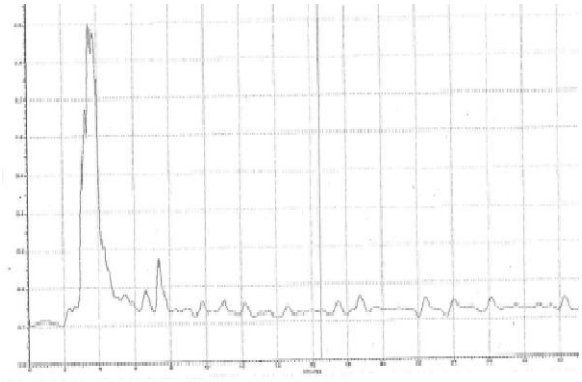

Figure 7: HPLC Chromatograph for Garlicon Tablet

Toxicity Study:

Acute and Sub-Acute study for Garlicon tablet were performed[1,2,3].

Acute toxicity study (OECD guideline, 423): Dose was selected by using acute toxicity study (OECD guideline, 423). The acute toxicity study for Garlicon tablet was performed using rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. To find out whether the drug is safe or not six groups of rats, containing six in each group, were given PM in the doses of 500, 1000 and 2000mg/kg orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the DRUG (Garlicon tablet) and therefore was found safe up to dose of 2000 mg/kg.

Dose selection: Dose was selected based on acute oral toxicity study done on Garlicon tablet was found to be safe up to the dose level 2000mg/kg. There was no behavioral abnormality and zero mortality was recorded till 48 h post treatment with no signs of acute toxicity. Therefore 1/10th of the dose 2000mg/kg of DRUG (Garlicon tablet) was selected i.e., 500mg/kg of DRUG as middle dose in rats. The following regime was followed: For rats- 500 mg/kg.

Volume of the injected drug solution: The volume of drug solution was calculated based upon the body weight of the animal.

Sub-acute Oral Toxicity Test (OECD Guideline 407): Repeated dose oral toxicity study was carried out according to OECD Guideline 407. The animals were divided into 4 groups of 2 animals each (8 females). Group 1 received 10 ml/kg body weight of normal saline and served as control. Groups 2, 3 and 4 received DRUG doses of 100, 500 and 2000 mg/kg body weight, respectively. Mortality, body weights, food and water

consumption as well as observation for general toxicity signs of the animals were evaluated daily for 28 days. Results shown in Table 14, 15, 16, 17 and 18. Animals used in the study

Species	Albino rats (Wistar strain)
Age	6-8 weeks
Weight	150-200 gm
Gender	Female

Table 14: Observations for Acute Oral Toxicity Study as Follows

Extract use	No. of animals	Limit of Extract	Duration of period	Sing of toxicity	Mortality
Aqueous extract (Garlicon tablet)	6 Rats (Females)	2000mg/kg	24 hour	No	No

Observation for Effect of Aqueous Extract of Garlicon Tablet on the Body Weight of Rats for

Acute Oral Toxicity Study:

Table 15: Effect of Aqueous Extract of Garlicon Tablet on the Body Weight of Rats

Groups	Treatment	Body Weights Of Rats		Results
		Before Treatment (gm)	After Treatment (gm)	
Group 1	500 mg/kg	200	194	No mortality
Group 2	1000 mg/kg	175	170	No mortality
Group 3	2000mg/kg	175	170	No mortality

No any significant change was observed in the body weights of rats.

Table 16: Observations for Sub-Acute Oral Toxicity Study

Extract use	No. of animals	Limit of Extract	Duration of period	Sign of toxicity	Mortality
Aqueous extract (Garlicon tablet)	8 rats (females)	2000mg/kg	28 days	No	No

Effect of Aqueous Extract of Garlicon Tablet on the Body Weight of Rats for Sub-Acute Oral Toxicity Study

Table 17: Effect of Aqueous Extract of Garlicon Tablet on the Body Weight of Rats

Groups	Treatment	Body Weights Of Rats		Results
		Before Treatment (gm)	After Treatment (gm)	
Control	Water for injection	200	190	No mortality
Group 1	100 mg/kg	175	170	No mortality
Group 2	500 mg/kg	175	172	No mortality
Group 3	2000mg/kg	180	175	No mortality

No any significant change was observed in the body weights of rats.

Observations for behavioural changes in rats for both Acute and Sub-Acute Toxicity Study

Table 18: Behavioural Changes in Rats

Observation	Control	Test
Skin and fur	Normal	Normal
Food	Normal	Normal
Eyes	Normal	Normal
Sleep	Normal	Normal
Mortality	Normal	Normal
Morbidity	Normal	Normal

DISCUSSION

All the raw materials were tested for total ash and acid insoluble ash values which were found to be within the limits which was indicated that there was absence of silica, especially sand and siliceous earth. Water and Alcohol soluble extractive values for Raw materials of Garlicon tablet was found to be within the limits which indicated that approximate measure of polar constituents of crude drug. Deterioration time depends upon the amount of water present in raw material of Garlicon tablet. If the water content is high, drug can be easily deteriorated due to fungus. It was found that the loss on drying at 105°C in raw material of Garlicon tablet within the limits. The flow ability of Raw materials of Garlicon Tablet was found to be good and angle of repose was also showed good flow ability, which was further confirmed by Hausner's ratio, and Carr's index whose values were also indicated good flow ability of Raw materials of Garlicon Tablet. The Heavy Metal contents in Garlicon Tablet were found to be within the limits.

The results obtained from phytochemical screening reveals that phyto-constituents like alkaloids which indicated there was presence of Nitrogenous compounds in drugs which had high pharmacological action, presence of tannins showed that there was presence of Non- of Nitrogenous compounds, there was presence of cardiac glycosides, presence of steroids indicated that it contained cyclopentano-perhydro-phenanthrene in their nucleus, also presence of terpenoids and phenols indicated that there was presence of hydrocarbon chain in the structure of compounds, flavonides indicated presence of phenolic compounds with aromatic ring and lastly presence of carbohydrates indicated that there was presence of optical active polyhydroxy aldehyde or polyhydroxy ketones.

By TLC of Garlicon Tablet R_f was found to be 0.78 which indicated that the formulation was in pure form. Garlicon extract was analysed using RP-HPLC at wavelength (λ_{max}) 220nm. HPLC chromatograph of Garlicon extract showed 11 intense peaks corresponding to different constituents of Garlicon extract. The chromatograph of 220nm showed more

intense peak as complex to λ_{max}. From HPLC analysis predicted that presence of 11 major constituents in the Garlicon extract. The showed 11 different probable constituents present in Garlicon extract and showed that there was presence of acidic compound mostly.

For toxicity study of Garlicon tablet it was observed that no significant changes were observed in body weight and behavioral pattern as well as the control animals was found to be normal.Safe dose for acute and sub- acute oral toxicity was found to be 2000mg/kg.

CONCLUSION

The main objective of the studies was to establish Quality standardization profile and to perform Toxicity study for Garlicon tablet. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles, physical and chemical standards. This study focused on standardization of a Polyherbal Ayurvedic formulation i.e. Garlicon tablet used to treat cardiovascular disease. As Garlicon was a polyherbal formulation which contained six different ingredients having different activity. Garlicon and its six different ingredients were standardized by checking their physical, chemical, microscopical parameters. This study confirmed identity and purity of all ingredients as well as Garlicon tablet also different analytical studies such as TLC, HPLC had performed for Garlicon tablet and this study was indicated that there was presence of different components in Garlicon tablet. So objective of study was focused on establishment of Quality Standardization profile. For most Herbal drugs toxicity are studies not done according to OECD guidelines. So present study mainly focused on Toxicity studies for Garlicon tablet. The Acute and Sub-Acute Oral Toxicity studies for Garlicon were performed. In these studies, Acute Sub-Acute Oral Toxicity was determined as per OECD guidelines. It was also observed that there was no mortality in any of the dose up to 2000gm/kg body weight. The administration of this Garlicon tablet did not show any significant changes in the body weight, indicated that it did not have any adverse effects on body weight. All groups were almost continuously observed for mortality and behavioural changes. There was no abnormality observed in any of these groups.

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