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Effect of Acute and Chronic Treatment of Common Spices in Swiss Albino Mice: A Safety Assessment Study

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Abstract: Spices viz., *Allium cepa* (AC), *Allium sativum* (AS), *Anethum graveolens* (AG), *Capsicum annuum* (CA) and *Carum carvi* (CC) were assessed for their safety. The safety evaluation study included acute toxicity (24 h), chronic toxicity (90 days), LD_{50} and/or Maximum Tolerance Dose (MTD) and genotoxicity determination. During chronic treatment, the body and organ weights of the mice showed no significant difference between the control and the treated group. The treatment of AC, AS, AG, CA and CC dose not induce any significant change in the frequency of micronucleated-polychromatic erythrocytes (PCE) and the ratio of PCE and non-chromatic erythrocytes (NCE), indicating lack of clastogenic activity except AG and CA which shows a significant increase in the frequency of micronuclei in PCE as compared to the control group ($p<0.05$). These findings suggest that orally administrated aqueous suspension of above mentioned spices is relatively safe.

Key words: Spices, toxicity, genotoxicity, LD_{50}, clastogenic effect, micronuclei

INTRODUCTION

Parallel with the recent increasing interest in alternative/herbal medicine for the prevention and treatment of various illnesses, there is an increasing concern about the safety of medicinal plants (Cao *et al*., 2008; Twaij *et al*., 1983). There are general and herb-specific concerns regarding medicinal plants and their ability to produce toxicity and adverse effects. Accidental herbal toxicity occurs, not only as a result of a lack of pharmaceutical quality control in harvesting and preparation but also because herbal remedies are believed to be harmless (Asiri *et al*., 2008; Gurib-Fakim, 2006; Ranjbar *et al*., 2011). Asian countries are richly endowed with a wide range of medicinal plants of which, some are consumed as condiments and spices. Reports suggested that spices and their chemical constituents may have diverse pharmacological activities and toxicity (Gurib-Fakim, 2006; Khan *et al*., 2006; Khan and Sultana, 2009; Saad *et al*., 2005). Spices may be in the form of dried seeds, fruits, roots, barks, leafs or vegetative used nutritionally in significant quantities as a food additive for the purpose of flavor, color or as a preservative that kills harmful bacteria or prevents their growth (Tsai *et al*., 2007; Gurib-Fakim, 2006; Krishna *et al*., 2009). Spices are aromatic and pungent herbal substances used as condiments. These substances generally give a characteristic smack, flavor, color and aroma (Gurib-Fakim, 2006; Khan and Balick, 2001).

For these qualities, spices are widely used in oriental cooking in many food items, in addition to their use in the ancient systems of medicine in the treatment of gastrointestinal disorders (Chung, 2006; Saad *et al*., 2005) many of these substances are also used as cosmetics, in perfumers or as vegetables. Spices are distinguished from herbs which are leafy, green plant parts used for flavoring purposes. Spices, however, are dried and often ground or grated into a powder. Extracts of herbs and spices are increasingly of interest in the food industry because they are known to retard the oxidative degradation of lipids and enhance the flavour. For ages, spices have been used by professionals in traditional medicine (Saad *et al*., 2005;

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Khan and Balick, 2001; Krishna *et al*., 2009; Ranjbar *et al*., 2011).

Many reports have suggested the folkloric uses of spices against gastrointestinal disturbances and revealed that some spices are non-detrimental to the gastric mucosa and have a beneficial adaptive cytoprotective response (Graham *et al*., 1988; Marotta and Floch, 1991). Several spices including ginger, cinnamon, turmeric, black pepper, saffron, dill, cumin, coriander, cardamom, mint and garlic are found to be carminatives, stomachic, stimulant and antispasmodics (Kirtikar and Basu, 1984; Leung, 1980; Said *et al*., 1996; Chopra *et al*., 1956; Atrooz, 2009).

Allium cepa (onion), *Allium sativum* (garlic), *Anethum graveolens* (dill), *Capsicum annuum* (chili) and *Carum carvi* (caraway) which are common components of different spices, have a wide application in traditional systems of medicine in several countries (Kirtikar and Basu, 1984; Said *et al*., 1996; Ogunlade *et al*., 2012; Nithya and Ramachandramurty, 2007; Ranjbar *et al*., 2011).

Allium cepa (AC) commonly known as onion is a natural part of the daily diet for most of the world's population (Mogren *et al*., 2008; Sohail *et al*., 2011). AC phenol compounds, particularly flavonols, are known to be potent free radical scavengers and antioxidants; they are considered to be protective against cardiovascular diseases and to contribute in the prevention of colorectal cancers in humans (Benkeblia, 2004; Fernandes *et al*., 2007; Prakash *et al*., 2007a; Ogunlade *et al*., 2012; Sohail *et al*., 2011). At least 25 different flavonols have been characterised in AC, quercetin and quercetin derivatives being the most predominant pigments. Quercetin-40-glucoside and quercetin-3, 40-diglucoside are the main AC flavonols (Bonaccorsi *et al*., 2005; Fossen *et al*., 1997; Prakash *et al*., 2007b).

Allium sativum (AS) commonly known as garlic is of particular interest due to its prophylactic and therapeutic actions (Benkeblia, 2004; Benkeblia, 2005; Prakash *et al*., 2007b). Newall *et al*. (1996) reported the oil of AS to suppress the gastrointestinal movements in mice induced by charcoal meal and castor oil. The antibacterial, antifungal and antioxidant activities of AS is reported to be due to presence of Sulphur and polyphenols (Biljana *et al*., 2008; Chung, 2006; Gorinstein *et al*., 2006b; Gorinstein *et al*., 2006a; Jastrzebski *et al*., 2007).

Anethum graveolens (AG) commonly known as dill, is a sparse looking plant with feathery leaves and tiny yellow flowers. Some pharmacological effects including antimicrobial (Delaquis *et al*., 2002), antihyperlipidaemic and cholesterol lowering activities have been reported (Yazdanparast and Alavi, 2001). As a folk remedy, AG is considered for some gastrointestinal disorders such as flatulence, indigestion, stomachache and colic (Delaquis *et al*., 2002; Duke, 2001). AG fruit has an antispasmodic effect on the smooth muscles of the gastrointestinal tract (Fleming, 2000). The herb has been frequently mentioned in Ayurvedic and other Indian Systems of Medicine prescriptions against a wide variety of ailments. It has remained a scientific curiosity that a single plant can have biological activities for such a large variety of ailments or diseases for which these prescriptions are employed (Delaquis *et al*., 2002; Duke, 2001; Fleming, 2000; Yazdanparast and Alavi, 2001; Malihezaman and Sara, 2007).

Capsicum annuum (CA) commonly known as red chili is used as a culinary coloring agent, flavoring vegetable or as a seasoning in cooking, in cheese, stuffed olives, processed meats (Duke, 2001). It is used against throat inflammations, antibacterial, stomach disorders, diarrhoea, amoebic infection, intestinal worms and as an antidote for poisons (Fleming, 2000; Heiser and Smith, 1953; Jansen, 1981). The principal constituents of chili are phenolic compounds, carotenoids, ascorbic acid, vitamin A, capsaicin, dihydrocapsaicin and nitrophenols (4-nitroguaiacol, 4,6-dinitroguaicol, nitrocapsaicin and nitrodihydro-capsaicin) (Duke, 2001; Fleming, 2000; Jansen, 1981). Chilies have been shown to reduce blood cholesterol level via conversion of cholesterol to bile acids and decreasing the tendency of the blood to clot (Heiser and Smith, 1953). Capsaicin was found to inhibit the lipid peroxidation-induced by ascorbic acid and ferrous sulphate (Burkill and Dalziel, 1985). Capsaicin and spices containing capsaicin have shown mutagenicity and carcinogenicity and as well as anti-mutagenicity and anti-carcinogenicity activities (Desai *et al*., 1973; Myers *et al*., 1987; Avci *et al*., 2005). CA-ingestion produces heartburn, nonburning chest discomfort, nausea, belching, distension and facial sweating in up to 42% of patients with presenting chronic upper abdominal pain (Desai *et al*., 1973). CA, when fed at 6% of the diet has been reported to produce liver tumors in rats (Myers *et al*., 1987).

Carum carvi (CC) seeds are known as cumin, seeds are widely used as a spice for culinary purposes and for flavouring bread biscuits, cakes, candies, cheese, curries, pickles, sausages, meat products, confectionery and liqueurs of kummel type. Constituents present in the oil are α-and β-pinene ρ-cymene, camphene, Δ3-carene, dihydrocarvone, β-fenchene, myrcene, α-and β-phellandrene, sabinene, α and γ-terpinene, α-thujene, terpinolene, tricyclene, d- and l-dihydropinol, l-neodihydrocarveol, l-isodihydrocarveol, carveol, d-dihydrocarveol, acetaldehyde and methyl alcohol. Furfurals have also been isolated from European CC oil

(Kirtikar and Basu, 1984; Leung, 1980). CC is used as carminative, mild stomachic, aromatic and diuretic remedy. Both the seeds and the essential oils (CC oil) are prescribed in flatulence colic and stomach derangements (Ene *et al*., 2008).

Herbal medicines have been used widely to treat a wide range of medical conditions. Recent years have witnessed an increase in their use but questions remain concerned about their quality, safety and efficacy (QSE) (Aggarwal *et al*., 2009). Since time immemorial spices have been used as food additives because of their flavouring properties and preservative action against their anti-microbial activities. However, there are some chemical constituents of spices which are known to be toxic, oxidative, mutagenic, cytotoxic and carcinogenic. (Jansen, 1981; Tsai *et al*., 2007). From the point of human consumption of spices and the possible genetic effects, it would be of interest to know if whole spices, rather than the isolated chemical constituents are genotoxic, because this is the form in which spices are used for dietary, culinary and medicinal purposes (Tsai *et al*., 2007). Hence, the objective of our present study was to evaluate the possible clastogenicity of spices as a whole. The present study on the genotoxicity of spices involved the conduct of micronucleus test, an assay which is universally approved and recommended to detect the genotoxicity and carcinogenicity of probable carcinogens.

Most of the spices used for dietary, culinary and medicinal purposes are relatively safe, if the dose is taken within the limits of their required use in food. Nevertheless, when the dose is beyond the nutritional or therapeutic use, the safety of spices requires a thorough evaluation on their toxicity (Jansen, 1981; Tsai *et al*., 2007). The toxic effects of spices used in traditional medicine are, generally not mentioned in the ancient literature, deliberately or inadvertently. However, recent researches have exposed some of the toxic implications of the plant derived food and medicine. The present study on the acute and chronic toxicity of spices was undertaken in view of their medicinal and nutritional importance, as well as, the toxic nature of some of their phytoconstituents.

There is limited information on spices toxicity in the literature in view of reported deleterious effects of some phytoconstituents of spices widely used. The present study was designed to evaluate general toxicity and possible genotoxic effects in mice to assess their safety.

MATERIALS AND METHODS

Chemicals: EDTA, Tris, Bovine Serum Albumin (BSA) and Tween 20 were obtained from Sigma Chemicals Co (St Louis, MO). All other chemicals were of the highest purity and commercially available.

Plant material and preparation of aqueous suspension: Spices were purchased from local market of Riyadh and their identities were confirmed by the expert taxonomist of Department of Pharmacognosy, where voucher specimens of the spices has been kept in the Herbarium for future reference. Spices were separately ground to a very fine powder and used as an aqueous suspension for the treatment in the different experiments.

Animals: Swiss albino mice of either sex, approximately of the same age, weighing 20 to 25 g and fed on standard chow diet were used. The aqueous suspensions of spices were freshly prepared before its administration. The protocol of animal studies was approved by the Research and Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Dose selection and route of administration: The regulatory guidelines on selection of dose for long term treatment require minimal toxicity to allow meaningful evaluation of the data (Newall *et al*., 1996). The doses selected for each spice were in accordance with the Maximum Tolerable Dose (MTD) and the dose that kills 50% of animals (LD_{50}) doses observed in our study, previous studies and preliminary experiments conducted on the pharmacological activity of each spice as shown in Table 1 (Al-Bekairi *et al*., 1991). The route of administration of the aqueous suspension of each spice was oral (gastric intubation) in all the experiments.

Table 1: Treatment regimen of experimental groups and their doses

		Doses for acute toxicity		Doses for chronic toxicity	
Treatment	LD^*_{50}/MTD**	Low (mg kg^{-1})	High (mg kg^{-1})	Low (mg kg^{-1} day^{-1})	High (mg kg^{-1} day^{-1})
Control	Vehicle only	Saline	Saline	Vehicle only	Vehicle only
Allium cepa	Different doses up to 30 g or LD_{50}	250	500	75	150
Allium sativum	Different doses up to 30 g or LD_{50}	250	500	25	50
Anethum graveolens	Different doses up to 30 g or LD_{50}	250	500	50	100
Capsicum annuum	Different doses up to 30 g or LD_{50}	250	500	25	50
Carum carvi	Different doses up to 30 g or LD_{50}	250	500	12.5	25

*LD_{50}: Lethal dose which killed 50% of the animals as determined by Karber's method; **MTD: Maximum tolerable dose, n = 10 $group^{-1}$

Table 2: Effect of different spices on general symptoms of toxicity and mortality rate after acute treatment in Swiss albino mice

		Mortality			
Groups	Doses	D/T	Latency (min)	Maximum dose	Toxic symptoms
Control	Vehicle	0/10	–		None
Allium cepa	250 mg kg^{-1}	0/10	--		None
	500 mg kg^{-1}	0/10	--		None
	30 g kg^{-1}	0/10	30-90	MTD**	Hypothermia, respiration, heart rate, piloerection, micturition, defecation
Allium sativum	250 mg kg^{-1}	0/10	-		None
	500 mg kg^{-1}	0/10	--		None
	25 g kg^{-1}	6/10	30-90	LD_{50}*	Hyperthermia, respiration, heart rate, piloerection, staggering, reflex impairment, tremors, muscle tone, itching, excitation, twitches, tremors, sedations, calmness
Anethum graveolens	250	0/10			None
	500	0/10			None
	30 g kg^{-1}	1/10	30-90	MTD**	Heart rate, piloerection, salivation, tremors, convulsions, sedation calmness,
Capsicum annuum	250	0/10	--		None
	500	0/10	--		None
	24.3	6/10	30-90	LD_{50}*	Hyperthermia, respiration, heart rate, defecation, writhing, straub tail, tremors, convultions, sedation calmness
Carum carvi	250	0/10	--		None
	500	0/10	--		None
	8.7 g kg^{-1}	5/10	30-90	LD_{50}*	Respiration, heart rate, salivation, reflex impairment, staggering, convulsions, muscle tone, sedation calmness

D/T: Dead/treated mice; None: No toxic symptoms were seen during the observation period; latency: time to death (in days) after the dose. *LD_{50} = Lethal dose which killed 50% of the animals as determined by Karber's Method; **MTD = Maximum tolerable dose, n = 10 $group^{-1}$

Acute toxicity studies: For acute toxicity study, 110 male Swiss abino mice were divided into eleven groups having 10 mice in each. Group 1 served as saline treated control and group 2-11 treated 250 and 500 mg kg^{-1} doses of AC, 250 and 500 mg kg^{-1} doses of AS, 250 and 500 mg kg^{-1} doses of AG, 250 and 500 mg kg^{-1} doses of CA and 250 and 500 mg kg^{-1} doses of CC, respectively. The general behavior of mice and signs of toxicity were observed continuously for 30 min after the oral treatment and then intermittently for 90 min and there after over a period of 24 h (Twaij *et al*., 1983). The mice were further observed once a day up to 14 days for following treatment for their behavioral changes and signs of toxicity and/or death and the latency of death.

Determination of MTD and LD_{50}: Determination of MTD and LD_{50} required 3-5 experimental trials for each spice. Each experiment constituted 10 mice. The LD_{50} value was determined according to the method of Karber's (Yeoh *et al*., 1995). All animals were sacrificed 24 h after treatment.

Chronic toxicity studies: Studies on chronic toxicity were undertaken on both sexes of Swiss albino mice to observe sex distinction of toxicity. For chronic toxicity study, 110 male and 110 female Swiss abino mice were divided into eleven groups having 10 mice in each as shown in the Table 2 and 3. Group 1 served as saline treated control and group 2-11 treated different doses of AC, AS, AG, CA and CC, respectively for 90 days. The doses selected for each spice were in accordance with MTD and LD_{50} doses of the different spices, previous studies (Gorinstein *et al*., 2006b; Gorinstein *et al*., 2006a; Jastrzebski *et al*., 2007). Animals were administered daily from 08:00 to 09:30 h and the observations to detect toxic symptoms started approximately 1 h later. Appearance and overt behaviour were recorded daily, so that any changes in the skin, eyes and mucous membranes are observed. Any disturbances in the respiration, circulation, autonomic or central nervous system and behaviour pattern were also observed. Surviving animals were sacrificed after 90 days of treatment. Prior to the sacrifice, animals were isolated in individual cages and fasted for 12 h, with water provided *ad libitum*. Then, they were terminally anaesthetized with diethyl ether. During the autopsy, the abdominal, thoracic and cranial cavities of all animals were examined. At necropsy, organ weights of the liver, heart, kidneys, spleen and testes/uterus of all animals were recorded. Organs were weighed using a Sartorius scale (Sartorius Universal, Goettingen, Germany). The percent ratios of the organ to body weight were determined (Newall *et al*., 1996). Samples of all the above-mentioned organs were preserved in 10% buffered formaldehyde for histopathological observations.

Genotoxicity: Experiments on genotoxicity were conducted to determine the clastogenic and mitodepressive potentials of the different spices. The genotoxicity studies involved indices on the frequency of micronucleated polychromatic erythrocytes, a measure for the assessment of clastogenicity and the ratio of polychromatic to normochromatic erythrocytes, a measure

Table 3: Effect of different spices on general symptoms of toxicity and mortality rate after chronic treatment in Swiss albino male and female mice

			Mortality		
Groups	Sex	Doses	D/T	Death time	Toxic symptoms
Control (Vehicle)	Male	0.3 mL	1/10	12th week	None
	Female	0.3 mL	0/10	--	None
Allium cepa	Male	75 mg kg^{-1}	1/10	10	None
		150 mg kg^{-1}	1/10	8	Mild hypothermia, Aggression, itching, alopecia
	Female	75 mg kg^{-1}	0/10	--	None
		150 mg kg^{-1}	1/10	12th week	Mild hypothermia, itching,
Allium sativum	Male	37.5 mg kg^{-1}	1/10	8th week	Aggression
		75 mg kg^{-1}	2/10	6, 10th week	Moderate hypothermia, respiration, heart rate, Aggression, excitation, itching, alopecia
	Female	37.5 mg kg^{-1}	0/10	--	None
		75 mg kg^{-1}	0/10	--	Mild respiration, heart rate, excitation, itching, alopecia
Anethum graveolens	Male	50 mg kg^{-1}	2/10	10, 12th week	None
		100 mg kg^{-1}	3/10	6,10,12th week	Moderate hyperthermia, respiration, heart rate, piloerection
	Female	50 mg kg^{-1}	1/10	10th week	None
		100 mg kg^{-1}	2/10	6, 10th week	Mild hyperthermia, respiration, heart rate, piloerection
Capsicum annuum	Male	25 mg kg^{-1}	2/10	8, 10	None
		50 mg kg^{-1}	2/10	6,10	Moderate hyperthermia, respiration, heart rate
	Female	25 mg kg^{-1}	1/10	8th week	None
		50 mg kg^{-1}	2/10	6, 10th week	Mild hyperthermia, respiration, heart rate
Carum carvi	Male	12.5 mg kg^{-1}	2/10	6,10th week	None
		25 mg kg^{-1}	3/10	6,10,12th week	Moderate hyperthermia, respiration, heart rate, aggression mild itching
	Female	12.5 mg kg^{-1}	1/10	8 week	None
		25 mg kg^{-1}	2/10	6, 10th week	Mild hyperthermia, respiration, heart rate

D/T: Dead/treated mice; none: No toxic symptoms were seen during the observation period; latency: Time to death (in days) after the dose 10 $group^{-1}$

to assess the effect on mitodepression and/or cytotoxicity (Moseberg and Hayes, 1989).

Statistical analysis: Values in tables are given as arithmetic means " standard error of the mean (SEM). Data were analyzed by using one-way analysis of variance (ANOVA) followed by Student's t-test.

RESULTS

Acute toxicity studies of spices: The acute treatment of AC, AS, AG, CA and CC at lower doses (250 and 500 mg kg^{-1}, shown in Table 1) showed no significant changes in the autonomic responses as shown in the Table 2. Symptoms observed at the higher dose (LD_{50}-MTD) includes changes in respiration, heart rate, hypothermia, piloerection and itching. The other symptoms observed at this dose included changes in motor activity and CNS excitation. LD_{50} of AS, CA, CC was found to be 25, 24.3 and 8.7 g kg^{-1} body weight, respectively and mortality observed at LD_{50} was found to be 6/10, 6/10 and 5/10, respectively.

Chronic toxicity studies of spices: Chronic treatment of spices revealed mild to moderate autonomic symptoms including the changes the rate of respiration, heart rate, hypothermia, piloerection, itching and alopecia. The other symptoms observed were injuries, inflammations on snout, eyes and aggressive behavior except with AG treatment. AG treated animals were observed to be calm and drowsy (Table 3).

The observations on body weight and rate of mortality after chronic treatment with spices revealed no significant difference as compared with control in both the sexes (Table 4 and 5). Chronic treatment of AC showed significant reduction in the weight of liver and spleen in male mice whereas female mice showed increase in the size of uterus (Table 6 and 7). AC is reported to decrease the levels of RBC and inhibit the activity of glucose-6-phosphatase dehydrogenase (Al-Bekairi *et al*., 1991). The exact mechanism of the AC-induced toxicity is not known, however, it may be related to the active ingredients such as alliin, saponins, phenolic acids (Burkill and Dalziel, 1985; Desai *et al*., 1973). CA treatment in male mice was found to reduce the size of testes in male mice and cause reduction in the size of uterus and increase in the size of kidney in female mice as revealed by the observations on visceral examination. These observations are confirmed by our data on the effect of CA on organ weight which showed significant reduction of weight of testes and uterus and increase in weight of kidney in female mice (Table 6). Chronic treatment of AS, AG and CC showed no significant difference in viscera of male and female mice as compared to the control group, respectively.

Genotoxicity studies of spices: The AC, AS and CC treatment did not induce any significant changes in the frequency of micronucleated-PCE (Polychromatic erythrocytes) and the ratio of PCE and NCE (Normochromatic erythrocytes) (Table 8), indicating lack of any clastogenic activity. Treatment with AG

Table 4: Effect of different spices on body weight after chronic treatment in male Swiss albino mice

Treatment and dose (mg kg^{-1} day^{-1}) b.wt.	Body weight (Mean±SE)/week												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12
Control (distilled water, 0.3 mL mouse day^{-1}	23.10±0.53	24.2±0.39	25.8±0.42	27.8±0.56	31.50±0.52	32.00±0.58	31.75±0.70	32.00±0.80	33.50±1.03	35.50±0.92	37.50±1.01	38.60±0.98	40.42±1.08
Allium cepa (75.0)	21.40±0.58	22.00±0.96	27.30±0.63	29.40±0.70	30.88±0.95	31.75±0.35	32.71±0.86	33.00±1.00	34.60±1.02	36.00±0.94	36.60±0.93	37.40±0.68	37.60±1.68
Allium cepa (150.0)	23.10±0.89	24.70±0.87	27.10±0.97	28.10±0.64	32.30±0.84	32.50±0.67	33.70±0.67	36.00±1.24	37.50±1.34	38.60±1.45	38.90±1.36	39.70±1.34	39.20±1.32
Allium sativum (37.5)	19.70±0.83	22.10±0.71	24.40±0.76	30.90±0.71	32.22±0.99	31.90±0.97	33.22±0.90	35.00±1.10	35.22±0.82	38.67±0.89	39.90±0.69	40.22±0.94	41.00±1.03
Allium sativum (75.0)	19.30±0.32	20.90±0.67	25.50±0.96	29.20±0.63	32.00±1.45	32.75±1.03	34.12±1.14	34.97±1.91	36.25±1.98	38.13±1.96	39.90±2.82	39.90±2.10	39.87±2.15
Anethum graveolens (50.0)	23.40±0.42	24.30±0.51	25.60±0.60	27.30±0.83	27.50±0.50	31.00±0.61	31.66±0.81	31.77±0.70	34.77±0.85	36.10±1.14	34.55±0.89	38.00±0.77	38.75±1.01
Anethum graveolens (100.0)	25.50±0.47	25.30±0.42	28.40±0.54	29.00±0.51	30.70±0.55	30.70±0.55	31.20±0.98	33.70±0.65	37.50±0.88	39.00±0.96	40.60±1.01	41.00±2.00	41.85±1.26
Capsicum annuum (25.0)	24.70±0.33	25.30±0.42	26.90±0.43	29.50±0.58	29.22±0.66	29.22±0.66	32.00±0.88	35.22±0.59	37.55±0.55	36.95±0.61	38.22±0.61	40.88±1.17	40.17±0.91
Capsicum annuum (50.0)	24.60±0.47	24.80±0.38	25.90±0.37	26.10±0.52	29.50±0.58	31.80±0.62	31.70±0.66	34.10±0.76	35.50±0.94	36.50±0.77	38.70±1.09	39.50±0.94	39.70±1.26
Carum carvi (12.5)	22.90±0.31	24.60±0.42	25.70±0.51	25.60±0.50	26.80±0.59	30.70±0.76	31.00±0.76	32.60±0.80	35.60±0.56	35.00±0.66	37.00±0.81	38.00±0.84	38.25±0.77
Carum carvi (25.0)	23.70±0.44	24.80±0.35	27.00±0.49	28.80±0.53	30.90±0.79	30.50±0.83	32.30±0.85	34.70±0.66	36.10±0.90	36.60±1.38	40.40±0.68	41.80±1.25	42.70±1.80

n = 10 group^{-1}; Value shows mean±standard error. No significant difference as compared with control (Student's t-test)

Table 5: Effect of different spices on body weight after chronic treatment in female Swiss albino mice

Treatment and dose (mg kg^{-1} day^{-1}) b.wt.	Body weight (Mean±SE)/week												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12
Control (distilled water, 0.3 mL/mouse/day	22.2±0.57	23.1±0.32	24.1±0.38	26.5±0.40	27.7±0.47	28.8±0.43	29.0±0.48	29.5±0.48	30.0±0.85	31.0±1.0	31.3±1.03	30.2±0.75	31.5±1.24
Allium cepa (75.0)	20.2±0.39	20.5±0.72	21.5±0.60	23.6±0.54	24.7±0.47	26.20±0.52	27.4±0.84	28.52±0.80	29.7±0.80	31.7±0.79	32.5±0.87	32.7±0.70	32.9±1.18
Allium cepa (150.0)	20.1±0.74	19.7±0.63	22.22±0.31	23.11±0.55	26.6±1.00	26.77±0.85	27.00±0.74	28.11±1.03	29.66±1.22	31.33±1.56	32.33±1.45	33.0±1.47	33.11±1.52
Allium sativum (37.5)	18.3±0.70	18.6±0.60	22.1±0.53	23.9±0.64	27.8±0.71	27.4±0.64	27.8±0.61	27.9±0.89	29.8±1.28	31.3±1.03	30.3±1.03	31.0±1.28	30.7±1.27
Allium sativum (75.0)	19.2±0.65	19.5±0.65	22.3±0.59	24.6±0.37	26.9±0.94	27.1±0.64	26.5±0.65	29.1±1.23	30.3±1.53	31.6±1.14	32.6±1.14	33.4±1.49	34.7±1.46
Anethum graveolens (50.0)	24.2±0.53	24.3±0.63	25.1±0.87	25.3±0.55	26.3±0.44	27.3±0.47	27.7±0.53	28.4±0.33	31.2±1.16	32.66±1.20	32.2±1.21	32.88±0.97	34.00±1.32
Anethum graveolens (100.0)	24.4±0.26	24.4±0.43	25.0±0.85	25.1±0.37	26.9±0.70	27.3±0.42	27.5±0.60	27.3±0.59	31.77±1.15	31.2±1.16	30.55±1.09	32.2±1.21	34.37±1.13
Capsicum annuum (25.0)	22.5±0.50	24.8±0.48	24.9±0.56	25.7±0.4	27.4±0.54	26.9±0.67	27.6±0.52	27.1±0.64	29.8±0.82	29.2±0.59	32.5±0.81	32.0±0.91	33.88±1.54
Capsicum annuum (50.0)	23.5±0.34	24.2±0.41	24.6±0.49	25.3±0.53	25.7±0.42	25.7±0.31	26.2±0.44	27.7±0.42	30.8±1.03	31.8±1.05	32.9±0.93	32.9±0.83	33.1±1.59
Carum carvi (12.5)	23.2±0.32	23.7±0.47	25.4±0.47	25.8±0.41	25.9±0.56	28.2±0.46	28.0±0.36	28.1±0.52	29.7±0.47	29.7±0.67	31.6±0.67	32.11±0.11	32.77±1.43
Carum carvi (25.0)	22.4±0.52	23.3±0.36	24.6±0.47	25.9±0.56	26.5±0.50	26.9±0.52	28.4±0.58	29.6±0.99	30.3±0.97	29.8±1.20	30.8±0.72	31.2±1.26	33.1±1.51

n = 10 group^{-1}; Value shows Mean±standard error. No significant difference as compared with control (Student's t-test)

Table 6: Effect of the different spices on average organ weight after chronic treatment in Swiss albino male mice

Treatment and dose (mg kg^{-1} b.wt. day^{-1})	Mean weight per 100 g b.wt. Mean±SE					
	Heart	Lungs	Liver	Kidney	Spleen	Testes
Control, distilled water (0.3 mL/mouse/day)	0.54±0.05	0.74±0.04	6.30±0.25	1.58±0.07	0.76±0.11	0.48±0.06
Allium cepa (75.0)	0.56±0.03	0.77±0.04	5.63±0.12*	1.40±0.07	0.72±0.09	0.47±0.07
Allium cepa (150.0)	0.57±0.04	0.74±0.03	5.50±0.17*	1.62±0.06	0.46±0.03*	0.59±0.03
Allium sativum (37.5)	0.63±0.06	0.69±0.02	6.39±0.12	1.42±0.08	0.55±0.05	0.52±0.04
Allium sativum (75.0)	0.61±0.08	0.73±0.03	6.42±0.20	1.69±0.05	0.88±0.05	0.88±0.29
Anethum graveolens (50.0)	0.67±0.06	0.69±0.02	5.86±0.47	1.53±0.05	0.79±0.16	0.60±0.06
Anethum graveolens (100.0)	0.63±0.06	0.71±0.08	6.05±0.51	1.36±0.09	0.88±0.18	0.56±0.03
Capsicum annuum (25.0)	0.66±0.09	0.71±0.03	6.42±0.28	1.34±0.07	1.10±0.10	0.40±0.03**
Capsicum annuum (50.0)	0.69±0.01	0.67±0.05	6.54±0.16	1.53±0.13	0.94±0.03	0.41±0.01**
Carum carvi (12.5)	0.62±0.04	0.79±0.06	6.65±0.30	1.50±0.08	0.94±0.08	0.52±0.06
Carum carvi (25.0)	0.76±0.12	0.68±0.03	6.16±0.40	1.41±0.07	0.89±0.10	0.61±0.02

n = 10 group^{-1}. Value shows Mean±Standard error, *p<0.05; **p<0.01 (Student's t-test)

Table 7: Effect of the different spices on average organ weight after chronic treatment in Swiss albino female mice

Treatment and dose (mg kg^{-1} b.wt. day^{-1})	Mean weight per 100 g b.wt. Mean±SE					
	Heart	Lungs	Liver	Kidney	Spleen	Uterus
Control, distilled water (0.3 mL/mouse/day)	0.57±0.02	0.69±0.04	5.3±0.22	1.03±0.02	0.49±0.05	0.62±0.05
Allium cepa (75.0)	0.56±0.04	0.68±0.02	5.3±0.34	1.11±0.60	0.59±0.08	0.78±0.07
Allium cepa (150.0)	0.58±0.04	0.73±0.03	4.9±0.15	1.12±0.05	0.43±0.02	0.80±0.11
Allium sativum (37.5)	0.52±0.03	0.78±0.11	4.57±0.32	1.05±0.08	0.59±0.08	0.66±0.06
Allium sativum (75.0)	0.59±0.02	0.72±0.04	5.02±0.31	1.11±0.04	0.51±0.06	0.72±0.06
Anethum graveolens (50.0)	0.71±0.03	0.81±0.01	5.89±0.32	1.26±0.08	0.48±0.04	0.86±0.15
Anethum graveolens (100.0)	0.68±0.04	0.85±0.05	5.98±0.27	1.12±0.02	0.65±0.17	0.98±0.33
Capsicum annuum (25.0)	0.58±0.04	0.70±0.04	5.57±0.45	1.31±0.03	0.80±0.17	0.65±0.08
Capsicum annuum (50.0)	0.70±0.06	0.86±0.08	5.65±0.31	1.44±0.05*	1.03±0.22	0.52±0.03*
Carum carvi (12.5)	0.69±0.03	0.86±0.07	6.04±0.25	1.26±0.08	0.53±0.02	0.65±0.03
Carum carvi (25.0)	0.73±0.04	0.73±0.03	5.89±0.23	1.30±0.10	0.77±0.08	0.76±0.18

n = 10 group^{-1}. Value shows Mean±Standard error, *p<0.05 (Student's t-test)

Table 8: Clastogenic effect of the different spices on femoral cells in Swiss albino mice

Treatment and dose (mg kg^{-1} b.wt. day^{-1})	Polychromatic erythrocytes (PCE) screened	Micronucleated polychromatic erythrocytes (%) (Mean±SE)	Normochromatic erythrocytes (NCE) screened	PCE/NCE ratio (Mean±SE)
Control (distilled water; 0.3 mL/mouse)	5035	0.29±0.03	4983	1.02±0.02
Allium cepa (250)	5009	0.31±0.02	5097	1.00±0.04
Allium cepa (500)	5500	0.30±0.03	5157	1.01±0.08
Allium sativum (250)	5000	0.29±0.03	5050	1.03±0.10
Allium sativum (500)	5210	0.30±0.03	5150	1.02±0.04
Anethum graveolens (250)	5000	0.32±0.03	4913	1.02±0.04
Anethum graveolens (500)	5200	0.47±0.06*	5200	1.01±0.04
Capsicum annuum (250)	5130	0.35±0.01	5210	0.98±0.03
Capsicum annuum (500)	5205	0.43±0.04*	5510	0.94±0.01*
Carum carvi (250)	4975	0.32±0.03	4900	1.02±0.03
Carum carvi (500)	5202	0.34±0.02	5105	1.02±0.03

n = 10 group^{-1}. Value shows Mean±Standard error, *p<0.05 (Student's t-test)

significantly increased the frequency of micronuclei in PCE. However, the ratio of PCE and NCE was not affected (Table 8). CA treatment significantly increased the frequency of micronucleated-PCE and decreased the ratio of PCE and NCE indicating the clastogenic and cytotoxic potential of CA.

DISCUSSION

The studies on the general signs and symptoms at acute lower doses (250 and 500 mg kg^{-1}) treatment with different spices showed no significant changes. Some of the severe symptoms like salivation, convulsions, staggering, straub tail, reflex impairement, tremors, excitation, waltzing movements, muscle tone, sedation and comatose condition were recorded in the acute toxicity studies only at the higher doses (LD_{50} and MTD). One interesting feature of AC and AG observed in the present study is that there was no mortality even up to a dose of 30 g kg^{-1} (MTD) which confirms the antioxidant activity of AC and AG observed in the earlier studies (Mehlman *et al*., 1997). In addition, the animals showed tremors, convulsions and drowsiness (Table 2). The LD_{50}/MTD values of AC, AS, AG, CA and CC indicate these spices to be safe natural drugs, because such a high dose is never encountered for its use as a diet or a medicine for human beings.

Chronic treatment with different spices showed mild changes in respiration, heart rate, body temperature, piloerection, itching and alopecia in both male and female mice. Male mice after the treatment with AC, AS, CA and CC showed aggression which caused physical injuries and inflammation. The aggressive behavior in male mice may be responsible for the injuries and inflammations. The aggression in male mice may be due to the effect of spices on the Cerbro-Spinal Fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (Mehlman *et al*., 1997). AG treated animals were observed to be calm and drowsy. AG has been reported to lower the blood pressure, dilate blood vessels, stimulate respiration and slow the heart rate. Furthermore, *in-vivo* administration of AG oil results in the genesis of three dangerous hallucinogenic amphetamines (Leung, 1980) (Table 3).

There was an increase in the mean body weight of male and female mice throughout the duration of 90 days treatment. The increase in body weight and lack of any effect on viscera, organ weight and mortality is attributed, to the antioxidative constituents present in different spices such as flavonoids (AC); amino acids, linalool (AS); anethol, eugenol, umbelliferone, flavonoids (AG); phenolic amides (CA); flavonoids (CC) (Bakkali *et al*., 2008; Moseberg and Hayes, 1989) (Table 4, 5).

The visceral examination showed increased size of uterus (AC, AS, AG, CC). This observation suggests the estrogenic potentials of these spices. CA was also found to reduce the size of the uterus as compared to the uterus observed in the normal mice. It is worthwhile to consider the influence of these spices on a possible disturbance of menstruation in women. The treatment with CA had an impact on the size of testes which were small as compared to the untreated animals. The use of CA may have some effects on the male and female sex hormones which need further investigations (Table 6, 7).

It has been reported that AC decreases the levels of RBC and inhibit the activity of glucose-6-phosphatase dehydrogenase (Al-Bekairi *et al*., 1991). The exact mechanism of the AC-induced toxicity is not known, however, it may be related to the active ingredients such as alliin, saponins, phenolic acids (Burkill and Dalziel, 1985; Desai *et al*., 1973). Generally, high doses of AS in humans are reported to cause anaemia due to both decreased haemoglobin synthesis and haemolysis (Newall *et al*., 1996) and inhibit the synthesis of prostaglandins (Ali *et al*., 1993). Allicin and Allistatin, present in AS are known to possess cytotoxic, bactericidal, germicidal and fungicidal properties (Benkeblia, 2005; Biljana *et al*., 2008; Chung, 2006; Gorinstein *et al*., 2006b; Gorinstein *et al*., 2006a; Newall *et al*., 1996). It has also been reported s-allyl cysteine sulfoxide, a sulphur containing amino acid of AS to decrease the activities of serum enzymes like alkaline phosphatase, acid phosphatase, lactate dehydrogenase and liver glucose-6-phosphatase. Furthermore, the liver and intestinal HMG-CoA reductase activity and liver hexokinase activity were significantly increased by s-allyl cysteine sulfoxide (Biljana *et al*., 2008; Chung, 2006; Newall *et al*., 1996). Nevertheless, carvone content in the volatile oils of CC may be responsible for the observed toxicity. Earlier studies have shown carvone to be toxic to *Drosophila melanogaster* (Duke, 2001). CA has been reported to cause IgE-mediated reactions, heart burn, chest discomfort, nausea, belching, distension and chronic abdominal pain in humans (Leung, 1980). The mechanism of CA-induced toxicity is not known. However, it appears to be related to its active principle, capsaicin which may produce cytotoxic reactive species (Phenoxy radical intermediates) under the influence of cytochrome P450 (Yeoh *et al*., 1995).

There is dearth of related literature on toxicity of spices and their constituents, however, the observed symptoms are attributed to toxic and oxidative phytoconstituents present in spices such as alliin, saponins, phenolic acids (AC), allicin, allistatin, s-allyl cysteine sulfoxide (AS), amphetamine like metabolites, D(+) carvone (AG), capsaicin (CA) and carvone (CC).

Our results on the clastogenic potentials of AG confirmed a previous report (Fukuoka *et al*., 1980) which showed AG exhibit mutagenicity in Salmonella typhimurium strains TA 98 and TA 100. However, the observed activity of AG appears to be related to its mutagenic principles such as isorhamnetin 3-sulphate (Persicarin) and quercetin 3-sulphate (Fukuoka *et al*., 1980). Present results are consistent with the findings of Villasenor and de-Ocampo (1994), who found isolate CF-1 of the *Capsicum frutescens* extract to be clastogenic in mice and *Salmonella typhimurium*. The exact mode of action of CA-induced clastogenicity is not known. However, it appears to be due to the biotransformation of capsaicin to reactive species (Surh and Lee, 1995) as a result of its interaction with other phytoconstituents present in CA. CA and AG were found to be mutagenic and CA showed significant cytotoxicity (Table 8). The exact mode of the observed clastogenicity and cytotoxicity after treatment with AG and CA is not known, however, it appears to be due to phytoconstituents such as quercetin 3-sulphate (AG); capsaicin (CA). The proliferative activity of PCE may be related to ornithine decarboxylase activity which is associated with the increase in nucleic acid synthesis and cell proliferation.

CONCLUSION

The increase in the mean body weight in chronic treatment and high values of LD_{50}/MTD of AC, AS, AG, CA and CC indicates that these spices are safe for human consumption. Effect of AC, AS, AG and CC on uterus shows the estrogenic potentials of these spices. The CA treatment reduced the weight and size of testes and uterus in male and female mice which needs further investigations. The mutagenic, cytotoxic and proliferative activity CA and AG observed in the present study warrants further studies on the possible carcinogenic potentials.

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