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Research Article

Toxicity Studies of *Pimpinella anisum* in Albino Mice

Aamir Mushtaq[1*], Fatima Habib[2], Umar Farooq Gohar[3], Abdul Malik[4] and Mobasher Ahmad[2]

[1]*Department of Pharmaceutical Sciences, Government College University Lahore, Pakistan*
[2]*Gulab Devi Institute of Pharmacy, Gulab Devi Educational Complex Lahore, Pakistan*
[3]*Institute of Industrial Biotechnology, Government College University Lahore, Pakistan*
[4]*Department of Pharmacology, College of Pharmacy, University of Sargodha, Sargodha, Pakistan*

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A Mushtaq and FH conducted experimental work. A Malik and A Mushtaq wrote the manuscript. UFG compiled the results. A Malik and UFG analysed the data. MA presented the concept of the study and supervised it.

Keywords
Pimpinella anisum, Anise, Chronic toxicity, Liver enzymes, Toxicology

Abstract | Keeping in view the extensive pharmacological application of *Pimpinella anisum* (Anise) for treatment of various diseases, the study is designed to explore its acute and chronic toxicity. The dried fruits/seeds of *P. anisum* were powdered and its methanolic extract (with 19 % yield) was prepared by cold extraction technique. Acute toxicity studies were performed in female albino mice by administration of single oral doses (4, 5, 6, 7 and 8 g/Kg/po) respectively, to individual groups (n=5) and after that the behavioral changes were observed. To find the chronic toxicity, the animals were divided in three groups (n=6) which were orally administered with normal saline, *P. anisum* methanolic extract 400 mg/Kg, and 800 mg/Kg respectively for three months. The results of acute toxicity study represented the LD_{50}, value as 4.1 g/Kg/po in albino mice and it was observed that the acute toxic doses produced behavioral changes like hyper-salivation, hyper-secretions, irritation, cyanosis and sedation in mice. Results of chronic toxicity indicated that plant extract did not affect the level of erythrocytes, leukocytes, thrombocytes, hemoglobin, hematocrit, creatinine and liver enzymes (SGOT, SGPT and TB) in mice. However, it significantly ($P \leq 0.05$) increased the level of ALP in mice but on the other hand it lowered the blood glucose and cholesterol levels significantly along with increase in body weight of mice. It is concluded that *P. anisum* methanolic extract has a broad therapeutic index and does not produce considerable liver, kidney and blood toxicity. So, its use in therapeutic doses is considered safe for the treatment of different disorders. However, more scientific work is needed to explore its fetotoxicity and genotoxicity.

Novelty Statement | In this article, we contributed a new thing by providing scientific evidences regarding acute and chronic toxicity of *Pimpinella anisum* that *P. anisum* is free from the toxic. It can be used in food and medicines quite safely.

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Introduction

Pimpinella anisum (Apiaceae) is commonly known as anise or aniseed and widely distributed in Pakistan, India, Egypt, Sri Lanka, Spain and Turkey. It is a well-known spice and aromatic herb and finds wide range of applications as a carminative tonic. It had been used as an appetizer and tranquilizer in traditional medicines (Kosalec *et al.*, 2005). In veterinary medicines, *P. anisum* seeds are administered to the animals to treat the intestinal worms (Marino *et al.*, 2001). A paste is made by putting *P. anisum* seeds powder in mustard oil and applied on the skin to minimize the urge of rashes in scabies (Andallu and Rajeshwari, 2011). It is used as a folk remedy to treat constipation (Heinrich, 2000), bronchial asthma (Andallu and Rajeshwari, 2011), skin infection, sore throat, colic,

Corresponding Author: Aamir Mushtaq
aamir_mushtaq@hotmail.com

urine born infections, bacterial, fungal and parasitic infections (Marino *et al.*, 2001). The scientific studies have been done on *P. anisum,* and it has been explored that its essential oils prevented tonic clonic seizures in albino mice (Pourgholami *et al.*, 1999). Pharmacologically, it has been proved that *P. anisum* is effective for various gynecological disorders like dysmenorrhea and host flushes in post menopause women (Karami *et al.*, 2008). It has been demonstrated that its antispasmodic and muscle relaxant action is due to anti-muscarinic activity (Boskabady and Ramazani-Assari, 2001) as well as due to activation of NO-cGMP pathway (Tirapelli *et al.*, 2007). Different other pharmacological activities of *P. anisum* have also been proved scientifically e.g., anti-ulcer (Al-Mofleh *et al.*, 2007), anti-hyperlipidimic, anti-diabetic (Rajeshwari *et al.*, 2011), anti-amnesic (Mushtaq *et al.*, 2020) and estrogenic activity (Karami *et al.*, 2008).

The phytochemical studies have shown that the essential oils constitute about 3 to 6% of ingredients of *P. anisum* seeds. Anethole (80 to 95% of essential oils) cis-anithole, trans-anithole, anisaldehyde and different terpenes are the chief ingredients of anisedd essential oils. In addition, carbohydrates, proteins, polyenes, scopolectin, estrols, estragole, glucide, carvone, palmitic acid, α-cuparene, β-caryophyllene, α-himachalene, β-bisabolene, terpene hydrocarbons, benzoic acid, petroselinic acid, neophytadiene, methylchavicol, di-hydrocarvyl acetate, anisaldehyde, eugenol, methyleugenol and polyacetylene etc have been isolated from anise (Shojaii and Abdollahi Fard, 2012).

Pimpinella anisum has been declared a safe aromatic spice by US, Food and Drug Administration. It is considered free of toxic effects when it is used as a spice in small quantities. However, it may be toxic for the living organisms if it is administered in the form of purified extracts. Studies have shown that different constituents of *P. anisum* like anethole (the chief essential oil of *Pimpinella anisum* seeds) produces acute and chronic toxicity in living organisms. It has been demonstrated that the anethole produces neurogenic problems like headache, seizures, cerebral congestion, muscle cramps and numbness, when it is consumed in toxic doses (De and De, 2022). No toxicity studies have been conducted for purified extracts of *P. anisum* which can confirm the potential hazards of the plant. So, this study was designed to find the acute and chronic toxic effect of methanolic extract of *P. anisum* dried fruits/seeds in albino mice. In our previous project we have found the pharmacological basis of anti-amnesic activity of *P. anisum* in albino mice (Mushtaq *et al.*, 2019).

Materials and Methods

Chemicals

All chemicals of commercial grade were used in the study including; methanol (Sigma-Aldrich-US), ethanol (Sigma-Aldrich-US), SGOT diagnostic kit (Human-Germany), SGPT diagnostic Kit (Human-Germany), ALP diagnostic Kit (Human-Germany), Total bilirubin diagnostic Kit (Human-Germany), cholesterol diagnostic Kit (Human-Germany), creatinine diagnostic Kit (Human-Germany), carboxy methyl cellulose (Sigma-Eldrich), glucose diagnostic Kit (Human-Germany).

Animals

We conducted experiments on Swiss albino mice (female gender) weighing 20±05 g. The animals were kept in standard polycarbonate cages in animal house of Punjab University College of Pharmacy, Punjab University Lahore. The bedding of animal cages was changed on a daily basis to maintain a neat and clean hygienic environment. The permission regarding use of animals was obtained from institutional research and ethics committee vide permission letter no: AEC/PUCP/1072/A. The animals had free access to food and water *ad-libitum*. They were provided with standard living conditions i.e., temperature; 37°C, humidity; 50% and equally divided light and dark time span; 12h each. The body weight of animals was measured daily and dose was administered as per body weight.

Plant material and extraction

Dried ripened fruits/seeds of *P. anisum* were purchased locally from the Market of Lahore. They were first identified by the botanist of GC University Lahore and the specimen was preserved in the herbarium of the University. The Specimen was given herbarium no: GC.Herb.Bot.3386. After identification, 700 g of dried fruits were taken and washed with water to remove the dust particles. They were dried in air and ground into fine powder material. After grinding, the powdered material was soaked in 3L of methanol for three consecutive days. After three days, the material was recovered by filtration and dried using a rotary evaporator (Heidolph, Germany) at temperature; 37°C, pressure; 50 Torr and rotation; 60 rpm. Finally, the semisolid paste was obtained and preserved in air tight glass container at −8°C in refrigerator. The yield of the extract was calculated by dividing weight of dried extract with total weight of dried powdered material.

Acute toxicity studies

To perform acute toxicity studies the OECD guidelines 423 were followed. To find the lethal dose range pilot study was performed initially on two animals for each dose. Then animals were divided into different groups n=5. Group-1 was orally administered with 0.9% NaCl solution in dose 10 ml/Kg. The remaining groups were orally administered with *P. anisum* methanolic extract (PaMe) in wide space dose ranges in ascending order i.e., 1, 2, 3, 4, 5 g/Kg respectively and the dose was increased until all the animals died at that dose. The animals which survived after administration of a single acute dose were observed

for 24 h to assess the signs of acute toxic reactions (El Hilaly *et al*., 2004). Then the Karber's method was applied to calculate LD_{50} (median lethal) of *P. anisum* methanolic extract (Akhila *et al*., 2007).

Chronic toxicity studies

To perform chronic toxicity studies, the animals were divided into two groups with six animals in each. They were acclimatized for one week before performing the studies. The animals in group-I were administered with 0.9 % normal saline (10 ml/Kg) daily for three months. Then the animals in group-II and III were given oral treatment with *P. anisum* methanolic extract (PaMe) in doses of 400 and 800 mg/Kg, respectively daily for three months. In our previous study the anti-amnesic activity of *P. anisum* methanolic extract was explored at dose 400 and 800 mg/Kg P.O (Mushtaq *et al*., 2020). So, 400 and 800 mg/Kg doses were considered as therapeutic doses and the chronic toxic effects were observed for these two doses (400 and 800 mg/Kg per oral) in this study.

Effect on body weight

The body weights of the animals were found on the daily basis before administration of the dose. Thus, the effects of PaME on body weight of the animals were also observed and the results were expressed in tabulated form.

Hematological studies

When the treatment period was completed, 24 h after the administration of last dose, blood was collected from the heart by cardiac puncture. The blood was collected in two types of vacutainers; one with EDTA (ethylene di-amine tetra acetic acid) and the other without EDTA. The anti-coagulated blood was reserved for the hematological analysis while the non EDTA blood was used for the serological studies. The animals were then sacrificed by using chloroform, immediately after the collection of blood. Non coagulated blood was used for the estimation of erythrocytes, leukocytes, thrombocytes, hematocrit and hemoglobin levels. Blood was subjected to auto analyzer Merck Microlab 300 made by Germany to assess the hematological parameters.

Serological studies

The non EDTA blood was centrifuged at 4000 rpm for five minutes and the serum was collected for the performance of serological studies. The serum was used for the analysis of creatinine, cholesterol, glucose, ALP, SGOT, SGPT and TB levels by using diagnostic kits. Standard procedures were followed by using auto analyzer Merck Microlab 300 made by Germany to assess serological parameters (El-Hilaly *et al*., 2004; Mushtaq *et al*., 2017).

Statistical analysis

The data were analyzed by using graph pad prism and values were expressed as mean±standard error of mean. ANOVA (One way) test was applied and given data sets were compared by applying Dunnett's test. The level of significance was considered as following; $P \leq 0.05$ = significant and denoted by α, $P \leq 0.01$= more significant and denoted by β, $P \leq 0.001$= highly significant and denoted by $^{\gamma}$, $P \geq 0.05$= non-significant and denoted by π.

Results and Discussion

Percentage yield

After extraction and drying, total of 132.5 g of PaMe (*P. anisum* methanolic extract) was obtained. The percentage yield was calculated as (132.5/700 x 100 = 18.92) almost 19%.

Acute toxicity study

The results of acute toxicity indicated that LD_{50} of PaMe was calculated as 4.1 g/Kg per oral as shown in Table 1. The previous studies have shown that *P. anisum* produced the highest anti-amnesic activity at a dose of 800 mg/Kg. Thus, it had a broad therapeutic index. It has been observed that the treatment of animals with acute toxic doses of plant extract produced irritation, hypersecretions and muscle spasms at a dose of 5 g/Kg administered orally. While in addition to all these behavioral changes the sedation, both hypnosis and cyanosis were observed in animals treated with acute toxic dose of 7 g/Kg per oral (Tables 2-4).

Table 1: Calculation of LD_{50} (median lethal dose) of methanolic extract of *P. anisum* (PaMe).

Groups	D (Dose difference)	M (Mortality)	M_1 (Mean mortality)	(Dx M_1)	Σ(Dx M_1)/n
G-I	0	0	0	0	9.5/5
G-II	4	0	0+0/2=0	0	
G-III	1	0	0+0/2=0	0	
G-IV	1	3	0+3/2=1.5	1.5	
G-V	1	4	3+4/2=3.5	3.5	
G-VI	1	5	4+5/2=4.5	4.5	

LD_{50}= Least lethal Dose - Σ(DxM_1)/n; LD_{50} = 6–1.9=4.1 g/Kg. G-1 was administered with 0.9 % NaCl solution, and G-II to VI were orally administered with PaMe in doses 4, 5, 6, 7 and 8 g, respectively. Each group comprised 5 animals (n=5).

Chronic toxicity study

The results of chronic toxicity studies indicated that the oral administration of PaMe in doses 400 and 800 mg/Kg produced negligible effects on hematology (erythrocytes, leukocytes, thrombocytes, hemoglobin and hematocrit levels) and serology (cholesterol, glucose, ALP, SGOT, TB and SGPT levels) of mice (Tables 6-7). A slight gain in body weights of mice was also observed (Table 5).

Pimpinella anisum, being an aromatic plant has been used extensively in the food industry as a flavoring and confectionery ingredient. In this concern its safety should

Table 2: Acute toxic effect of PaMe 5g/Kg in albino mice.

Behavioral changes	Observation after treatment						
	0h	1h	2h	4h	8h	12h	24h
Seizures	–	–	–	–	–	–	–
Sedation	–	–	–	+	+	–	–
Hyper stimulation	–	–	–	+	+	–	–
Hyper secretions	–	+++	++	++	+	–	–
Muscle rigidity	–	++	++	++	+	–	–
Cyanosis	–	–	–	–	–	–	–
Depression	–	–	–	–	–	–	–
Irritation	–	+++	++	+++	++	+	+
Hypnosis	–	–	–	–	–	–	–
Spasticity	–	++	++	++	++	+	+
Hyper salivation	–	++	++	++	++	+	+
Hyperactivity	–	–	–	–	–	–	–

Animals were orally administered with single acute dose (5g/Kg) of PaMe. Before administration of acute dose, all the signs were absent in animals. + refers to slightly present, ++ refers to moderately present and +++ indicates highly presence of the characteristics.

Table 3: Acute toxic effect of PaMe 6g/Kg in albino mice.

Behavioral Changes	Observation after treatment						
	0h	1h	2h	4h	8h	12h	24h
Seizures	–	–	–	–	–	–	–
Sedation	–	–	+	+	+	–	–
Hyper stimulation	–	–	+	+	+	–	–
Hyper secretions	–	+++	++	++	+	–	–
Muscle rigidity	+	+++	+++	+++	+	–	–
Cyanosis	–	–	–	–	–	–	–
Depression	–	–	–	–	–	–	–
Irritation	–	+++	++	+++	++	+	+
Hypnosis	–	–	–	–	–	–	–
Spasticity	–	++	++	++	++	+	+
Hyper salivation	–	++	++	++	++	+	+
Hyperactivity	–	+	+	–	–	–	–

Animals were orally administered with single acute dose (6g/Kg) of PaMe. Before administration of acute dose, all the signs were absent in animals. + refers to slightly present, ++ refers to moderately present and +++ indicates highly presence of the characteristics.

Table 4: Acute toxic effect of PaMe 7g/Kg in albino mice.

Behavioral changes	Observation after treatment						
	0h	1h	2h	4h	8h	12h	24h
Seizures	–	–	–	–	–	–	–
Sedation	–	++	++	+	+	–	–
Hyper stimulation	–	–	+	+	+	+	–
Hyper secretions	+++	+++	++	++	+	+	–
Muscle rigidity	++	+++	+++	+++	+	–	–
Cyanosis	+	+	+	+	+	+	–
Depression	–	–	–	–	–	–	–
Irritation	–	+++	++	+++	++	+	+
Hypnosis	–	+	+	+	+	–	–
Spasticity	–	++	++	++	++	+	+
Hyper salivation	–	++	++	++	++	+	+
Hyperactivity	–	+	+	+	+	–	–

Animals were orally administered with single acute dose (7g/Kg) of PaMe. Before administration of acute dose, all the signs were absent in animals. + refers to slightly present, ++ refers to moderately present and +++ indicates highly presence of the characteristics.

be of prime importance to avoid any serious health problems. It has been reported that some of the constituents of essential oils of anise produce severe toxicity in insects and may be used as insecticides to prevent crop damage (Marcus and Lichtenstein, 1979). They are considered toxic and may be used as fumigants to kill insects (Tu *et al.*, 2018). They may also produce a few adverse effects including pulmonary edema, seizures, convulsions, nausea and vomiting when absorbed topically. The anethole is the principal ingredient of anise and may produce skin irritability and rashes when applied externally on skin. It has also been reported that it may also cause erythema, vesiculation and dermal scaling in sensitive people (Ozguven, 2012).

Although the anise plant is considered safe and used widely in folk medicines for the treatment of different kind of disorders (Janahmadi *et al.*, 2008) but still there is a need to identify any systemic adverse effects to ensure its safety. No scientific study has been conducted on *P. anisum* so far, which could ensure its safe and toxicity free therapeutic profile. Acute toxicity studies were performed on female albino mice in order find the effect of acute toxic doses on the behavior of mice. In addition to the calculation of LD_{50} of plant extract, the behavioral changes in mice was also observed. The significance of observing the behavioral characteristics in acute toxicity studies is that the toxic dose of any substance may affect the neurological patterns in living organisms which are subsequently observed as behavioral changes. It was observed that when animals were treated with acute toxic doses of plant extract behavioral changes were observed including hypersecretion, irritation, spasticity, hypersalivation and muscular rigidity. However, it did not produce seizures, hyperstimulation, hyperactivity, cyanosis and hypnosis in mice. It is clear from the results of the behavioral study that the plant did not produce central nervous system stimulation at tolerable acute toxic doses but at higher doses it induced sedation, hypnosis and cyanosis in mice.

In order to explore its renal toxicity, plasma creatinine level was used as a marker and our study indicated that PaMe (*P. anisum* methanolic extract) did not affect the creatinine levels in mice. The administration of animals with PaMe 800 mg/Kg did not produce a significant elevation in the creatinine levels in mice (Table 7). Creatinine is an important marker to assess kidney function and increased plasma concentration indicates potential kidney damage (Gowda *et al.*, 2010). Similarly, past studies have shown that ethanolic extract of *P. anisum* possesses nephro-protective activity which prevents renal damage by promoting glomerular filtration rate and by preventing the renal glomerular vasoconstriction (Changizi-Ashtiyani *et al.*, 2017). Methanolic extract contains more constituents than ethanolic extracts: thus, the current study provided the clear evidence that the plant has not any nephrotoxic effect in mice.

Table 5: Effect of chronic doses of PaMe on body weight of mice.

Groups	Body weight of mice (g)			
	Start of study	After 30 days	After 60 days	After 90 days
G-I (0.9 % NaCl 10 ml/Kg)	22.71±0.61	29.53±0.97	39.41±0.91	49.81±0.84
G-II (PaMe 400 mg/Kg)	21.11±0.29$^{\pi}$	30.39±0.88$^{\pi}$	42.66±0.81$^{\pi}$	54.19±0.99$^{\alpha}$
G-III (PaMe 800 mg/Kg)	20.19±0.54$^{\pi}$	33.57±0.93$^{\alpha}$	46.77±0.87$^{\beta}$	55.86±0.89$^{\alpha}$

Animals were orally administered with extract in above said doses. Values of G-II and III were compared with G-I, $^{\alpha}$ denotes to $P \leq 0.05$, $^{\beta}$ indicates $P \leq 0.01$ while $^{\pi}$ refers P ≥ 0.05.

Table 6: Effect of chronic doses of PaMe on mice hematology.

Groups	Parameters of hematology				
	Erythrocytes (million/mm^3)	Leukocytes (cells/mm^3)	Platelets (cells/ mm^3)	Hematocrit (% Vol)	Hemoglobin (g/dL)
G-I	7.80±0.22	7.48±0.23x10^3	8.69±0.30x10^4	40.84±0.91	14.03±0.47
G-II	8.01±0.39$^{\pi}$	7.92±0.33x10$^{3\pi}$	8.21±0.54x10$^{4\pi}$	41.49±0.87$^{\pi}$	13.19±0.73$^{\pi}$
G-III	7.41±0.43$^{\pi}$	7.24±0.48x10$^{3\pi}$	8.93±0.39x10$^{4\,\pi}$	40.27±0.68$^{\pi}$	14.33±0.39$^{\pi}$

Values of G-II and III were compared with G-I, $^{\pi}$ indicates $P \geq 0.05$

Table 7: Effect of chronic doses of PaMe on mice serology.

Groups	Parameters of serology						
	Glucose (Mg/dL)	Cholesterol (Mg/dL)	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)	TB (IU/L)	Creatinine (Mg/dL)
G-I	111.21±2.93	189.91±2.41	169.17± 4.42	75.41±5.19	71.24±3.01	0.69±0.03	0.21±0.02
G-II	104.51±4.41$^{\pi}$	176.31±3.95$^{\pi}$	201.29±7.12$^{\alpha}$	67.77±5.29$^{\pi}$	66.35±5.19$^{\pi}$	0.65±0.02$^{\pi}$	0.19±0.05$^{\pi}$
G-III	94.77±3.62$^{\alpha}$	160.09±2.81$^{\alpha}$	197.35±5.23$^{\alpha}$	69.17±4.21$^{\pi}$	61.41±4.63$^{\pi}$	0.63±0.05$^{\pi}$	0.18±0.03$^{\pi}$

ALP = Alkaline Phosphatase, SGOT = serum glutamic-oxaloacetic transaminase, SGPT = serum glutamic-pyruvic transaminase, TB= Total Bilirubin. Values of G-II and III were compared with G-I, $^{\alpha}$ refers to $P \leq 0.05$, while $^{\pi}$ indicates $P \geq 0.05$

It has also been shown that chronic treatment of mice with PaMe 800 mg/Kg/po did not elevate the levels of hepatic enzymes (SGOT, SGPT and TB) in mice. However, ALP level was observed to be slightly increased as shown in Table 7. The significant increase in ALP level indicates that it may produce hepatic damage in toxic doses but at therapeutic doses no significant hepatotoxicity occurs. These liver enzymes are used as biomarkers to identify hepatic cell damage. The levels of hepatic enzymes (ALT, SGOT, SGPT and TB) were significantly increased when the hepatic cellular damage takes place. The past studies have also shown that the *P. anisum* prevented hepatic cell damage in albino rats by reducing oxidative burden in hepatocytes (Cengiz *et al*., 2008).

Blood cells particularly RBCs are highly sensitive to certain drugs. Thus, it is very important to investigate the effect of the extract on blood cells. The results of hematological studies of our plant extract have shown that it did not produce any blood toxicity. The levels of erythrocytes, leukocytes, thrombocytes, hemoglobin and hematocrits remained unaffected after prolonged treatment with the extract (Table 6). Previously, it has been reported that alcoholic extracts of *P. anism* not only increased the levels of LH (luteinizing hormone) and FSH (follicle stimulating hormone) in goats but also prevented the destruction of blood cells (Hussein *et al*., 2020). The plant possesses strong antioxidant potential which prevents the damage caused by oxygen free radicals (Mushtaq *et al*., 2020). Thus, the use of the plant extract can be considered quite safe in terms of blood cell toxicity.

Natural and herbal products have the potential to reduce serum cholesterol and glucose levels and hence can be used in treatment of diabetes and hypercholesterolemia (Mughal *et al*., 2020). Our results have shown that circulating glucose and cholesterol were reduced significantly with extract treatment ($P \leq 0.05$) (Table 7). Phytochemical studies have shown that the anise plant possesses a high concentration of palmitic acid which not only prevents hypercholesterolemia but also treats hyperglycemia (Maedler *et al*., 2003). Similarly, oleic acid is a key ingredient of *P. anisum* which is reported to prevent absorption of saturated fatty acids from the intestinal brush border (Aspenstrom-Fagerlund *et al*., 2012). Hence it lowers cholesterol levels in serum. Research has also shown that β-sitosterol has been isolated from *P. anisum* which possesses many biological activities including lipid lowering and hypoglycemic potential (Yuk *et al*., 2007; Farzaneh *et al*., 2018). The hypoglycemic and lipid lowering effect observed after chronic administration of methanolic extract of *P. anisum* may be due to these phytochemical constituents.

The effect of the plant extract on body weight of animals indicated that the significant ($P \leq 0.05$) weight gain in animals was observed after the chronic treatment with the plant extract (Table 5). Similarly, acute toxic studies indicated that the plant extract increased the secretions in animals (Tables 2-4). Thus, it can be assumed that the plant extract increased digestion and food intake in animals which increased the body weight. It has been proven that the administration of broiler chickens with anise seeds potentially increased their feed intake and ultimately the chickens gained bodyweight. It has also been shown that anise seeds increase the gastric juice secretion and improve appetite and its use to prevent colic, bloating and nausea has been well established (Ibrahim and Abaas, 2007). The results indicated that the chronic treatment of animals with the methanolic extract of *P. anisum* improves the healthy body weight of the animals and reduces the body fat. The animals treated with a chronic therapeutic dose of 800 mg/Kg produced higher significant weight gain compared with animals treated with a chronic dose of 400 mg/Kg. It is clear from the above that methanolic extract of *P. anisum* is free from hepatic, renal and blood toxicity effects when used at lower therapeutic doses i.e 400 mg/Kg per oral. But it can produce some hepatic damage at higher doses as observed in animals treated with PaMe (800 mg/Kg per oral). Similarly, the results of the acute toxicity study indicated that the plant is is toxic at high doses and lethal at very high doses (LD_{50} = 4.1 g/Kg per oral).

Conclusions and Recommendations

It is concluded that *P. anisum* (dried fruits/seeds) doses are not toxic for blood, liver and kidney of the mice, when used in therapeutic doses. Its use in foods and medicines is considerably considered safe. However more extensive work is needed to identify the effects of this plant on gene regulation during fetal and postnatal life.

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Conflict of interest

The authors have declared no conflict of interest.

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