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Research Paper

Edible *Tagetes patula* Flower and Its Flavanoid Patuletin: Pre-clinical Evidence of Selective Systemic Toxicity

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ABSTRACT

Background: Phytotherapy is a common practice around the world, including Pakistan. One such medicinal plant is *Tagetes patula*. Its flower is edible and used for various local illnesses, especially cancer. However, they lack the toxicological evaluation of this plant, including its active flavonoid i.e. Patuletin, which is addressed in the present investigation.

Methods: Wistar rats of either sex (150 g) were intraperitoneally treated (acute [1 day], subacute [7 days], and repeated dose [28 days]) with *T. patula* flower methanolic extract (1 and 2 g/kg) and patuletin (5, 30 and 60 mg/kg). Weight changes in rats were observed. Additionally, the hematological and biochemical (hepatic and renal function) indicators were assessed using respective analyzers. Histological examination of the liver and kidney was also performed following hematoxylin and eosin staining.

Results: Our data showed that none of the treatments caused a significant alteration in the weight of rats compared to the control. Among the various hematological parameters, the white blood cell count was significantly reduced by both extract and patuletin treatments. However, the enhanced levels of aspartate aminotransferase along with morphological changes in the liver were observed only in patuletin-exposed animals.

Conclusion: The present study demonstrated the toxic potential of *T. patula* flower methanolic extract and its active flavonoid i.e. patuletin. It further recommends the implementation of a regulatory check over its consumption to protect fellow citizens from the harmful effects.

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Introduction

Humans are blessed with a wealth of plants, which have been used to treat various diseases for centuries. The history of herbal medicines is as old as the history itself. Their usage dates back over 4,000 years, especially in countries like China and India [1]. According to the World Health Organization (WHO), it is estimated that 80% of the world's population relies on herbal medicine and their use as an alternative medicine is expanding rapidly [2]. The belief that herbal products are safe is vanishing after the emergence of scientific reports regarding their systemic toxicities. Cardiac toxicity is associated with the consumption of herbs, such as *Glycyrrhiza glabra* [3] and *Ephedra sinica* [4]. The administration of *Sauropus androgynus* is associated with the occurrence of chronic obstructive pulmonary disease [5]. The *Aristolochia fangchi* utilization was attributed to renal toxicity and carcinogenicity [6]. The famous *Ginkgo biloba* caused hematological toxicities [7], while the popular *Kava kava* led to hepatotoxicity [8]. It is worth noting that the commonly used *Zingiber officinale* was linked with fetal toxicity [9]. The aforementioned reports advocate that herbs also possess the potential to induce toxicity in humans.

The use of herbs as alternative medicine is extremely popular in Pakistan due to their affordability, availability, and belief in safety [10]. The herbal medicines are widely sold over-the-counter as reflected by 52,600 registered Unani practitioners [11] and estimated market sales of 5 billion rupees [12]. Several reports revealed the toxicity of herbs consumed in the country, such as heavy metals (zinc [Zn], copper [Cu], chromium [Cr], nickel [Ni], cobalt [Co], cadmium [Cd], lead [Pb], manganese [Mn] and iron [Fe]) were greater than permissible amounts in some (*Achyranthes aspera*, *Alternanthera pungens*, *Brassica campestris*, *Cannabis sativa*, *Convolvulus arvensis*, *Hordeum vulgare*, *Justicia adhatoda*, *Parthenium hysterophorus*, *Ricinus communis*, *Withania somnifera*) of the traditional plants of Haripur district [13]. Some of the industrial herbal products with heavy metal contents beyond the allowed limits were analyzed [14].

Tagetes patula (common name = French Marigold) is among the plants with substantial therapeutic value. It is an edible plant traditionally used as a medicine for managing colics, diarrhea, vomiting, fever, skin, and hepatic diseases. It is worth mentioning that traditional healers have been utilizing its petals to treat cancers [15]. To support this, it was scientifically proven to be effective against many cancers, including lung cancer [16]. Patuletin is a flavonoid, which is considered to be the major therapeutic moiety residing in the Tagetes flower [17]. However, they lack the toxicological evaluation of this plant, including its active flavonoid i.e. Patuletin. This study was conducted to pre-clinically assess the toxic potential of Tagetes flower and patuletin.

Materials and Methods

Chemicals

The following chemicals were used in the study: Sodium chloride, EDTA, isopropyl alcohol, paraformaldehyde, paraffin, hematoxyline, and eosin were provided by Sigma Aldrich (USA).

Animals

Wistar rats of either sex (150 g) were obtained from the animal house facility of H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi. The animals were acclimatized to laboratory conditions a week before conducting experiments. Animals were maintained on a 12 h dark/light cycle at about 25 ˚C and allowed access to a standard laboratory diet and water during the experiments.

Test substances

The *T. patula* methanolic extract and its flavonoid i.e. patuletin was provided by Prof. Shaheen Faizi from the International Center for Chemical and Biological Sciences, University of Karachi, and Pakistan. The details about the preparation of said extract and isolation of patuletin were previously reported [16].

Treatments

The rats were divided into 6 groups of 9 animals each as follows: Control (saline), Tagetes extract (1 or 2 g/kg), and patuletin (5, 30, or 60 mg/kg). Rats were given the respective treatment (intraperitoneal) for 28 days. The changes in the weight were observed. Three animals from each group were euthanized on day 1 (acute), 14 (sub-acute), and 28 (repeated dose) for toxicological evaluations.

Hematological and biochemical parameters

The blood was collected through cardiac puncture and collected in non-heparinized and ethylene diamine tetra acetic acid (EDTA)-containing tubes for the biochemical and hematological parameters, respectfully. Hematological parameters include hemoglobin (Hb) concentration, hematocrit (HCT), red blood cells (RBC), total

leukocyte (white blood cells [WBC]), and platelet count, whereas, biochemical tests included aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and serum creatinine. The tests were determined using automatic hematology and chemical analyzers at the diagnostic laboratory of Dr. Panjwani Centre for Molecular Medicine and Drug Research, Karachi University, Karachi, Pakistan.

Histopathology

After sacrificing the animals (highest dose and day 28 only), the liver and kidney were collected for histopathological examination. The tissues were washed and immediately fixed with 10% formalin for 24 h. Later, the tissues were dehydrated (serial isopropyl solutions), embedded (paraffin), sliced (4 µm thickness), and stained with hematoxylin and eosin dye for microscopic examination (Nikon, Japan). The microscopic features (x40) of the organs (liver and kidney) were compared with the control group.

Statistical analysis

The data are presented as Mean±SE (n=3 per group). Comparisons between different groups were performed using a one-way analysis of variance followed by a post hoc test (least significant difference) using SPSS software, version 19, (Chicago, IL, USA). The minimum level of significance was set at $P<0.05$.

Results

Weight variation

No statistical difference was observed in the weight of animals among various treatments with control for 28 days (Figure 1). Additionally, no signs of toxicity, such as tremors, salivation, lacrimation, diarrhea, and dermal abnormalities were observed.

Hematology

The hematological parameters i.e. Hb, HCT, RBC, and platelets count showed no significant change in all treatment groups compared to the control (Table 1). However, the WBC count was significantly reduced following 28 days (repeated dose) of treatment of Tagetes extract (1 and 2 g/kg), while patuletin treatment caused its significant dose-dependent decline upon acute, sub-acute, and repeated administration compared to control.

Hepatic and renal function tests

The biochemical indicators for hepatic function revealed a significant increase in the levels of AST following patuletin treatment compared to the control (Table 2). During acute treatment, only the highest tested dose of patuletin i.e. 60 mg/kg could induce the aforementioned increase, while it was found to be elevated at lower doses i.e. 5 and 30 mg/kg in sub-acute and repeated dose groups. The other indicator of hepatic function i.e.

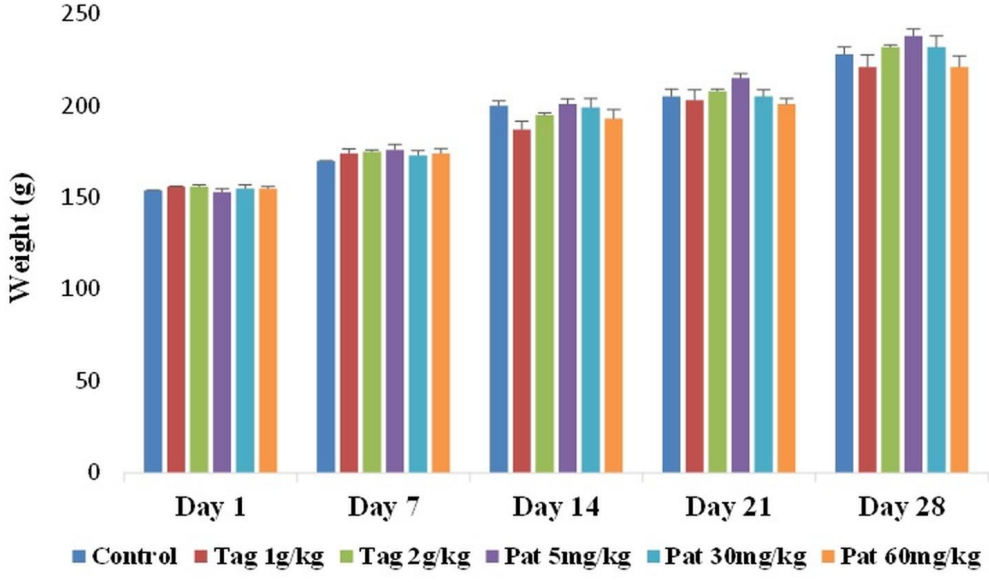

Figure 1. Effect of Tagetes extract and patuletin on the weight of rats

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Notes: The figure depicts the Mean±SE of weight of rats (n=3) following Tagetes (1 or 2 g/kg) and patuletin (5, 30 or 60 mg/kg) treatments daily for 28 days. No statistical difference was observed in weight of treated rats with control (saline group).

Table 1. Effect of Tagetes methanolic extract and patuletin on hematology of rats

Duration	Treatment	Dose	Hb (g/dL)	RBC Count (10^9 cells/mL)	HCT	WBC Count (10^9 cells/L)	Platelets Count (10^9 cells/L)
1 day	Control	-	13±0.2	8.2±0.1	40±1.4	9.6±1.9	1224±63
	Tagetes extract	1 g/kg	12±0.6	8.9±0.1	41±1.2	10±2.0	1201±19
		2 g/kg	14±1.0	9.3±0.6	42±1.0	9.3±0.2	1089±90
		5 mg/kg	12±0.6	7.3±0.4	41±1.0	11±0.2	934±106
	Patuletin	30 mg/kg	12±0.5	7.4±0.3	39±2.4	5.7±1.0**	983±188
		60 mg/kg	12±0.1	7.5±0.3	39±1.0	4.9±0.3**	798±154
14 days	Control	-	11±0.4	6.1±0.2	12±1.6	13±0.2	885±48
	Tagetes extract	1 g/kg	12±0.1	6.3±0.1	12±0.4	13±0.2	696±20
		2 g/kg	12±0.7	7.1±0.6	11±0.3	13±0.2	902±178
		5 mg/kg	11±0.6	6.3±0.6	40±1.2	12±0.1	664±175
	Patuletin	30 mg/kg	11±0.5	6.0±0.3	38±3.7	6.0±1.0***	759±92
		60 mg/kg	11±0.1	6.0±0.1	37±1.2	5.0±0.3***	657±116
28 days	Control	-	12±0.1	6.2±0.1	42±1.0	13±1.0	837±40
	Tagetes extract	1 g/kg	12±0.2	6.2±0.1	44±0.3	4.2±0.5***	781±42
		2 g/kg	11±0.1	6.0±0.1	41±0.3	3.0±0.3***	780±41
		5 mg/kg	12±0.1	6.3±0.1	41±0.1	9.0±0.5***	783±138
	Patuletin	30 mg/kg	12±0.4	6.0±0.5	41±0.5	7.7±1.0***	738±112
		60 mg/kg	12±0.5	6.2±0.4	40±1.3	4.9±0.4***	923±62

Abbreviations: Hb: Hemoglobin; RBC: Red blood cells; HCT: Hematocrit; WBC: White blood cells.

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**P<0.01, ***P<0.005.

ALT remained un-altered in any of the treatment groups. The biochemical indicators of renal function i.e. BUN and creatinine remained unchanged in any of the treatment groups compared to the control.

Histopathology

The macroscopic examination of the vital organs of treated animals revealed no abnormalities in color and texture compared to the organs of the control group. The light microscopy examination of the sections of control livers revealed normal tissue with intact architecture (Figure 2). In the case of the liver treated with Tagetes extract, intact architecture was found. However, in one of the patuletin treatment groups (60 mg/kg for 28 days), the liver tissue had dilated veins and moderate inflammation around blood vessels.

The microscopic examination of the kidneys of control and treated animals revealed a similar picture i.e. glomeruli and renal tubules were unremarkable. No mesangial proliferation, vascular thickening, sclerosis, hemorrhage, and tubular atrophy were observed.

Discussion

The use of medicinal plants for healing purposes is globally common due to the belief of the common man in their safety compared to allopathic medicines. However, an emerging set of scientific data is diluting this belief by attributing various toxicities to the use of these natural products. The literature revealed the scarcity of reports addressing the toxicological actions of these natural products. Considering these facts, the present study

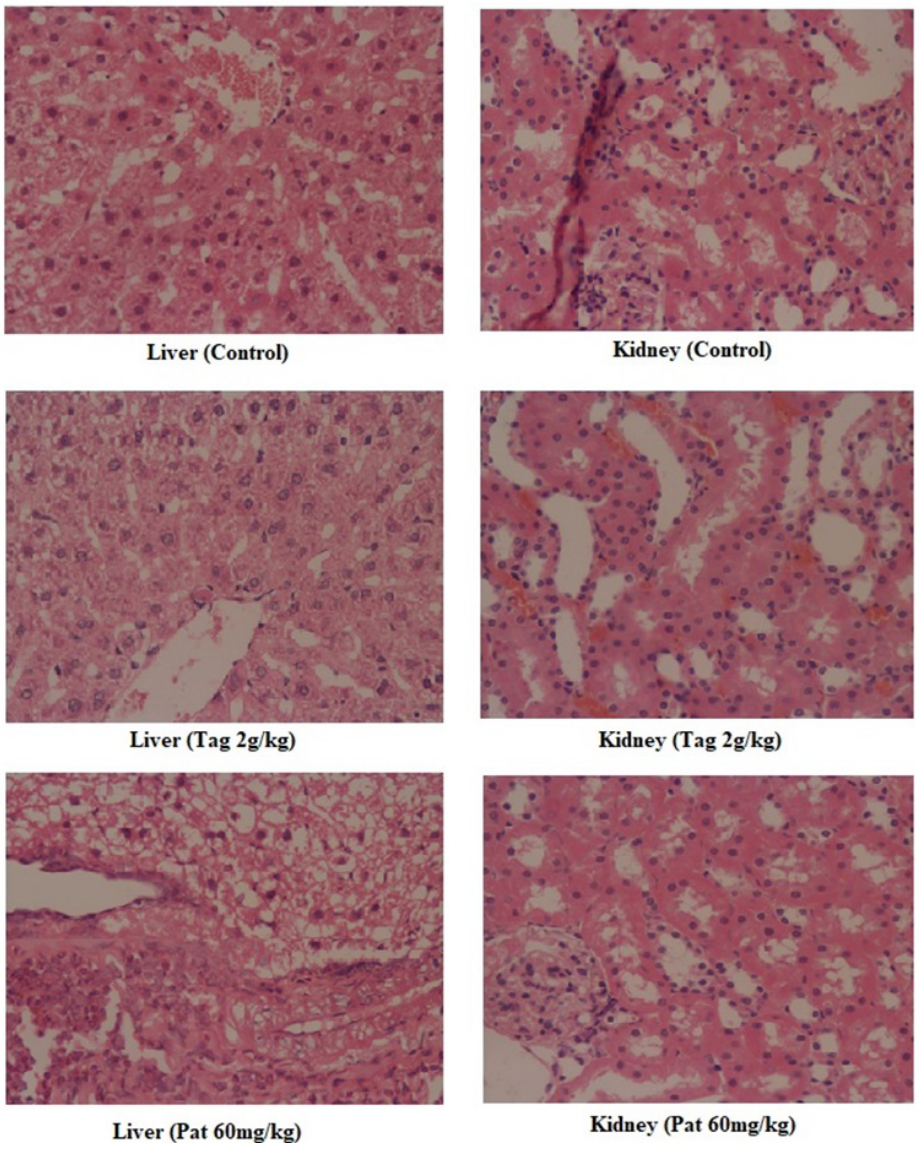

Figure 2. Microscopic examination of liver and kidney

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Notes: The figure depicts the images (x40) of the liver and kidney of control and treated animals (Tagetes extract [2 g/kg] and patuletin [60 mg/kg]) of repeated dose group. The light microscopy examination of a section of control livers revealed normal tissue with intact architecture. The Tagetes-exposed liver exhibits rare lymphocytes and occasional plasma cells in the hepatic portal tracts, while patuletin-treated liver tissue revealed dilated veins and focal moderate inflammation around blood vessels. However, the overall architecture was intact without any signs of necrosis, fibrosis, and steatosis. The microscopic examination of the kidney revealed intact architecture and unremarkable changes in any of the treatment groups as compared to the control.

was designed to assess the systemic toxicity of *T. patula* and its active flavonoid i.e. patuletin.

T. patula flower is edible [18]. Presumably, our weight variation data showed no significant alterations by Tagetes extract and patuletin treatments compared to the control (Figure 1). This supports the safety belief of natural products. In conformity, the hematological assessment (Hb, HCT, RBC count, WBC count, and platelet count) revealed that most parameters remained unaffected by both Tagetes extract and patuletin treatments (Table 1). However, both of them caused a significant decline in the WBC count with the difference that this change became significant with time in the case of extract, while it was significant even upon the acute administration of patuletin. This advocates that the WBC count-reducing action of Tagetes extract was most likely due to the presence of patuletin residing in it. To the best of our knowledge, no report regarding such action of patuletin was found in the literature. However, flavonoids are reported

Table 2. Effect of Tagetes extract and patuletin on biochemical indicators of hepatic and renal function

Duration	Treatment	Dose	Hepatic Indicators		Renal Indicators	
			AST (U/L)	ALT (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
1 day	Control	-	180±6	57±6	15±1	0.15±0.01
	Tagetes extract	1 g/kg	180±5	54±5	15±0.9	0.15±0.01
		2 g/kg	189±14	64±6	15±0.6	0.13±0.01
		5 mg/kg	231±17	54±5	15±0.9	0.2±0.01
	Patuletin	30 mg/kg	241±29	58±7	16±0.6	0.2±0.01
		60 mg/kg	279±14**	63±4	14±0.6	0.2±0.01
14 days	Control	-	142±18	71±10	15±0	0.5±0.08
	Tagetes extract	1 g/kg	137±16	71±2	16±0.6	0.4±0.01
		2 g/kg	132±7	63±7	15±1	0.4±0.02
		5 mg/kg	231±17*	53±5	14±0.6	0.5±0.06
	Patuletin	30 mg/kg	237±32*	57±5	14±0.7	0.7±0.03
		60 mg/kg	245±24**	53±6	15±0.9	0.7±0.03
28 days	Control	-	172±12	84±5	14±0.7	0.4±0.02
	Tagetes extract	1 g/kg	156±16	65±6	13±0.9	0.4±0.03
		2 g/kg	140±13	99±25	12±0.9	0.5±0.03
		5 mg/kg	211±8*	79±11	12±0.3	0.5±0.03
	Patuletin	30 mg/kg	223±6*	73±7	14±0.7	0.5±0.05
		60 mg/kg	258±17***	86±4	12±1	0.5±0.03

Abbreviations: BUN: Blood urea nitrogen; ALT: Alanine transferase; AST: Aspartate transferase.

*P<0.05, **P<0.01, ***P<0.005.

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to decrease the proliferation of immune cells, which in turn contributes to its beneficial action in inflammation, auto-immune disorders, and cancer [19]. Consistent with our results, the ability of *T. patula* flower methanolic extract and patuletin to produce toxicity was also reported earlier [20]. Hence, this outcome warrants further investigations to delineate the potential underlying mechanisms underlying the aforementioned toxicities.

The biochemical investigations revealed that Tagetes extract did not significantly alter any of the hepatic (AST and ALT) and renal (BUN and creatinine) function indicators under study compared to the control (Table 2). However, the patuletin was found to significantly increase the level of AST, the effect which became more obvious over time even at the lowest tested dose of 5 mg/kg. In conformity, the histological examination of patuletin exposed liver also revealed traces of hepatotoxicity (Figure 2). The search of the literature revealed a handful of reports suggesting the hepato-protective potential of flavonoids [21]. However, a report suggests that flavonoids, depending upon dose, can turn into hepatotoxic phytochemicals [22]. However, further work is required to explain the unusual hepatotoxic action of patuletin. On the other hand, the biochemical indicators of renal function as well as histological examination of kidneys did not reveal any remarkable effects. Hence, it can be deduced that patuletin is not harmful to the kidneys.

Conclusion

The present study demonstrates the systemic toxic potential of *T. patula* flower methanolic extract and its active flavonoid patuletin. Hence, their use should be regulated to protect users from detrimental effects.

The primary limitation of the study was the usage of an un-standardized extract of *T. patula*. Furthermore, the present study involves only a single extract type i.e. methanolic, which may not have drawn all constituents from the plant. Additionally, the regulatory guidelines suggest assessing toxicity in at least two species. However, the present study was conducted only in rats. Moreover, the study only addressed systemic toxicity, while other types of toxicity assessment, such as genotoxicity, reproductive toxicity, and carcinogenicity are crucial in understanding the overall safety of test substances.

Future studies will be conducted to address the aforesaid limitations. Additionally, the plant is edible and commonly used therefore; a clinical study will be performed to evaluate the toxicity in humans.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethical Committee of the Institute for the Care and Use of Laboratory Animals, Dow University of Health Sciences, Karachi, Pakistan (IRB- 1121/DUHS/Approval/2018/208).

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Authors' contributions

Conceptualization: Ahsana Dar Farooq; Data curation and writing manuscript: Muhammad Kashif; Formal analysis: Hazar Khan, Asma Khawer, Hafiz Syed Imran ul Haq, Muhammad Aitmaud uddolah Khan, Mudassar Azhar, and Shaheen Faizi.

Conflict of interest

The authors declared no conflict of interest.

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