INTERNATIONAL JOURNAL OF DRUG DEVELOPMENT AND RESEARCH

INT.J.DRUG DEV & RES., SEP-DEC 2009;1(1):161-165

RESEARCH ARTICLE

TOXICOLOGICAL EVALUATION OF CHLOROFORM FRACTION OF FLOWER OF *TAGETES ERECTA* L. ON RATS

*[1]FARJANA NIKKON, [1]M. ROWSHANUL HABIB, [1]ZAHANGIR ALAM SAUD, [1]M. REZAUL KARIM, [2]APURBA KUMAR ROY, [2] SHAHRIAR ZAMAN

[1]Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh.

[2]Department of Genetic Engineering and Biotechnology, Rajshahi University, Rajshahi-6205, Bangladesh.

ABSTRACT

Tagetes erecta Linn. has been used in folk medicine for the cure of eye diseases, colds conjunctivitis, rheumatism, coughs, bleeding piles and ulcers. In addition, in our previous study, we have reported the potent antimicrobial, cytotoxic and insecticidal activities of chloroform fraction of Tagetes erecta flower. However, its other pharmacological effects have not yet been elucidated clearly. The aim of this study was to investigate the subacute toxicity of chloroform fraction prepared from ethanol extract of Tagetes erecta flower by solvent-solvent partitioning method. The subacute toxicity of chloroform fraction was evaluated on Long Evan's rats at 200 and 400 mg/kg doses and the results obtained from chloroform fraction treated rats were compared with untreated controls. Treatment of chloroform fraction at 200 and 400 mg/kg doses, did not make any significant alterations on the hematological and biochemical parameters of rats when data were compared with that of untreated controls. Histopathological examination also showed no detectable changes in liver, kidney, heart and lung of chloroform fraction treated rats. This study revealed that the chloroform fraction of Tagetes erecta had no toxic effects. The results validate the traditional use of this plant in indigenous system of medicine.

KEY WORDS: *Acute toxicity test, Chloroform fraction and Tagetes erecta*

INTRODUCTION

The plant *Tagetes erecta* Linn., locally known as "*Genda Phul*" (Marigold) belongs to the family Asteraceae (Compositae). It is a stout, branching herb, native of Maxico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics including Bangladesh and India[1]. It is very popular as a garden plant and yields a strongly aromatic essential oil (tagetes oil), which is mainly used for the compounding of high-grade perfumes[2]. Different parts of this plant including flower are used in folk medicine to cure various diseases. Leaves are used as anticeptic and in kidney troubles, muscular pain, piles and applied to boils and carbuncles. The flower is useful in fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and is also employed in diseases of the eyes[1, 3]. They are said to purify blood and flower juice is given as a remedy for

*Corresponding Author: e-mail: f.nikkon@gmail.com ;Tel: .+88-0721-751007 Fax:+88-0721-750064

Received on: 15-10-09 ; Accepted on: 28-12-09

bleeding piles and also used in rheumatism, colds and bronchitis[1, 3].

Phytochemical studies of its different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpeniods[4]. The plant *T. erecta* has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallat[3]. Previously in our laboratory we found that the flower of *Tagetes erecta* have antibacterial, antifungal, cytotoxic (against brine shrimp nauplii) and insecticidal activity (against *Tribolium castaneum* and *Culex quinquefasciatus*) and the potency of the chloroform fraction was higher than that of the ethanol extract or petroleum ether fraction of flower of *Tagetes erecta*[5]. These lead us to evaluate the subacute toxicity of the chloroform fraction of the flower of *Tagetes erecta* on rats.

MATERIALS AND METHODS

Plant collection

Fresh flowers of *Tagetes erecta* were collected from the adjoining areas of Rajshahi University Campus, during the month of December to January and taxonomically identified by Professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh, where a voucher specimen (No. J. Sultana 23, collection date 17.01.1994) has been deposited.

Extraction, fractionation and TLC screening

The fresh flowers of *Tagetes erecta* were sun dried for 7 days and finally in an electrical oven below 60^{o}C for 48 hours. The dried plant materials (1 kg) were then extracted in room temperature with ethanol (5.0 *l*). The filtrate was concentrated and fractionated with petroleum ether and chloroform. The solvents were evaporated by rotary evaporator at 40^{o}C under reduced pressure to afford a brownish syrupy suspension of ethanol extract (50.0 g), petroleum ether fraction (18.6 g) and chloroform fraction (23.8 g). All extract/fractions were run on pre-coated silica gel plate using petroleum ether and ethyl acetate (9:1 and 7:5) as the mobile phase and vanillin-H_2SO_4 reagent was used as spray reagent. Ethanol extract of flower gave positive test for glycosides, terpenoids and flavonoids but the chloroform and petroleum ether fractions mainly showed the presence of terpenoids and flavonoids[6].

Animals

Long Evan's rats (104.0-105.5 g) were collected from the Animal Research Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B). The rats were acclimatized in cleaned iron cages for two weeks before treatment. They were fed standard dry pellet diet (Collected from ICDDR,B) and sterilized water, under standard conditions of a 12 hrs dark-light cycle, 60±10% humidity and a temperature of 21.5±2°C. These protocols were approved by the Institutional Animal Care and Use Committee of UNICAMP, which follows the recommendations of the Canadian Council on Animal Care[7].

Determination of median lethal dose (LD_{50})

In order to determine the LD_{50}, Long Evan rats of average weight 104.6 g, were divided into 3 groups (5 animals in each) and chloroform fraction was intraperitoneally administered in group 1, 2 and 3 at 3200, 6400 and 12800 mg/kg doses, respectively. Death was monitored over a period of 24 hrs and LD_{50} was then determined using the method of Lorke[8]. The LD_{50} value for chloroform fraction was found to be 8964.8 mg/kg body weight on rats.

Subacute toxicity studies

A subacute toxicity study was carried out for a period of 14 days[8]. Twenty four (24) healthy Long Evan rats of average weight 105.2 g, were randomly divided into four groups of six animals in each. Rats in group 1 were regarded as untreated control. Rats in group 2 were given (i.p) 0.2 ml of vehicle (5.6 ml distilled water plus 5 drops of Tween-20) for 14 days with 24-hrs intervals and designated as vehicle control. Chloroform fraction was intraperitoneally administered to the rats of group 3 and 4 at 200 and 400 mg/kg body weight, respectively, for 14 days with 24-hrs intervals. A measured amount of fresh

*Corresponding Author: e-mail: f.nikkon@gmail.com ;Tel: .+88-0721-751007 Fax:+88-0721-750064

Received on: 15-10-09 : Accepted on: 28-12-09

food was supplied daily at 10.00 a.m and the general well-being and behavior of the animals were observed daily, throughout the study. For the hematological study, total RBC (red blood cells), total WBC (white blood cells), differential count of WBC (neutrophil, lymphocyte, monocyte, eosinophil), platelet, hemoglobin (%) and ESR (erythrocytes sedimentation rate) were determined by standard procedures[9-12] at the end of the treatment.

The rats were sacrificed after 14 days of the start of the experiment, by chloroform anesthesia and blood was collected from the jugular veins of each of the animals. Serum was separated by centrifugation at 4000 rpm for 10 minutes. Serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SALP), urea, bilirubin and creatinine were determined in an Bioanalyzer (Microlab 200) using commercial kits (Boehringer Mannheim GmbH Diagnostica, UK). Histopathological studies of the liver, kidney, heart and lung were performed using haematoxylin-eosin stain and D. P. X mounting fluid[13]. The samples were examined under a microscope at the Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh.

Statistical analysis

Results are presented as mean ± SD (Standard Deviation). Student's *t*-test was used for comparison between the untreated and treated groups. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Results of this study revealed that, both of the untreated and chloroform fractions (200 and 400 mg/kg) treated rats showed no signs of tremor, convulsion and reflex abnormalities. No muscular numbness of the hind and fore legs, salivation or diarrhoea was observed. Table No. 1 shows the average body weights of the rats before and after the treatment. No significant changes in body weights of treated rats were observed when compared with untreated control groups. The hematological profiles such as total RBC, total WBC, differential count of WBC, platelet, hemoglobin and ESR were examined at the 14th day of treatment. In both untreated and treated rats, almost all hematological parameters were increased insignificantly and no clinical abnormalities were observed at the end of 14th day (Table No. 2). No significant changes of any biochemical parameters such as SGPT, SGOT, SALP, bilirubin, creatinine and blood urea of experimental rats were observed (Table No. 3). Histopathological examinations of liver, heart, lung and kidney of the controls and treated rats were examined after the experimental period and no detectable changes in the histopathology of these organs were observed under microscope (data and photographs are not included).

Table 1:*Effect of chloroform fraction of Tagetes erecta flower on the body weight of rats.*

Group	Treatment	Body weight (g)	
		Before treatment	After treatment
1	-	104.25 ± 0.50	112.75 ± 1.26
2	Vehicle	105.50 ± 0.96	114.5 ± 2.38
3	200 mg/kg	104.00 ± 0.96	113.0 ± 1.41
4	400 mg/kg	105.00 ± 0.50	113.75 ± 1.30

Values are expressed as mean ± SD (Standard Deviation).

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Table No. 2

Effect of chloroform fraction of *Tagetes erecta* flower on hematological parameters of rats.

	Group 1	Group 2	Group 3	Group 4
		Treatment		
Parameters	-	Vehicle	200 mg/kg	400 mg/kg
RBC (Cells mL^{-1} x 10^6)	5.0 ± 0.08	5.05 ± 0.06	5.0 ± 0.16	5.15 ± 0.06
WBC (Cells mL^{-1} x 10^3)	7.02 ± 0.05	7.15 ± 0.12	7.0 ± 0.09	7.50 ± 0.09*
Neutrophil (%)	54.7 ± 2.06	56.75 ± 1.50	50.7 ± 2.06	54.7 ± 1.50
Lymphocyte (%)	31.7 ± 1.26	32.7 ± 0.96	31.8 ± 1.26	32.2 ± 0.96
Monocyte (%)	4.7 ± 0.96	4.0 ± 0.82	4.5 ± 0.96	4.2 ± 0.82
Eosinophil (%)	1.7 ± 0.50	1.7 ± 0.50	1.7 ± 0.50	1.7 ± 0.50
Platelet Cells (Cells mL^{-1} x 10^6)	305.0 ± 1.0	310.0 ± 9.1	305.0 ± 5.7	310.0 ± 9.1
Hemoglobin (%)	13.25 ± 0.50	13.5 ± 0.58	13.2 ± 0.96	13.1 ± 0.31
ESR (mm/1st hour)	13.25 ±1.26	13.75 ± 0.50	13.8 ± 1.29	13.25 ± 1.26

Values are expressed as mean ± SD (Standard Deviation); *P<0.05: against group 1 and ^{t}P<0.05: against group 2.

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed[14]. Interest in medicinal plants as a reemerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs[15].

In this study chloroform fraction of flower of *Tagetes erecta* was used for subacute toxicity test on rats. From the biochemical, hematological and histopathological examinations, it was confirmed that chloroform fractions had no toxic effect on cellular structure, i.e., they do not cause degeneration of the cells of these organs.

CONCLUSION

Chloroform fraction of ethanol extract of *Tagetes erecta* flower was biologically more active than that of ethanol extract or petroleum ether fraction as evaluated in previous experiments[5]. The results of the present study suggest that the chloroform fraction is not acutely toxic to the rats thereby providing a support to the use of *Tagetes erecta* flower in indigenous system of medicine. However, further long-term toxicological studies (chronic toxicity), are needed in order to establish it as medicine.

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Table 3 :*Effect of chloroform fraction of Tagetes erecta flower on biochemical parameters of rats.*

Parameters	Group 1	Group 2	Group 3	Group 4
	Treatment			
	-	Vehicle	200 mg/kg	400 mg/kg
SGPT (IU/L)	12.25 ± 0.50	12.50 ± 0.58	12.85 ± 0.50	12.75 ± 0.50
SGOT (IU/L)	14 ± 0.82	14.25 ± 0.50	14.25 ± 0.17	14.50 ± 0.50
SALP (IU/L)	10.25 ± 0.12	10.25 ± 0.50	10.25 ± 0.14	10.5 ± 0.58
Bilirubin (mmol/dL)	0.37 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
Creatinine (mg/dL)	0.58 ± 0.01	0.59 ± 0.05	0.60 ± 0.03	0.60 ± 0.07
Blood urea (mg/dL)	17.75 ± 0.96	17.25 ± 2.08	17.75 ± 1.50	17.55 ± 1.29

Values are expressed as mean ± SD (Standard Deviation).

AKNOWLEDGEMENTS

The authors are thankful to Department of Pathology, Rajshahi Medical College and Dr. Md. Anwar Habib, Assistant Professor, Dept. of Pathology, Rajshahi Medical College for providing laboratory facilities.

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Source of Support: NIL

Conflict of Interest: NONE

Corresponding Author: e-mail: f.nikkon@gmail.com ;Tel: .+88-0721-751007 Fax:+88-0721-750064

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