International Journal of Animal and Veterinary Advances 6(3): 92-96, 2014
DOI:10.19026/ijava.6.5624
ISSN: 2041-2894; e-ISSN: 2041-2908

Submitted: January 27, 2014 Accepted: February 10, 2014 Published: June 20, 2014

Research Article

Toxicity of *Ruta graveolens* Seeds' Extracts on Male Wistar Rats

[1]Shama, I.Y. Adam, [1]Najla, N.A. Ahmed, [2]A.M. Eltayeb, [2]H. Saad and [2]K.A. Taha
[1]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, Al Neelain University, P.O. Box 12702, Khartoum,
[2]Department of Botany, Omdurman Islamic University, P.O. Box 382, Omdurman, Sudan

Abstract: The multipurpose medicinal plant, *Ruta graveolens*, used widly as a traditional treatment for many diseases. The present study was carried out on Wistar rats to evaluate the toxicity of ethanolic and aqueous extracts of *R. graveolens* seeds at two different doses 50 and 200 mg/kg/day orally for 4 weeks. The rats were allotted to five groups, each of six rats. One group served as control. Two groups were given aqueous extract at 50 and 200 mg/kg/day and other two groups were given methanolic extract at 50 and 200 mg/kg/day orally for 4 weeks. The mortality and weight gain, serobiochemical and hematological parameters were recorded in addition to pathological changes. Study showed that, it has a toxic effects reflected in depression on body weight and alterations in some serum parameters which accompanied with fatty changes and beginning of hemorrhage in liver and kidneys, also alterations in globulins and urea concentration in groups 3 and 5 were observed. Animals that received the lowest concentration treatment suffered no significant hematological alteration. From the current experiment we concluded that toxicity from oral administration of 200 mg/kg/day of *R. graveolen*s seeds extracts for 4 weeks was sever as evidenced by consistent extensive damage to liver and kidney exemplified by necrosis, fatty changes, heomorrhage, congestion and depression in growth. No significant changes were observed for all the measured parameters at concentrations 50 mg/kg/day for 4 weeks administered orally. Consumption of *R. graveolen*s seeds extract at 50 mg/kg/day was not toxic and safe for use.

Keywords: Rats, *Ruta graveolens*, serobiochemical and pathological changes, toxicity

INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases, though relatively little knowledge about their mode of action is available (Ratheesh and Halen, 2007; Patil and Patil, 2011).

Ruta graveolens L. (Rutaceae), locally known as Sazab, is widely distributed in various geographical regions of Sudan and some parts of other Afro Asian countries (El Agraa *et al*., 2002) as a cultivated plant such as ornamental plant (Salvador *et al*., 2009; Tian-Shung *et al*., 2003) and is used in traditional medicine as a remedy for a variety of disorders from cramps to hysteria, helminthoses, edema, hypertension, skin conditions and diseases of the womb (El Agraa *et al*., 2002; Preethi *et al*., 2006; El-Sherbeny *et al*., 2007). It is also used as insect repellent and as antidote for toxins (Freire *et al*., 2010). Antipyretic and diuretic (Tian-Shung *et al*., 2003). Antitumour activity (Preethi *et al*., 2006). Antibacterial, antifungal, anti-parasitic, purgative, antioxidant, hepatoprotective, hypotensive, to improve eyesight, against epilepsy, immune stimulant and anti-inflammatory and for the treatment of the malaria and skin afflictions (De Freitas *et al*., 2005; Issa and Masoud, 2012).

The chemical composition is large and varied. Phytochemical investigations have demonstrated the presence of more than 100 chemical compounds have been found in all parts of the plant, including fats, oils, flavonoids, furoquinolone, alkaloids, Glycosides, essential oils, Terpenoids, Steriods, Sterols, coumarins, Tannins, Phenols, Saponin, pyranocoumarin Cardioglycosides, Carbohydrates, Amino Acids, Protein and others. All parts of the plant contains the active principles, although they are mostly encountered in leaves (Rajeshwari *et al*., 2011; Khare, 2007; Inna *et al*., 2004; Sinshemko *et al*., 2000; De-Feo *et al*., 2002).

There is a lack of information about the toxicity of *Ruta graveolens* seeds to animals. So according to what have been stated above, this study was conducted to determine possible toxic effects of the mentioned plant by investigating the effect of various oral doses of its seeds extract (ethanolic and aqueous) on the growth, biochemical, hematological and pathological

Corresponding Author: Shama, I.Y. Adam, Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, Al Neelain University, P.O. Box 12702, Khartoum, Sudan, Tel.: + 249912617716; Fax: +249 83 771154

characteristics of Wistar rats and subsequently to evaluate whether its ethnopharmacological uses may have possible side effects.

MATERIALS AND METHODS

Plant material: *Ruta graveolens* seeds (Fig. 1) were obtained from a local market in Khartoum (March, 2012), Sudan. The plant seeds were cleaned and coarsely ground through mechanical grinder and the methanol and aqueous extracts were prepared and used in this study.

Preparation of plant extracts: Hundred grams of the coarsely powdered seeds were weighed and subjected to extraction with 500 mL petroleum ether (60-80°C) for 2 h through Soxhlet apparatus, then, the extract was separated from solvent using rotary evaporator, the plant residues were further dried, weighted and extracted with 500 mL methanol (99.8%) for 2 h, then, the extract was separated from solvent using rotary evaporator, the plant residues were further dried, weighted and extracted with 500 mL distilled water over night at room temperature (25-30°C), filtered and further dried by freeze drier (Mudeser, 2004; Culei, 1989).

Experimental design: Thirty male Wistar rats with average body weight ranged from 160 to 165 g were used in this study. The rats were apparently clinically healthy and housed within the premises of El-Neelain University Animals House under standard husbandry conditions and water provided *ad. Libitum.* Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee. The rats were divided randomly to five groups, each of 6 rats. Group 1 continued to be fed the normal diet and used as control, groups 2 and 3 were given ethanol extract of seeds part of plant at 50 and 200 mg/kg/day via the oral route through cathedral tube, respectively. The rats in Groups 4 and 5 were given aqueous extract at 50 and 200 mg/kg/day via the oral route through cathedral tube, respectively. All rats were dosed their designated experimental oral doses for 4 weeks. Average, body weight and body weight gain for each group were recorded. Blood samples were collected at slaughter. At necropsy, all rats were examined to identify gross lesions and specimens of the liver, kidney, heart and intestines were immediately fixed in 10% neutral buffered formalin then stored for histopathology study.

Haematological parameters: The blood samples for measurement of Complete Blood Count (CBC) were collected in EDTA anticoagulant blood containers and examined for Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), White Blood Cells (WBCs) and differential WBCs count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), immediately analyzed through automated Haematology analyzer (Human GambH, max-planck-Ring21, D-65205 wiesbaden, Germany).

Fig. 1: Seeds of *Ruta graveolens*

Serobiochemical parameters: Blood samples for the serobiochemical parameters were collected in heparinized containers and centrifuged immediately at 3500 rpm for 5 min, serum was separated in a new plain sample container labeled according to the study group, rat number, time and date of collection and the parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and concentrations of total protein, albumin, globulin, bilirubin, cholesterol and urea, were analyzed by Roche diagnostic Hitachi 902 analyser (Germany, 1996).

Pathological methods: Necropsy was conducted to study the effects of the plant extracts on the histology of some vital organs, namely, liver, kidneys, heart, spleen and small intestines. After scarifying the rats, specimens of about 1 cm^3 of each organ were immediately fixed in 10% neutral buffered formalin to prevent the post mortem changes. The fixed tissues were dehydrated via transferring them into different concentration of alcohol solutions for 17.5 h in an automated tissue processor, after which the tissues were passed through a solution of xylene. Following clearance in xylene, the sections were then infiltrated and embedded in molten paraffin wax, then sections of 5 µm thickness were cut using a rotary microtome, floated onto clean slides coated with 2% formaldehyde for proper cementing of the sections to the slides and were then stained with Haematoxylin and eosin stains (H and E) (Andrew *et al*., 2008).

Statistical analysis: Statistical Package for Social Science (SPSS) was used for the analysis of the data, the values of body weight and biochemical estimations were expressed as mean±standard error of mean (S.E.M.) and analyzed through hoc Duncan's simple t-test. Differences between groups were considered significant at $p<0.05$ levels (Snedecor and Cochran, 1989).

RESULTS

Growth changes: Body weight and body weight gain of rats given daily oral doses of *R.* graveolens seeds extracts (ethanolic and aqueous) were recorded and the value of Body weight analysis in ethanol extract at 50

Table 1: Body weight and body weight gain in rats orally given *Ruta graveolens* seeds extract for 4 weeks

	Parameters		
Treatment groups	Body weight (g) 0 week	Body weight (g) After 4 weeks	Body weight gain (g) After 4 weeks
1.Control	163.2±5.7	189.8±3.7	26.3±6.2
2.50 mg/kg/day ethanol extract	163.5±4.9	185.0±2.1	21.5±5.1^{NS}
3.200 mg/kg/day ethanol extract	162.0±3.6	182.0±2.6	19.0±4.2*
4.50 mg/kg/day aqueous extract	165.0±5.4	188.5±4.1	23.5±5.2^{NS}
5.200 mg/kg/day aqueous extract	162.5±6.3	180.5±5.2	17.5±3.5*

Values are expressed as mean±S.E; NS = Not Significant; *Significant = ($p<0.05$)

Table 2: Hematological analysis of rats given *Ruta graveolens* ethanolic and aqueous extracts for 4 weeks

	Groups				
Parameters	1.Control	2. *Ruta graveolens* (50 mg/kg/day) ethanolic extract	3. *Ruta graveolens* (200 mg/kg/day) ethanolic extract	4. *Ruta graveolens* (50 mg/kg/day) aqueous extract	5. *Ruta graveolens* (200 mg/kg/day) aqueous extract
Hb (g/dl)	11.5±2.0	11.8±0.4^{NS}	10.0±1.1^{NS}	12.1±0.25^{NS}	10.34±1.3^{NS}
RBCs ($X10^6mm^3$)	6.4±1.2	6.5±0.2^{NS}	4.7±0.5*	7.0 ±0.15^{NS}	5.3±0.7*
PCV (%)	36.7±6.7	36.9±1.7^{NS}	32.7±3.2 *	39.8±1.15 ^{NS}	33.3±5.4*
MCV (m^3)	57.6±0.6	56.8±0.9^{NS}	55.7±0.6^{NS}	56.5±1.92 ^{NS}	56.2±1.7 ^{NS}
MCH (pg)	18.1±0.3	18.2±0.3^{NS}	17.6±0.4^{NS}	17.2±0.54^{NS}	16.8±0.9 ^{NS}
MCHC (%)	31.4±0.4	32.2±0.8^{NS}	31.5±0.5^{NS}	30.5±0.32^{NS}	29.9±1.5^{NS}
WBCs ($X10^3mm^3$)	5.2±2.0	5.3±0.7^{NS}	5.3±1.2^{NS}	4.4 ±0.50^{NS}	3.7±0.6*
Lymphocytes (%)	56.7±9.3	61.1±5.7^{NS}	59.6±2.9N^{S}	52.3±4.82^{NS}	62.7±0.8^{NS}
Neutrophils (%)	43.3±9.3	39.0±5.7^{NS}	40.4±2.9^{NS}	47.7 ±4.82^{NS}	37.3±0.8^{NS}

Value are expressed as mean±S.E; NS = not significant; *Significant = ($p<0.05$)

Table 3: Serobiochemical analysis of rats given *Ruta graveolens* ethanolic and aqueous extracts for 4 weeks

	Groups				
Parameters	1.Control	2. *Ruta graveolens* (50 mg/kg/day) ethanolic extract	3. *Ruta graveolens* (200 mg/kg/day) ethanolic extract	4. *Ruta graveolens* (50 mg/kg/day) aqueous extract	5. *Ruta graveolens* (200 mg/kg/day) aqueous extract
AST(iu)	166.0±9.4	205.1±8.4*	232.7±9.0*	190.2±8.3*	198.3±5.5 *
ALT(iu)	67.3±8.0	67.0±9.4 ^{NS}	93.0±8.4*	61.4±4.8^{NS}	87.4±5.1*
ALP(iu)	218.8±5.3	136.0±8.8*	168.3±8.0*	247.3±6.9*	222.0±5.3^{NS}
Total protein (g/dL)	8.9±0.3	7.9±0.3^{NS}	7.6±0.6^{NS}	8.0±0.4^{NS}	7.8±0.2^{NS}
Albumin (g/dL)	5.0±0.5	4.8±0.1^{NS}	4.7±0.4 ^{NS}	5.0±0.1^{NS}	5.0±0.4^{NS}
Globulin (g/dL)	3.9±0.2	3.1±0.4^{NS}	2.9±0.5 *	3.0±3.3 ^{NS}	2.8±0.4*
Bilirobin (mg/dL)	0.3±0.0	0.3±0.1^{NS}	0.4±0.2 ^{NS}	0.2±0.0^{NS}	0.3±0.0^{NS}
Cholesterol (mg/dL)	66.8±6.2	69.3±4.0^{NS}	72.0±7.7^{NS}	63.0±2.5^{NS}	67.8±5.1^{NS}
Urea (mg/dL)	58.5±2.6	60.5±4.9 ^{NS}	79.8±5.3*	59.8±5.1 ^{NS}	71.8±3.5*

Values are expressed as mean±S.E; NS = not significant; *Significant = ($p<0.05$)

and 200 mg/kg/day (Groups 2 and 3), respectively, aqueous extract at 50 and 200 mg/kg/day (Groups 4 and 5), respectively for 4 weeks are presented in Table 1. The body weight in groups 3 and 5 were depressed compared to control, while groups 2 and 4 showed no significant changes after 4 weeks.

Hematological changes: Hematological changes for rats given daily oral doses of *R. graveolens* seeds extracts ethanolic extract at 50 and 200 mg/kg/day (Groups 2 and 3), respectively and aqueous extract at 50 and 200 mg/kg/day (Groups 4 and 5) respectively for 4 weeks are presented in Table 2. The hematological parameters showed no significant changes among all dosed groups except for groups 3 and 5 for WBCs, RBC and PCV parameters which showed significant decrease ($p<0.05$) when compared with control (G1).

Serobiochemical changes: Serobiochemical changes for rats given daily oral doses of *R graveolens* seeds extracts for 4 weeks are presented in Table 3. The value of AST activity in all groups and ALT, urea concentrations in groups 3 and 5 were significantly increased compared to control (G1). Groups 3 and 5 showed significant decrease ($p<0.05$) in globulin concentrations when compared to control. No significant changes occurred among all groups regarding Total protein, bilirubin and cholesterol concentrations.

DISCUSSION

Utilization of natural products as pharmacological tools could lead to a number of therapeutically active metabolites present in the plants. *Ruta graveolens* has been used ethanomedicinally as a therapeutic agent for a variety of diseases. But there are no available pharmacological records about the toxic effects in this plant (Rita *et al*., 2000; Pulok *et al*., 2010).

This study was aimed to investigate the effects of methanolic and aqueous extracts of *R. graveolens* seeds at concentration 50 and 200 mg/kg/day for 4 weeks on haematological, serobiochemical and histopathological profile in Wistar rats and subsequently to evaluate

whether its ethnopharmacological uses may have possible side effects, which is common with the use of most chemotherapeutic agents.

The body weights of the animals were found to be affected by seed extracts as they lost considerable weight in dose dependant manner when compared to the control group.

Administration of seeds extracts of *R. graveolens* to rats produced fatty change and necrosis in the liver attributed to the increased activity of AST, ALT and the concentration of globulin resulting from in ability of hepatocytes to synthetize albumin insufficiency or over excretion in urine in renal dysfunction. Increases in the activity of enzymes are attributed to the damage in liver. There is no change in total protein and the decrease in body weight is due to malabsorption which resulted from intestinal disquamination or damage in liver. Increase in the concentration of urea is due to inability of renal tubule to excrete waste product in renal insufficiency. The decrease in RBCs and PCV with no change in MCV or MCHC indicated normocytic normochromic anaemia in groups 3 and 5.

The susceptibility of animals fed plant materials seems dependent on the types of active principles in the plant and the rate of their metabolic conversion in the liver and their consequent excretion (Raghav *et al*., 2006., Oliva *et al*., 2003; Gandhi *et al*., 1991).

Reported study of toxicity in 7-8 month old Nubian goats orally administrated at 5 g/kg b.w per day of *R. graveolens* leaves (El Agraa *et al*., 2002) and 10% *R. graveolens* in the diet to Bovans chicks when fed for 2 weeks (Nesreen and EI-Adam, 2007). *R. graveolens* use might cause multi-organ toxicity (Seak and Lin, 2007).

Leaves and their oils are toxic in large doses (over 100 mL of the oil or 120 mg of the leaf) and in this study the seeds are toxic when consumed at dose of 200 mg/kg/day and not toxic and safe for use when used at 50 mg/kg/day for 4 weeks, although Lana and Julai (2014) has reported that, lower doses of rue oil can cause gastroenteritis, salivation, swelling of the tongue, feeble slow pulse and coldness of extremities (Lana and Julai, 2014).

The *R. graveolens* stimulating abrtificent, hepatotoxicity and contradicted in kidney and intestins (Khare, 2007). Moreover, the essential oil have effect on central nervous system lead to depressant and high doses has becomes a narcotic (El-Sherbeny *et al*., 2007).

From these *in vivo* studies, we can conclude that the prepared seeds extracts of *R. graveolens* in the administered doses showed an increase in AST and ALT, activities as well as some pathological changes in liver and kidney which indicate that the plant extracts have the potential to cause toxicity at dose dependant manner. But the exact chemical compound (s) responsible for these effects in the plant extracts still remains speculative, therefore more detailed studies using different doses must be conducted for the assessment of safety and lethal doses. Also we strongly recommend pregnant women to avoid consumption of *R. graveolens* parts for the sake of their babies and their own health.

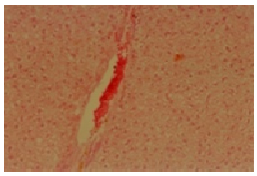

Fig. 2a: Liver of a rat received daily oral doses of *R. graveolens* seeds aqueous extract at 200 mg/kg/day for 4 weeks, showing cytoplasmic vacuolation of the centrilobular hepatocytes and isolated cell necrosis and hemorrhage H&E x100

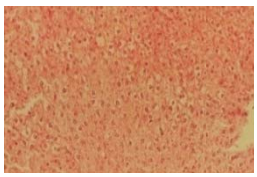

Fig. 2b: Liver of a rat received daily oral doses of *R. graveolens* seeds aqueous extract at 200 mg/kg/day for 4 weeks, showing fatty cytoplasmic vaculation of the entralobular hepatocytes. H&E x100

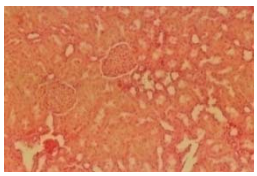

Fig. 3a: Kidney of a rat received daily oral doses of *R. graveolens* seeds ethanolic extract at 200 mg/kg/day for 4 weeks, showing fatty change of the renal tubules in cortex. H&E x100

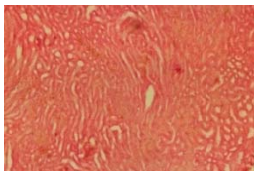

Fig. 3b: Kidney of a rat received daily oral doses of *R. graveolens* seeds ethanolic extract at 200 mg/kg/day for 4 weeks, showing glomerular alteration, fatty change and dilatation H&E x100

Pathological changes: After 4 weeks of treatment with the daily oral doses of *R graveolens* seeds extracts, there were lesions in the liver and kidney of rats given methanolic extract at 200 mg/kg/day (Group 3) and in aqueous extract at 200 mg/kg/day (Group 5), there is fatty cytoplasmic vaculation of the centirlobular hepatocytes, cell necrosis and hemorrhage in the liver of rats 200 mg/kg/day givn aqueous extract (Fig. 2a and b) and glomerular alteration, dilatation and fatty change in the kidney at dose of 200 mg/kg ethanolic extract (Fig. 3a and b).

REFERENCES

Andrew, H.F., K.A. Jacobson, J. Rose and R. Zeller, 2008. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc., pdb.prot4986. DOI: 10.1101/pdb.prot4986.

Culei, J., 1989. Methodology for Analysis of Vegetable Drugs: Practical Manual on the Industrial Utilization of Medicinal and Aromatic Plants. Bucharest Office of the Joint UNIDO, Romania, pp: 2-67.

De-Feo, V., F. De-Simone and F. Senatore, 2002. Potential allelochemicals from the essential oil of *Ruta graveolens*. Phytochemistry, 61: 573-578.

De Freitas, T.G., P.M. Augusto and T. Montanari, 2005. Effect of *Ruta graveolens* L. on pregnant mice. Contraception, 71(1): 74-77.

El Agraa, S.E.I., S.M.A. Elbadwi and S.E.I. Adam, 2002. Preliminary observations on experimental *Ruta graveolens* toxicosis in Nubian goats. Trop. Anim. Health Pro., 34(42): 271-281.

El-Sherbeny, S.E., M.Y. Khalil and M.S. Hussein, 2007. Growth and productivity of *Ruta graveolens* under different foliar fertilizers application. J. Appl. Sci. Res., 3(5): 399-407.

Freire, R.B., H.R. Borba and C.D. Coelho, 2010. *Ruta graveolens* L. toxicity in vampirolepis nana infected mice. India J. Pharmacol., 42(6): 345-350.

Gandhi, M., R. Lal, A. Sankaranarayanan and P.L. Sharma, 1991. Post-coital antifertility action of Ruta graveolens in female rats and hamsters. J. Ethnopharmacol., 34: 49-59.

Inna, K., K. Irina and S. Bernd, 2004. Specific accumulation and revised structures of acridone alkaloid glucosides in the tips of transformed root of *Ruta graveolens*. Phytochemistry, 65(8): 1095-1100.

Issa, G.A. and H.K. Masoud, 2012. Effect of aquatic, methanolic and ethanoliclic extracts of *Ruta graveolens* on some mycotoxigenic fungi. American-Eurasian J. Agric. Environ. Sci., 12(6): 729-732.

Khare, C.P., 2007. Indian Medicinal Plants: An Illustrated Dictionary. Springer, New York, USA.

Lana, D. and W. Julia, 2014. *Ruta Graveolens* in Boston Healing Landscape Project. A Program for the Study of Cultural, Therapeutic and Religious Pluralism. School of Medicine, Boston University, Retrieved from: https:// www.bu.edu/bhlp/Clinical/cross-cultural/herbal_index/herbs/Ruta%20Graveolens.html. (Accessed on: January 5, 2014)

Mudeser, M.H., 2004. Extraction Methods. Medicinal and Aromatic Research Institutes, Khartoum, Sudan.

Nesreen, I.E.S. and S. EI-Adam, 2007. The effect of low levels of dietary *Ruta graveolens* and solenostemma argel or their mixture on bovans chicks. Asian J. Anim. Vet. Adv., 2: 27-31.

Oliva, A., K.M. Meepagala, D.E. Wedge, D. Harries, A.L. Hale, G. Aliotta *et al*., 2003. Natural fungicides from *Ruta graveolens* L. leaves, including a new quinolone alkaloid. J. Agr. Food Chem., 51: 890-6.

Patil, S.J. and S.B. Patil, 2011. Toxicity studies on hepatic, nephric and endocrine organs of *citrus medica* seeds extract on of female albino mice. J. Glob. Pharma Technol., 3(1): 14-21.

Preethi, K.C., K. Girija and K. Ramadasan, 2006. Anti-tumour activity of *Ruta graveolens* extract. Asian Pac. J. Cancer P., 7: 439.

Pulok, K., P. Mukherjee, S. Venkatesh and Ponnusankar, 2010. Ethnopharmacology and integrative medicine: Let the history tell the future. J. Ayurveda Integr. Med., 1(2): 100-109.

Raghav, S.K., B. Gupta, C. Agrawal, K. Goswami and H.R. Das, 2006. Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. J. Ethnopharmacol., 104: 234-239.

Rajeshwari, S., A. Balakrishnan, M. Thenmozhi and R. Venckatesh, 2011. Preliminary phytochemical analysis of *Aegle marmelos*, *Ruta graveolens*, *Opuntia dellini*, *Euphorbia royleana* and *Euphorbia antiquorum*. Int. J. Pharm. Sci. Res., 2(1): 132-136.

Ratheesh, M. and A. Halen, 2007. Anti-inflammatory activity of *Ruta graveolens* L. on carrageen induced paw edema in wistar male rats. Afr. J. Biotechnol., 6(10): 1209-1211.

Rita, Z., R. Andrea, Eduardo and E.B. Argan˜araz, 2000. Perinatal toxicology of *Ruta chalepensi*s (Rutaceae) in mice. J. Ethnopharmacol., 69: 93-98.

Salvador, A.A.S., A.F.P. Maria, M.A.F. Francisco, S.F. Cristina and S.O. Salvio, 2009. Phytophotodermatitis due to *Ruta graveolens* prescribed for fibromyalgia. Med. Rheumatol., 48(11): 1401.

Seak, C.J. and C.C. Lin, 2007. *Ruta graveolens* intoxication. Clin. Toxicol. (Phila), 45(2): 173-175.

Sinshemko, E.E., R. Acosin and J.R. Martinez, 2000. High-resolution gas chromatographic analysis of the secondary metabolites obtained by sub critical fluid extraction from Colombian rue (*Ruta graveolens* L.). J. Biochem. Bioph. Meth., 43: 379-390.

Snedecor, G.W. and W.C. Cochran, 1989. Statistical Methods. 8th Edn., Iowa State University Press, Ames, Iowa.

Tian-Shung, W., S. Li-Shian, W. Jhi- Joung, I. Song-Chou, C. Hsien-Chang, C. Yuh-Pan, K. Yao-Haur, C. Ya-Ling and T. Che-Ming, 2003. Cytotoxic and antiplatelet aggregation principles of *Ruta graveolens*. J. Chinese Chem. Soc., 50(1): 171-178.