academicJournals

Vol. 7(32) pp. 2282-2290, 29 August, 2013
DOI 10.5897/AJPP12.1165
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

*Full Length Research Paper*

# The acute and long-term safety evaluation of aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima*

**M. A. Mandour[1]*, S. A. Al-Shami[1], M. M. Al-Eknah[2], Y. A. Hussein[2] and I. M. El-Ashmawy[3,4]**

[1]Department of Veterinary, Public Health and Animal Husbandry, King Faisal University, Kingdom of Saudi Arabia.
[2]Department of Clinical Studies, College of Veterinary Medicine and Animal Resources, King Faisal University, Kingdom of Saudi Arabia.
[3]Department of Pharmacology, Faculty of Veterinary Medicine, Alexandria University, Egypt.
[3]Department of Veterinary Medicine, Faculty of Agricultural and Veterinary Medicine, Qassim University, Saudi Arabia.

Accepted 30 August, 2013

**This study was designed to explore the safety and side effects of the different extracts (water, ethanolic and methanolic) of *Achillea fragrantissima* given acutely or on repeated doses (125 and 250 mg/kg) in rats. Acute and subchronic toxicity, as well as reproductive (fertility, embryotoxicity and teratogenecity, peri-and postnatal study) effects were recorded on treated and control rats. Daily administration of the plant extract revealed no significant changes on the body weights, heart rates, and other physiological parameters. The plant extract induced a significant increase in total proteins and globulins in rats. It did not induce any abnormal liver and kidney functional changes as demonstrated by serum biochemical analysis in rats. Interestingly, the plant extract induced a significant decrease in alkaline phosphatase (ALP), urea and creatinine. Significant decrease in blood glucose level was detected in animals receiving 250 mg/kg of the extract. The plant extract did not affect fertility. Dosed males showed comparable data with the controls when dosed at 250 mg/kg b.wt. It did not cause any embryotoxic, teratogenic or any deleterious effects on the dosed females and their offspring. Litter size, survival rate and weight gain were comparable between groups. In conclusion, *A. fragrantissima* extract is a well tolerated substance and had a wide safety margin. The tested plant extracts did not induce any toxic effects even on repeated administration in rats for 2 months. Additionally, no evidences of impaired fertility, or teratogenic potentials at higher doses up to several times the recommended maximum human doses were detected.**

**Key words:** *Achillea fragrantissima*, physiological parameters, fertility, embryotoxicity, teratogenicity.

## INTRODUCTION

The use of herbal medicine has become more prevalent, and the past few decades have witnessed a rapidly increasing demand worldwide. The range of medicinal plants is very diverse and it has been estimated that around 70,000 different plant species have been used at least once during the history of traditional medicine

*Corresponding author. E-mail: free2mandour@yahoo.com. Tel: +966543372292.

(Ghasemi, 2002). According to a World Health Organization (WHO) report, around four billion people (80% of the world´s population) use herbal medicine (Ghasemi, 2002), with eleven different bioclimatic regions and around 7,500 different plant species. Therefore, many folk remedies from plant origin are tested for itspotential antioxidant and hepatoprotective liver damage in experimental animal model. Medicinal plant which at least one of its parts contains substances, can be used for therapeutic purposes. *Achillea* is one of the most important genera of the Compositae family and comprises more than 115 species (Saeidnia et al., 2009). Several effects such as anti-inflammatory (Benedek et al., 2007), antihypertensive and anti-hyperlipidemia (Asgary et al., 2000), and antitumoral (Csupor-Löffler et al., 2009) have been reported for *Achillea*. It is widely used in traditional medicine for gastrointestinal disorders, and there are some reports of its effects on gastrointestinal tract such as antispasmotic (Lemmens-Gruber et al., 2006). *Achillea* Sch. Bip. is considered an important medicinal species with high content of essential oils, and is thoroughly studied.

The plant is overexploited by collection for folk medicinal uses, it has the Arabic common name Qaysoom and it is considered endangered in Al-Gouf and Al-Qaseem, Kingdom of Saudi Arabia (KSA). It is used for the treatment of gastro-intestinal disturbances, cough and was reported as carminative, anthelmintic and antiseptic to various infections for the urinary tract (Aboutable et al., 1986; Sincich, 2002). Neither acute nor subchronic toxocity were noticed in mice with ethanolic extracts of *Achillea fragrantissima* (Fleisher and Fleisher, 1993). Moreover, insecticidal and rodenticidal activities of *A*. *fragrantissima* oils were demonstrated by Hifnawy et al. (2001).

The herb contains essential oil (0.81%) and consists of 59 components of which α and β-thujone, α- pinene, β-pinene, limonene, 1,8-cineole, linalool,carvacrol, eugenol, artemisia ketone, palustrol, sabinene hydrate, α-terpineol and santolina alcohol are the major constituents. Its tannin content reaches 8% such as resorcin, phloro-glucin, methyl phloroglucin, and pyrocatechol. Flavonoids were also reported, such as afroside, cirsimartin, chrysoplenol and cirsiliol.

Also, fatty acids, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic as well as sesquiterpene lactones as achilloide A, in addition to taraxasterol and pseudotaraxa-sterol acetates have been identified (Elgamal et al., 1991; Moustafa et al., 1995; Shalaby and Richter, 1964).

A few studies have been published in the literature regarding the properties of *A. fragrantissima* extract in the kingdom of Saudi Arabia. Therefore, the present study was designed to explore the safety and side effects of the different extracts of *A. fragrantissima* given acutely or on repeated doses in rats.

## MATERIALS AND METHODS

### Animals

Fifteen male Sprague Dawley rats 2 to 3 month age (175 to 200 g) were obtained from the closed random bred colony maintained in the animal house at College of Veterinary Medicine and Animal Resources, King Faisal University, Kingdom of Saudi Arabia. The rats were acclimatized for a period of 15 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. They were maintained on food and water *ad libitum* and housed in groups of four in isolated cages. Rats in all groups received humane care in compliance with the animal care guidelines of the National Institute of Health, and the local ethical committee approved this study.

### Plant description and identfication

*Achillea* is one of the most important genera of the Compositae family. Synonyms: *Santolina fragrantissima* Forssk. Common names: Lavender cotton (English); Garda-robe, aurone gemelle, santoline (French); Cypressengarbe (German); Guardaroba, abrotano femmina, santolina (Italian); Qaysūm (Arabic), other vernacular names: quaysūn, baytaram, bu'aythirān. Description: Fragrant chamaephyte, 50 to 100 cm, many-stemmed from a woody base. Leaves small, oblong or ovate, generally more ovate than in the other Syrian species of *Achillea*. Heads discoid, involucre 5 mm, oblong-ovoid. Flowering: May to August. Habitat: Dry areas, steppe, and desert (El-Shatoury et al., 2009). Bedouin name: gaySuum, qaySuum. Status: Not at risk. This is a low shrub with woody older stems; the stems are white, woolly with hairs, the leaves oblong with an undulate margin; there are clusters of small yellow flower heads, and the flowers lack ray florets. The name gives away its intensely fragrant nature. It is a southern Middle East speciality, but is extremely common in Sinai and hence not at risk (Photo by Mike James (2001) Wadi Gebal). Distribution: Arabian peninsula, most rainy districts of Saudi Arabian Kingdom, especially Al-Jouf, South West of Skaka, Ryadah as well as al Qaseem. *A. fragrantissima* were collected at the flowering stage, dried in the shade, and the leaves were separated from the stem and ground in a grinder into fine powder using electric blender.

### Preparation of the crude extracts

Water crude extract was prepared by boiling 100 g of dry powder with 300 ml distilled water for 10 to 15 min, sieved and then the crude extract was evaporated until a paste was obtained and then dried. Solid crude extract was weighted and 10 g dissolved into 100 ml distilled water according to Chaplins'ka and Golovkin (1962).

Ethanolic and methanolic crude extract was prepared by soaking 50 g of dry powder each in 300 ml of each ethanol 95% and methanol 95%, with intermittent shaking till ethanolic and methanolic extracts were obtained, then the crude extract was evaporated until paste was obtained under vacuum using the rotary evaporator. The paste was weighed and diluted in 10 g with 100 ml Tween 80 solution 1% as a solvent.

### Acute toxicity studies

Six Sprague Dawley rat groups (6 rats/group) were administered different single doses (1.00, 2, 4 mg/kg bwt) of *A. fragrantissima* crude extract 125, 250, and 500, respectively. Doses of the extract

and the vehicle at the same volumes were given orally by stomach tube to adult albino rats. Clinical signs, symptoms and mortality were recorded during a 14-days observation period. The $LD_{50}$ values were calculated.

**Subchronic toxicity studies**

These studies were carried out in Sprague Dawley rats (2 to 3 month age). Four groups each of 10 mature male albino rats (160 to 185 g) were used. The drug was given by stomach tube once daily at a volume of 10 ml/kg bwt for a period of 2 months at two dose levels (from each extract), 125 and 250 mg/kg bwt. Meanwhile, rats of the control group were given only the vehicle. The rats were fed with standard feed and provided with water *ad libitum*. During the experiment, all animals were observed daily for general conditions and behavior. Body weight and food consumption in rats were recorded at week intervals.

After 2 weeks, 1 and 2 months from drug administration, rats from each group were anesthetized with light ether, and blood samples were drawn from their retro-orbital plexus before they were killed by decapitation. Two blood samples were collected from each animal. The first sample was collected on disodium salt of ethylene diamine tetra-acetic acid (EDTA) for hematological studies. The second blood sample was collected without anticoagulant for obtaining serum and kept frozen at -20°C until used for biochemical analysis. Hematological parameters [red blood corpuscels (RBCS), white blood corpuscles (WBCS), packed cell volume (PCV), and hemoglobin (HB)] were investigated according to Dacie and Lewis (1984) using Vet scan 5 HM-machine *ABaxis* USA analyzer (2010), and serum analysis for liver and renal functions [serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] activities were measured colourimetrically according to the method described by Reitman and Frankel (1957).

Alkaline phosphatase (ALP) was measured colourimetrically according to the method described by Kind and King (1954). Total protein and albumin levels were measured by the colourimetric method (Doumas et al., 1971), serum globulin level was determined by subtracting the albumin value from total protein value of the same sample as described by Coles (1974). Serum urea activity was measured by the enzymatic colourimetric method as described by Coulomb and Farreau (1963). Serum creatinine activity was measured by the colourimetric kinetic method as described by Husdan and Rapoport (1968), gamma glutamyl transferase (GGT), glucose and cholesterol by Tietz (1986) were recorded using commercial kits. Kits for all biochemical studies were obtained from BioSystems S.A. Costa Brava 30, Barcelona (Spain). At the end of the experiment (after 2 months), animals were weighed and anesthetized with ether for blood collection. Gross, pathologic changes, and weights of several organs were also recorded.

**Reproductive studies**

***Study of fertility***

A fertility study was carried out in 80 male and female Sprague Dawley rats. The extract was given at the dose of 250 mg/kg bwt. once daily to males and females, for respectively 35 and 14 days prior to mating. Dosed males and females were each mated with non dosed counterparts.

Dosed female rats were further treated throughout the gestation period. Control rats received the vehicle only. On day 20 of pregnancy the female rats were sacrificed and fetuses were delivered by caesarean section for further examination.

***Study of embryotoxicity and teratogenecity***

Three groups of 10 female Sprague Dawley rats each received the tested extracts at doses of 0 and 250 mg/kg bwt twice daily from day 6 to day 15 of pregnancy. Rats were sacrificed on day 20 of pregnancy. Fetuses were delivered by caesarean section. Fetal skeleton and visceral organs were examined. Each fetus was individually identified, weighed, sexed, and given a gross examination for external malformations/variations including observation for palatal defects. Approximately one-half of the fetuses in each litter were evaluated for visceral malformations/variations (Staples, 1974). The fetuses selected for visceral examination were injected abdominally with 0.2 ml Bouin's solution and then placed in Bouin's fixative for overnight, then turned to formalin solution 10% and subsequently sectioned and examined (Wilson, 1965). Whereas, the other fetuses were eviscerated and processed; the ossified skeletal structures were stained with alizarin red S and the cartilagenus parts were stained with alcian blue stain (Dawson, 1976; El-Ashmawy et al., 2011).

***Peri-and postnatal study***

In the peri-and postnatal study, 10 pregnant Sprague Dawley rats received the plant extracts at the dose 250 mg/kg bwt once daily. Dosing was started from day 16 of gestation and continued throughout the 3-week-lactation period. Ten other pregnant rats were used as controls. Observations on the offspring were made at birth and at day 4, 14 and 21 after birth.

**Statistical analyses**

Data were analyzed by the general linear model (GLM) procedure (SAS, 2004). The least square mean (LSM) + standard errors were calculated and tested for significance using the "t" test (Steel and Torrie, 1960).

## RESULTS

### Acute toxicity studies

The plant extract overdosing was tolerated in rats, up to 3 g /kg bwt, resulting in no fatality, or any signs of toxicity or change in behavior over 14 days following its administration by gavage.

### Subchronic toxicity studies

Daily administration of the plant extract by gavage daily at doses of 125 and 250 mg/kg in rats, for 2 months revealed no significant changes on the body weights, heart rates, and other physiological parameters and revealed no histological alterations in different organs, and the following results were obtained:

1. The plant extract did not induce any significant adverse changes in blood hematological parameters in rats (Tables 1 and 2).

**Table 1.** Hematological parameters of rats given aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima* by gavage once daily for 2 months.

| Treatment | Parameter | | |
|---|---|---|---|
| | Hb (g/dl) | PCV% | RBCS × $10^6$/Cmm |
| Control (vehicle) | 11.9±0.32 | 40.0±0.61 | 6.02±0.25 |
| **Aqueous extract (250 mg/kg)** | | | |
| 2nd week | 11.6±0.40 | 40.2±0.60 | 6.15±0.21 |
| 1st month | 12.0±0.68 | 40.4±1.41 | 6.2±0.36 |
| 2nd month | 11.8±0.56 | 40.8±1.31 | 6.28±0.44 |
| **Methanolic extract (250 mg/kg)** | | | |
| 2nd week | 12.0±0.56 | 40.0±1.36 | 5.88±0.34 |
| 1st month | 12.2±0.56 | 41.0±2.0 | 5.9±0.33 |
| 2nd month | 11.4±0.57 | 41.6±2.39 | 6.4±0.25 |
| **Ethanolic extract (250 mg/kg)** | | | |
| 2nd week | 12.2±0.64 | 40.1±1.15 | 6.3±0.54 |
| 1st month | 11.5±0.52 | 40.0±1.05 | 5.86±0.36 |
| 2nd month | 11.8±0.88 | 39.2±0.9 | 6.3±0.27 |

Values are mean ± SEN = 6 animals.

**Table 2.** Hematological and biochemical parameters of rats given aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima* by gavage once daily for 2 months.

| Treatment | Parameter | | |
|---|---|---|---|
| | WBCS × $10^3$/Cmm | Glucose (mg/dl) | Cholesterol (mg/dl) |
| Control (vehicle) | 7.1±0.24 | 66.6±2.09 | 59.6±1.29 |
| **Aqueous extract (250 mg/kg)** | | | |
| 2nd week | 7.2±0.31 | 65.2±1.16 | 59.8±1.59 |
| 1st month | 6.92±0.49 | 66.2±1.93 | 60.6±1.29 |
| 2nd month | 6.8±0.37 | 63.0±3.04 | 56.8±2.78 |
| **Methanolic extract (250 mg/kg)** | | | |
| 2nd week | 6.80±0.50 | 59.0±2.12 | 57.4±1.89 |
| 1st month | 7.00±0.44 | 58.8±2.25 | 51.8±1.07* |
| 2nd month | 6.6±0.51 | 57.6±2.62 | 49.2±1.07* |
| **Ethanolic extract (250 mg/kg)** | | | |
| 2nd week | 7.1±0.45 | 56.4±2.06 | 53.8±2.54 |
| 1st month | 6.8±0.37 | 57.2±2.96 | 51.0±1.52* |
| 2nd month | 6.4±0.51 | 53.6±2.38* | 49.6±1.03* |

Values are mean ± SEN = 6 animals. *Significantly different compared to control ($P < 0.05$).

2. The plant extract induced a significant increase in total proteins and globulins in rats (Table 3), yet it did not exceed the normal reference range in all animals.
3. The plant extract did not induce any abnormal liver and

**Table 3.** Serum protein profile of rats given aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima* by gavage once daily for 2 months.

| Treatment | Parameter | | |
|---|---|---|---|
| | Protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) |
| Control (vehicle) | 5.08±0.24 | 3.26±0.27 | 1.82±0.15 |
| **Aqueous extract (250 mg/kg)** | | | |
| 2nd week | 5.12±0.12 | 3.2±0.14 | 1.92±0.08 |
| 1st month | 5.2±0.22 | 3.28±0.18 | 1.92±0.08 |
| 2nd month | 5.28±0.21 | 3.5±0.22 | 1.78±0.19 |
| **Methanolic extract (250 mg/kg)** | | | |
| 2nd week | 5.64±0.27 | 3.66±0.29 | 1.98±0.02 |
| 1st month | 5.48±0.33 | 3.24±0.44 | 2.24±0.24* |
| 2nd month | 5.48±0.21 | 2.88±0.41 | 2.6±0.24* |
| **Ethanolic extract (250 mg/kg)** | | | |
| 2nd week | 5.52±0.26 | 3.34±0.23 | 2.18±0.22 |
| 1st month | 6.0±0.27* | 3.0±0.27 | 3.0±0.31* |
| 2nd month | 5.7±0.37* | 3.0±0.31 | 2.7±0.20* |

Values are mean ± S.E. N = 6 animals.*Significantly different compared to control ($P < 0.05$).

kidney functional changes as demonstrated by serum biochemical analysis in rats. Interestingly, the plant extract induced a significant decrease in ALP, urea & creatinine in rats (Tables 3 to 5).

4. Although no significant change in blood glucose level was observed in animals receiving the plant extract at the level of 125 g/kg, significant decrease in blood glucose level occurred in animals receiving 250 mg/kg (Table 2).

5. Light microscopic examination of the different organs in rats revealed no significant alterations as compared to the control animals.

## Reproductive studies

### *Fertility study in rats*

The plant extract did not affect fertility. Dosed males showed comparable data with the controls when dosed at 250 mg/kg bwt (Table 6).

### *Embryo toxicity and teratogenicity study in rats*

The plant extract did not cause any embryotoxic or teratogenic effect (Table 6 and Figure 1).

### *Peri- and postnatal study*

The plant extract did not cause any deleterious effects on the dosed females and their offspring. Litter size, survival rate and weight gain were comparable between groups (Table 7).

## DISCUSSION

The data concerning the effect of the *A. fragrantissima* extract in rats revealed no significant changes on the body weights, heart rates, and other physiological parameters and revealed no histological alterations in different organs. In the present investigation, it was observed that the medicinal plant treatment did not cause significant reduction in the rat body weight when compared to control. This shows the absence of toxic side effect of the plant in the tested animals. The same result has been found in the administration of *Alstonia scholaris* bark extract (Gupta et al., 2002), *Strychnos potatorum* seed extract (Gupta et al., 2006), *Tuniperus phoenica* (Shkukani et al., 2007) to male rats and *Achillea millefolium* flower extract (Montanari et al., 1998) to male mice.

Blood hematology picture, PCV, and Hb did not show

**Table 4.** Effect of administration of aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima* by gavage once daily for 2 months on serum AST, ALT, and ALP in rats.

| Treatment | Parameter | | |
|---|---|---|---|
| | AST (U/L) | ALT (U/L) | ALP (U/L) |
| Control (vehicle) | 50.2±1.56 | 19.2±0.58 | 81.4±1.12 |
| **Aqueous extract (250 mg/kg)** | | | |
| 2nd week | 49.4±2.36 | 16.6±0.98 | 83.0±1.52 |
| 1st month | 47.8±2.71 | 16.6±0.92 | 82.4±1.60 |
| 2nd month | 46.8±2.01 | 16. 6±0.46 | 81.2±1.85 |
| **Methanolic extract (250 mg/kg)** | | | |
| 2nd week | 48.0±0.44 | 18.0±0.71 | 80.6±2.16 |
| 1st month | 46.4±2.71 | 17.4±0.68 | 76.2±2.44 |
| 2nd month | 45.0±2.10 | 16.8±1.16 | 73.6±2.73* |
| **Ethanolic extract (250 mg/kg)** | | | |
| 2nd week | 45.6±3.06 | 17.4±0.60 | 79.2±1.46 |
| 1st month | 44.6±2.93 | 14.0±0.71* | 74.4±2.32 |
| 2nd month | 44.6±2.93 | 14.0±0.71* | 71.6±2.91* |

Values are mean ± S.E. N = 6 animals. *Significantly different compared to control ($P < 0.05$).

**Table 5.** Effect of administration of aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima* by gavage once daily for 2 months on serum GGT, urea and creatinine in rats.

| Treatment | Parameter | | |
|---|---|---|---|
| | GGT (U/L) | Urea (mg/dl) | Creatinine (mg/dl) |
| Control (vehicle) | 12.0±0.71 | 19.2±0.67 | 0.56±0.05 |
| **Aqueous extract (250 mg/kg)** | | | |
| 2nd week | 11.8±0.86 | 19.8±1.07 | 0.58±0.07 |
| 1st month | 11.2±1.16 | 19.4±1.75 | 0.56±0.05 |
| 2nd month | 12.5±1.07 | 18.6±2.07 | 0.58±0.06 |
| **Methanolic extract (250 mg/kg)** | | | |
| 2nd week | 12.2±0.73 | 19.0±0.89 | 0.58±0.03 |
| 1st month | 12.2±0.80 | 19.0±1.23 | 0.54±0.03 |
| 2nd month | 11.4±0.98 | 15.2±1.07* | 0.58±0.03 |
| **Ethanolic extract (250 mg/kg)** | | | |
| 2nd week | 12.6±0.81 | 17.6±2.32 | 0.58±0.08 |
| 1st month | 12.2±1.24 | 16.2±1.80 | 0.58±0.08 |
| 2nd month | 11.0±0.98 | 15.6±0.93* | 0.40±0.04* |

Values are mean ± S.E. N = 6 animals. *Significantly different compared to control $p < 0.05$.

**Table 6.** Effect of the administration of aqueous extract of *Achillea fragrantissima* by gavage on fertility in rats.

| Parameter | Dosage group (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | | 250 | |
| Adult rat data | C-M + T-F | T-M + C-F | C-M + T-F | T-M + C-F |
| No. of treated males (n) | 0 | 10 | 0 | 10 |
| No. of treated females (n) | 10 | 0 | 10 | 0 |
| No. of pregnant rats (n) | 8 | 9 | 9 | 9 |
| No. of surviving females (n) | 10 | 10 | 10 | 10 |
| Fertility index (%) | 80 | 90 | 90 | 90 |
| **Litter data** | | | | |
| Average no. of implantations/No. of pregnancies | 8.9 | 9.1 | 8.8 | 9.2 |
| No. of alive fetuses/No. of implantations (%) | 99.2 | 98.4 | 98.0 | 99.0 |
| No. of resorbed fetuses/No. of implantations (%) | 1.5 | 1.2 | 1.2 | 1.3 |
| Average weight at birth (g) | 5.5 | 5.2 | 5.6 | 5.4 |
| Abnormalities (%) | - | - | - | - |

C = Control, M = male, T = treated, F = female.

**Table 7.** Effect of the administration of ethanolic extract of *Achillea fragrantissima* by gavage on the development and viability of first generation ($F_1$) pups during the pre-weaning period in rats.

| Parameter | Dosage group (mg/kg) | |
|---|---|---|
| | 0 | 250 |
| **Mean number of pups/litter** | | |
| Born | 8.5±0.3 | 8.6±0.4 |
| Born alive | 8.0±0.4 | 8.4±0.3 |
| Sex ratio (males) | 51.0±2.4 | 49.5±3.0 |
| **Mean pup weight (g)/litter** | | |
| Postnatal day O (PND) males | 5.4±0.2 | 5.3±0.1 |
| Females | 5.1±0.1 | 5.6±0.1 |
| PND 4 males | 9.0±0.4 | 8.5±0.2 |
| Females | 8.3±0.1 | 8.2±0.1 |
| PND 14 males | 29.5±0.9 | 30.0±0.8 |
| Females | 28.2±0.7 | 28.5±0.6 |
| PND 21 males | 47.2±1.1 | 48.2±1.1 |
| Females | 43.7±1.4 | 44.0±2.3 |
| $\text{Survival (\%)} = \frac{\text{No. of pups alive on PND 21}}{\text{No. of pups alive on PND 0}} \times 100$ | 95.0±4.2 | 97.3±3.2 |

Values are mean ± SEN = 10 animals.

significance difference between all treated groups when compared with the control. Therefore, the extract of *A. fragrantissima* may not have had any adverse effect on the bone marrow, liver, kidney and haemoglobin

metabolism, since the value of red blood cells are notaffected (Young and Maciejewski, 1997). Similar results were demonstrated by Emadi et al. (2007) and Basavaraj et al. (2011) in the hematological picture of animals and birds.

Serum AST and ALT being the most sensitive hepatic markers employed in the diagnosis of hepatic damage (Sallie et al., 1991) were not significantly different between the treated and control group. Previous study has shown extraction of medicinal plants to be an effective antioxidant under *in vitro* conditions. Rasekh et al. (2001) have demonstrated no changes in these enzymes in male rats, and El-Bagir et al., (2010) indicated that medicinal plants were safe to be included in rat diet as reflected on the above unchanged liver and kidney function biomarkers. Moreover, the non significant change in the levels of AST and ALT is suggestive of the fact that decoction is successful in quenching the free radicals inhibiting lipid peroxidation and protecting the membrane lipids from oxidative damage in the liver of rats (Suboh et al., 2004).

Although no significant changes in blood glucose and cholesterol levels were observed in animals receiving the plant extract at the level of 125 g/kg, significant decrease in blood glucose level occurred in animals receiving 250 mg/kg. A finding that would be helpful while studying cardiovascular diseases such as arteriosclerosis that are caused as a result of hyperlipidemia elevate mortality% (Francle, 1995); so reducing serum hyperlipidemia is very important. A 1% reduction in serum cholesterol concentration results in a 2% reduction in the prevalence of coronary artery diseases (Francle, 1995).

In addition, present investigation demonstrated that oral administration of *Achilea millefolium* extract at different doses caused no significant change in fertility parameters in male rats. The animal model used in this work has previously been with minor changes used by several researchers to assess the effect of various extracts obtained from medical plants on reproductive functions in males (Yinusa et al., 2005; Parandin et al., 2008). The present results disagree with the previous studies that *A. millefolium* (200 mg/kg/day intraperitoneally for 20 days) and different variety of *Achillea santolina* (300 mg/kg intrapertonealy for 20 days) have an antispermatogenic and degenerative changes on mice testes (Montanari et al., 1998; Golalipour et al., 2004).

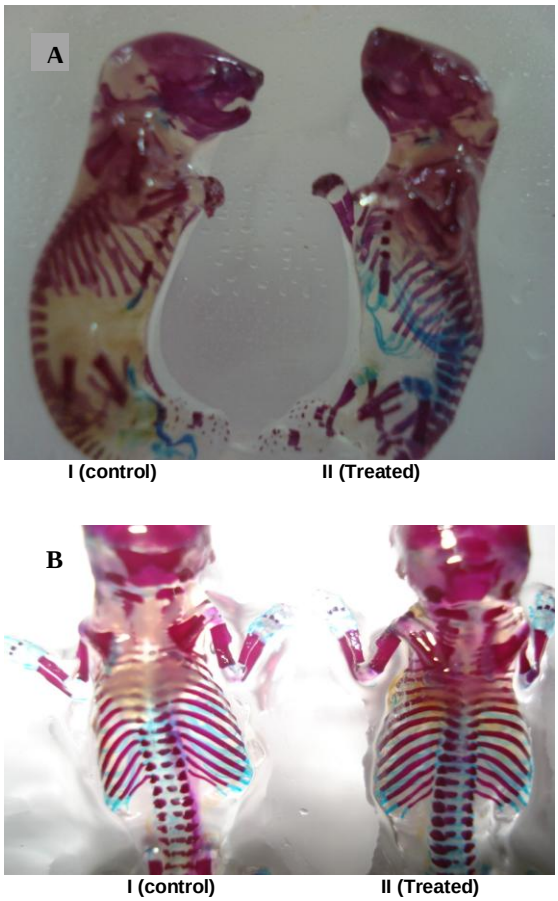


**Figure 1.** Effect of the administration of ethanolic extract of *Achillea fragrantissima* by gavage once daily from day 6 to day 15 of pregnancy on the fetal skeleton and cartilage development. (A) Normal ossifications of sternebrae and skull; (B) normal cartilage development ossifications of skull, vertebrae ,ribs and absence of fused ribs.

## Conclusion

The study showed that the tested material named *A. fragrantissima* extract is a well tolerated substance and had a wide safety margin as demonstrated from studies performed in this report. The study also confirmed that the tested plant extracts did not induce any toxic effects even on repeated administration in rats for 2 months.

Additionally, the study confirmed that *A. fragrantissima* extract had no evidences of impaired fertility or teratogenic potentials at higher doses up to several times the recommended maximum human doses.

## ACKNOWLEDGEMENT

This project was financially supported by Deanship of Scientific Research, King Faisal University, Ministry of Higher Education, Kingdom of Saudi Arabia.

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