

## Research Article



## Acute Toxicity of *Vitex agnus castus* Methanol Extract

Hayder B Sahib<sup>1\*</sup>, Adeeb A. AL-Zubaudy<sup>1</sup>, Shallal M. Hussain<sup>2</sup>, Ghaith Ali Jasim<sup>3</sup>, Ban J Qasim<sup>4</sup>, sawsan S. Al Rawi<sup>5</sup>

<sup>1</sup>Al-Nahrain University/ College of Medicine/ Pharmacology Department.

<sup>2</sup>Al-Mustansiriya University- Iraqi Centre for cancer and medical research.

<sup>3</sup>Al-Mustansiriya University / College of Pharmacy/ Pharmacology Department.

<sup>4</sup>Al-Nahrain University/ College of Medicine/ Pathology Department.

<sup>5</sup>University Sains Malaysia/ School of Pharmacy/ Pharmacology Department.

\*Corresponding author's E-mail: [haider\\_bahaa@yahoo.com](mailto:haider_bahaa@yahoo.com)

Accepted on: 27-03-2014; Finalized on: 31-05-2014.

### ABSTRACT

*Vitex agnus castus* widely use worldwide and traditionally used in Iraq for gynaecological issues. Extensive search has been done on this herb revealed that this herb has potential anti-angiogenesis activity. The objective of this study is to find the extent of herb safety, to be formulated later; and use by people as an anti angiogenic drug. Methanol extracts which showed potential anti-angiogenic activity has been tested against two animal species mice and rats for testing its safety. Toxicity of the extract was evaluated in Swiss albino mice by feeding the animals with serial doses of the extract between 1.0 to 20.0 g/kg body weight orally and observed continuously for the first 4 h and every hourly for the next 24 h, then 6 hourly for 48 h (72 h, acute toxicity) results collected after two weeks. Rats were also fed with 5000mg/kg extract The toxicity in the animals was carried out by assessing the effects on biochemical parameters, body weight and histopathological study for liver, heart, lung, stomach, spleen, sex organs for both male and female, and renal organs following oral administration of methanol extract. The median acute toxicity value (LD<sub>50</sub>) of *Vitex agnus castus leaves methanol* extract was found to be 17.21g/kg body weight. The extract reduced cholesterol level significantly. Other biochemical finding show no significant different in comparison to control. The body weight was observed in all the groups treated with the extract. No significant weight changes occur throughout the study. The LD<sub>50</sub> value indicated the drug to be quite safe in one dose treatment. The study also showed that the extract had good hypolipidemic effects.

**Keywords:** Acute toxicity of Herbs, Anti angiogenic herb, Lethal dose for *Vitex agnus castus*, *Vitex agnus castus*.

### INTRODUCTION

**V**itex agnus-castus Known as chasteberry or emmenagogue herb. It is widely known to cause homeopathic regulation of female endocrine system to ameliorate symptoms of post menopause sign (PMS), amenorrhea, and infertility, these pharmacological action are due to estrogenic effects of phytoestrogens present in *Vitex*.<sup>1</sup> This herb also blocks the production of prolactin from pituitary, shortening luteal phase and antagonizing hormonal imbalance (low progesterone synthesis in luteal phase).<sup>2</sup> Moreover, *Vitex agnus castus* induce cell death, (tumoricidal) action is established in ovarian, cervical, breast, gastric, colon, lung cancer cells *via* oxidative stress related induction of pro-apoptotic caspase 3,8,9 hydroxy oxidase (a reduction in BCL-2, BCL-XL and Bid protein, increased Bad gene expression and induced DNA fragmentation).<sup>3</sup> However, it showed side effects of nausea, headache, and rashes. The herb is approved by German Commission E with warnings of chaste tree fruit with irregularities of menstrual cycle; mastodynia under the doses of 30-40 mg per day in aqueous-alcoholic extracts.<sup>4</sup>

Angiogenesis processes can be described as developmental or disease-associated, although both types share many mechanistic features.<sup>5</sup> The differences might only be related to their regulatory control.<sup>6</sup> The aim of this study is to investigate the safety of methanol extract of *Vitex agnus castus* leaves, which showed the

highest anti-angiogenic activity in previous study done by Hayder et al.<sup>7</sup> This study will be used as a mean to identify its suitability for human administration.

### MATERIALS AND METHODS

#### Acute toxicity

The toxicity study was carried out using thirty-five (35) male and female Swiss albino mice weighing 20 – 25 g each. The animals were randomly distributed into one control group and six treated groups, containing five animals per group, provided with water and food and were allowed to adapt to the laboratory conditions for seven days before the experiment. After depriving the animals' food overnight, the control group received 0.3 ml of 2% Tween 80 solution orally while each treated group received orally the *Vitex agnus castus* methanol extract; prepared by dispersing 8.0 g in 10 ml volume of 2% Tween 80 in the doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the extract, to observe any death or changes in general behavior and other physiological activities the experiment last for fourteen days.<sup>8,9</sup> The study has been approved by the animal ethics committee of al Nahrain University/ college of Medicine/ Iraq.



### Acute toxicity study

The acute oral toxicity was evaluated following the World Health Organization (WHO) guideline.<sup>10</sup> Briefly, rats were divided into two groups of ten (five males, five females). The treated group was orally given the aqueous extract in a single dose of 5,000 mg/kg body weight, while the control group received only water vehicle. The animals were monitored for apparent signs of toxicity for 14 days. The animals that died within this period were subjected to necropsies. All rats were weighted and sacrificed on the 15th day after administration, and then the vital organs including heart, lungs, livers, kidneys, spleen, stomach and sex organs were grossly and histopathological examined.

### Experimental design and analysis of data

The experiment design used for this study was Rationalized Complete Block Design (RCBD). The results were reported as means  $\pm$  standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey test comparison t-test (2-tailed) was used to compare between treatments groups. The differences between the means are considered significant at the 5% confidence level. The statistical analysis was carried out by using SPSS 16.0, the level of significance was set at  $P < 0.05$ .

### RESULTS

#### LD<sub>50</sub> Identification for the methanol leaves extract of *Vitex agnus castus*

Tables 1 and 2 Show the number of dead and survive mice after 14 days of experiment for female and male mice respectively. Serial concentrations of methanol extract have been administered for each group of mice. Figure 1 shows the LD<sub>50</sub> which represent the dose required to kill fifty percent of mice.

**Table 1:** The numbers of groups, dose administered and numbers of death between female mice and percentage of death

Group	No. Mice	Dose of Extract g/kg	No. of Dead Mice's	% Cumulative of Dead Mice's
1	5	20	3	60
2	5	15	2	40
3	5	10	1	20
4	5	5	0	0
5	5	2.5	0	0
6	5	1	0	0
7	5	control	0	0

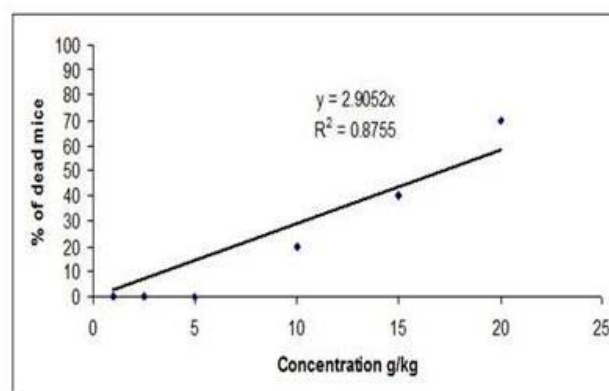
#### Acute toxicity study for vitex agnus castus methanol leaves on rats

After the rats were orally given a single dose of the methanol extract from the dried leaves of *Vitex agnus castus* at 5,000 mg/kg, neither signs of toxicity nor death of rats were observed during 14 days of the acute toxicity experimental period. The alterations of body weight gain

and organ weights from the control would reflect the toxicity of the substance, there was no significant difference in organ weight between treated and untreated (control) animals with the absence of any morphological changes. Table 3 showed the body weight gain changes during the experiment time for male and female respectively. Data expressed as mean  $\pm$  SD, each group has five rats. The weigh for male and female were measured every seven days. Table 4 and 5 showed the internal organs weight for male and female rats respectively. Figure 2 showed the histopathology examinations further confirmed that the extract did not cause any tissue damage. The internal organs revealed no histo-pathological abnormality relative to the control. Table 6 showed the changes in blood serum profile for the rats in comparison to their control. There were no significant differences between groups (male and female) relative to their control groups ( $P > 0.05$ ) except for that of cholesterol, there were a significant decrease in the lipid levels in comparison to their control ( $P < 0.05$ ).

**Table 2:** The numbers of groups, dose administered and numbers of death between male mice and percentage of death

Group	No. Mice	Dose of Extracts g/kg	No. of Dead Mice	% Cumulative of Dead Mice
1	5	20	4	80
2	5	15	2	40
3	5	10	1	20
4	5	5	0	0
5	5	2.5	0	0
6	5	1	0	0
7	5	control	0	0



**Figure 1:** The LD<sub>50</sub> of the methanol leaves extract on mice. The LD<sub>50</sub> calculated through the equation  $Y = 2.9052x$  and it was 17.21g/kg

Tissue sections were prepared and stained with hematoxylin and eosin. Images were digitally acquired using a 20 $\times$  objective. (A) Lung of rat exposed to the extract displaying no capillary congestion. No necrosis of alveolar epithelial cells. No interstitial or intra-alveolar edema or hemorrhage. No interstitial inflammatory cell infiltration. No hyaline membranes lining the alveolar ducts (B) Lung from vehicle control. (C) Kidney tissue

exposed to the extract no interstitial inflammatory cell infiltrate or fibrosis. No tubular dilation contraction or atrophy of their lining epithelium. No tubular thyroidization or neutrophils. No vascular changes. Normal glomeruli (D), The Kidney control. (E) Heart exposed to the extract no interstitial or perivascular inflammatory cell infiltrate. No myocyte injury or necrosis. (F) Heart control. (G) Liver received the extract showed no hepatocyte hydropic degeneration, fatty change, degeneration or necrosis. No interstitial inflammation or fibrosis. (H) was Liver control. (I) Stomach tissue which receive the extract showed no hemorrhage, edema no acute or chronic inflammatory cell infiltrate. No erosion or ulceration. (J) Stomach tissue

control. (K) Spleen tissue treated with the extract showed normal white and red bulb, no congestion of splenic cords, no reactive follicle hyperplasia, no neutrophil infiltration in the red bulb, no fibrosis. (L) The spleen tissue control. (M) Testes tissue received the extract and showed Normal Density of the seminiferous tubules in the section. Normal spermatogenesis, no tubular atrophy. No hyperplasia of Leydig cells. No thickening of Basement membrane of the seminiferous tubules. (N) Testes tissue control. (O) showed the ovary tissue treated with the extract Normal stages of maturation of vesicular follicles no changes in the ovarian stroma. (P) The image showed the ovary tissue control which receives the vehicle used to dissolve the extract with in preparing the 5000mg/kg.

**Table 3:** The weight of the animals (male and female) during the 14 days of experiments, the data showed no significant Weight reduction in animals ( $P>0.05$ ). The changes in weight represented by mean  $\pm$  SD. Each group has five animals (M, F, CM, CF represent Male, Female, control male and control female respectively).

Day Zero				Day 7				Day 14			
M	F	CM	CF	M	F	CM	CF	M	F	CM	CF
155	180	200	216	185	200	200	216	255	185	250	255
150	190	220	157	164	172	220	157	190	227	280	235
185	165	190	226	200	215	190	226	200	230	200	160
200	190	185	180	190	205	185	180	185	205	190	155
190	220	225	190	185	185	225	190	168	170	220	200
M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD	M $\pm$ SD
176 $\pm$ 22.1	189 $\pm$ 20.1	204 $\pm$ 17.8	193.8 $\pm$ 27.7	184.7 $\pm$ 15.1	198 $\pm$ 18.4	198.7 $\pm$ 15.4	194.7 $\pm$ 31.9	207.5 $\pm$ 32.2	211.7 $\pm$ 21	230 $\pm$ 42.4	201.2 $\pm$ 51.2

**Table 4:** Weight (g) for the internal organs for male group and their control Albino rats after 15 days of acute toxicity experiment. There were no significant differences between internal organ weight of male and their control ( $P>0.05$ ).

Internal organ	Male Rat	Control Male rat
Heart	1.05 $\pm$ 0.11	1.382 $\pm$ 0.03
Lung	2.42 $\pm$ 0.15	2.39 $\pm$ 0.4
Liver	8.5 $\pm$ 0.86	10.3 $\pm$ 1
Kidney	1.7 $\pm$ 0.31	2.16 $\pm$ 0.07
Stomach	2.06 $\pm$ 0.32	1.58 $\pm$ 0.3
Spleen	1.19 $\pm$ 0.05	1.94 $\pm$ 0.4
Intestine	3.7 $\pm$ 0.24	3.76 $\pm$ 0.1
Sex organs	4.82 $\pm$ 0.65	4.24 $\pm$ 0.5

**Table 5:** Weight (g) for the internal organs for female group and their control Albino rats after 15 days of acute toxicity experiment weight in gram. There were no significant differences between internal organ weight of male and their control ( $P>0.05$ ).

Internal organ	Female	Control Female
Heart	0.9 $\pm$ 0.09	1.06 $\pm$ 0.1
Lung	1.7 $\pm$ 0.17	1.79 $\pm$ 0.1
Liver	7.4 $\pm$ 0.6	8.92 $\pm$ 0.1
Kidney	1.5 $\pm$ 0.2	1.73 $\pm$ 0.2
Stomach	1.4 $\pm$ 0.2	1.64 $\pm$ 0.1
Spleen	0.9 $\pm$ 0.05	0.87 $\pm$ 0.06
Intestine	3.3 $\pm$ 0.07	3.42 $\pm$ 0.2
Sex organs	0.2 $\pm$ 0.1	0.20 $\pm$ 0.03

## DISCUSSION

LD<sub>50</sub> is a standard measurement of acute toxicity that is stated in gram (g) of extract per kilogram (kg) of body weight. LD<sub>50</sub> represents the individual dose required to kill fifty percent of a population of test animals (e.g., rats, fish, mice, cockroaches). Because LD<sub>50</sub> values are standard measurements, it is possible to compare relative toxicities among same species. The lower the LD<sub>50</sub> dose, the more toxic.<sup>11</sup> LD<sub>50</sub> as a mean to identify the dose that should be tested *in vivo* study and to clinical trial later on; as the starting concentration is 10% of the LD<sub>50</sub>.<sup>12</sup> After the rats were orally given a single dose of the water extract from the dried leaf extract of *Vitex agnus castus* at 5,000 mg/kg, neither signs of toxicity nor death of rats were observed during 14 days of the acute toxicity experimental period.<sup>12</sup> The results showed that the methanol extract has very safe LD<sub>50</sub> (17.21g/kg) orally. This study disagreed with the study issued by Tandon and Gupta 2004, they showed that the LD<sub>50</sub> of the ethanol extract of *Vitex agnus castus* was 7.58g/kg.<sup>13</sup> However, the differences are significant between the two results, the differences may be due to the nature of lands where the plant has planted and the solvents used in extraction process as in Tandon study ethanol used to extract the leaf while this study methanol used.

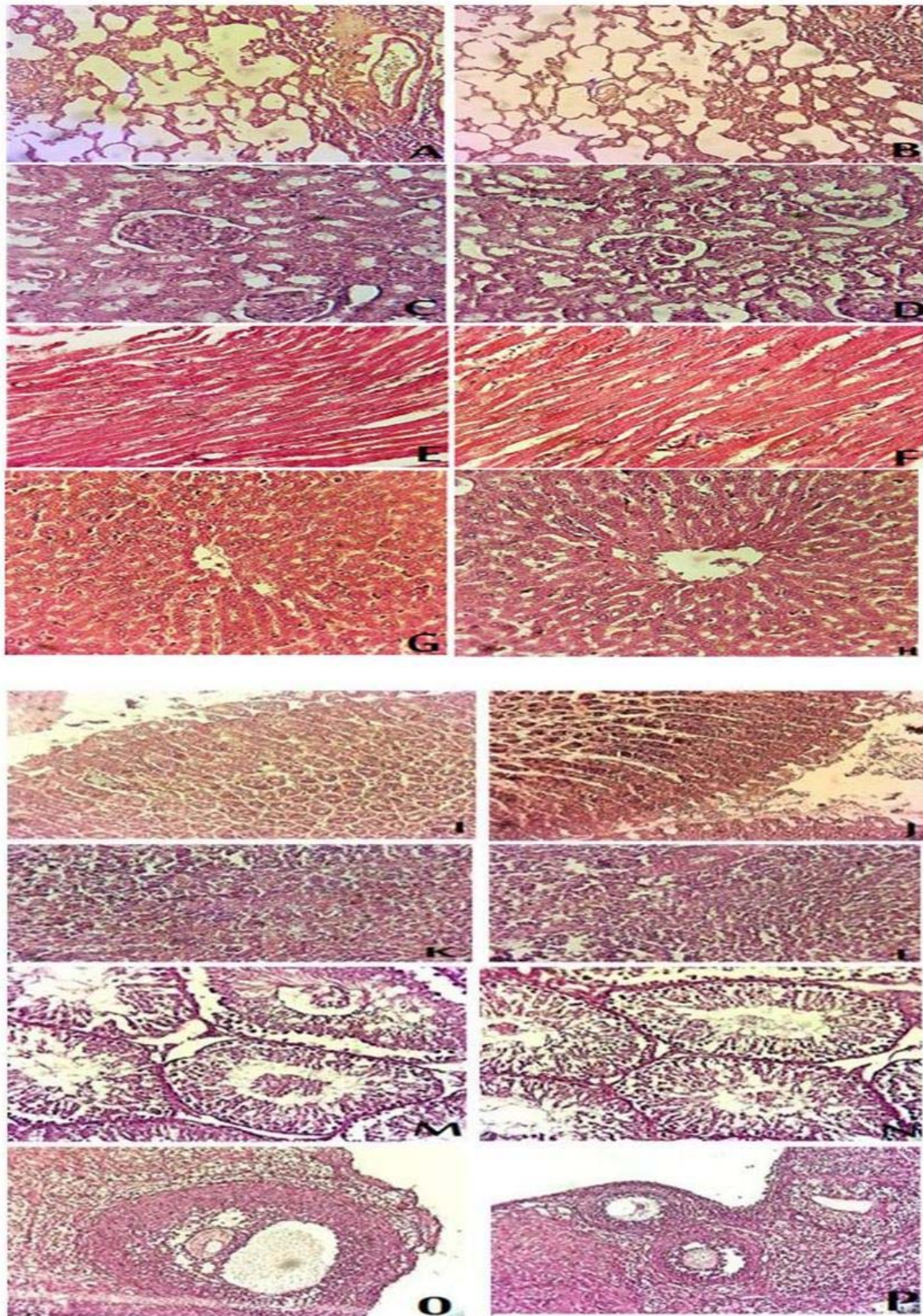
### Acute toxicity

The animals' model used in this experiment was rats. The alterations of body weight and organs weights from the control would reflect the toxicity of the substance.<sup>14</sup> The



data showed no significant difference in organ weight between treated and untreated (control) animals with absence of any morphological changes.<sup>15</sup> The extract showed no sign of toxicity at 5g/kg of the *Vitex agnus castus* methanol extract concentration. Blood profile showed no significant changes in comparisons to the

control groups for both male and female rats. However, there was significant reduction in cholesterol level in comparison to the control, this finding agreed with a previous study done by Mustafa abd al Majeed 2007. This reduction may be attributed to the existence of flavonoids.<sup>16</sup>



**Figure 2:** Histopathology of rat (male and Female) exposed to 5000mg/kg of *Vitex agnus castus* methanol extract

**Table 6:** Showing the serum profile for the rats received 5000mg/kg after 14 days. Sample has triplicate the results represented as mean  $\pm$ SD. there were no significant differences between each group and its control except that for lipid as it showed significant reduction

Serum profile	Female Rats	Female control	Male rats	Male control
Glucose	100 $\pm$ 5.5	98 $\pm$ 4.7	99 $\pm$ 8.7	97.9 $\pm$ 2.8
urea	29 $\pm$ 3.2	23 $\pm$ 5.5	28 $\pm$ 0.20	30 $\pm$ 1.4
Creatinine	0.54 $\pm$ 0.02	0.49 $\pm$ 0.05	0.51 $\pm$ 0.03	0.55 $\pm$ 0.002
Ca <sup>++</sup>	10.6 $\pm$ 1.1	10.3 $\pm$ 0.45	12.6 $\pm$ 1.1	11.98 $\pm$ 2.1
Phosphate	4.4 $\pm$ 0.88	4.6 $\pm$ 0.65	5.9 $\pm$ 1.02	6.6 $\pm$ 2.8
cholesterol	59 $\pm$ 1.9	98 $\pm$ 2.3	62 $\pm$ 8.8	90 $\pm$ 2.6
Triglyceride	33 $\pm$ 1.9	59 $\pm$ 4.4	52 $\pm$ 4.7	82 $\pm$ 3.9
HDL	7.2 $\pm$ 0.54	5.3 $\pm$ 3.9	6.2 $\pm$ 1.4	6.2 $\pm$ 1.1
total protien	6.6 $\pm$ 0.45	5.9 $\pm$ 0.04	3.1 $\pm$ 0.5	3.1 $\pm$ 0.3
Albumin	0.1 $\pm$ 0.003	0.1 $\pm$ 0.004	0.1 $\pm$ 0.001	0.1 $\pm$ 0.45
Bilirubin	0.1 $\pm$ 0.03	0.1 $\pm$ 0.003	0.1 $\pm$ 0.001	0.1 $\pm$ 0.001
Alk Phosph	157 $\pm$ 10.6	155 $\pm$ 4.7	160 $\pm$ 10.9	170 $\pm$ 11.9
ALT	28 $\pm$ 3.2	27 $\pm$ 3.3	42 $\pm$ 5.5	45 $\pm$ 4.9
AST	102 $\pm$ 8.88	99 $\pm$ 6.9	107 $\pm$ 4.4	105 $\pm$ 4.9

However no changes were in other blood profile and this may be due to the differences in the solvent used in extraction. This study pointed out that blood cholesterol levels were reduced by using a plant extract this action may attributed to the existence of flavonoids.<sup>16</sup> Flavonoids possess potent antioxidant and anti-inflammatory activity. Flavonoids are known to regulate cholesterol production and exert a potent hypocholesterolemic effect via suppression of the HMG-CoA reductase enzyme.<sup>17</sup> Unlike statin drugs, which reduce the activity of the HMG-CoA reductase enzyme<sup>[18]</sup>. Flavonoids appear to reduce total cholesterol levels by a novel post-transcriptional mechanism that regulates the degradation rate of the HMG-CoA reductase enzyme.<sup>18</sup> Other studies have demonstrated the antioxidant and anti-inflammatory benefits of flavonoids, and when combined with the current findings of a significant and repeatable reduction of total cholesterol and triglycerides, they lend further support to the overall cardio-protective benefits of these natural extracts.<sup>19</sup> Further studies on this subject are highly recommended. In conclusion this extract is safe to be sending for formulation for to be tested on human as antiangiogenic herb.

## REFERENCES

- Brazier NC, Levine MA, Drug-herb interaction among commonly used conventional medicines: a compendium for health care professionals, *Am J Ther*, 10(3), 2003, 163-169.
- Butterweck V, Winterhoff H, Herkenham M, St John's wort hypericin, and imipramine: a comparative analysis of mRNA levels in brain areas involved in HPA axis control following short-term and long-term administration in normal and stressed rats, *Mol Psychiatry*, 6(5), 2001, 547-564.
- Mc Guffin, American Herbal Products Association's Botanical Safety Handbook, CRC Press Inc. and HerbalGram Copyright American Botanical Council, 48, 2000, 42.
- Nieves BJ, D'Amore PA, Brayan BA, The function of vascular endothelial growth factor, *Biofactors*, 35(4), 2009, 332-337.
- Hinsbergh V, Vmw P KW R, Role of fibrin and plasminogen activators in repair associated angiogenesis: *In vitro* study with human endothelial cells, *EXS Begul*, 79, 1997, 391-411.
- Sahib HB, Aisha AF, Yam Mun Fe, Ismail Z, Z Asmawi, S Salhimi M, Abdul Majid AMS, Anti-angiogenic and antioxidant properties of *Orthosiphon stamineus* Benth, methanolic leaves extract, *International journal of pharmacology*, 5(2), 2009, 162-167.
- Hayder B. Sahib, Adeeb A Al-Zubaidy, Shallal M Hussain, Ghaith Ali Jassim, The Anti Angiogenic activity of *Vitex agnus castus* leaves extracts, *International Journal of Pharmacy and Pharmaceutical Sciences*, 6, 2, 2014, 863-869.
- Shah Ayub MA, Garg SK, Garg KM, Subacute toxicity studies on Pendimethalin in rats, *Indian J. Pharm.*, 29, 1997, 322-324.
- Bürger C, Fischer DR, Cordenunzi DA, Batschauer de Borba AP, Filho VC, Soaresdos Santos AR, Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasiliensis*) (Asteraceae) in mice, *J. Pharm. Sci.*, (www.cspCanada.org), 8(2), 2005, 370-373.
- World Health Organization (WHO), General guidelines for methodologies on research and evaluation of traditional medicine, Switzerland, 2000.
- Amein Al-Ali, Abdul Aziz Alkhawajah, Mohammad Akram Randhawa, Nisar Ahmed Shaikh, Oral and intra-peritoneal LD<sub>50</sub> of thymoquinone, an active principle of *NIGELLA SATIVA*, in mice and rats, *J Ayub Med Coll Abbottabad*, 20(2), 2008.



12. Francesco Di Pierro, Anna Borsetto Menghi MD, Angela Barreca MD, Maurizio Lucarelli MD, Andrea Calandrelli MD, Green Select® Phytosome as an Adjunct to a Low-Calorie Diet for Treatment of Obesity, A Clinical Trial Alternative Medicine Review, 14, 2009, 2.
13. Tandon V, Gupta RK, Histomorphological changes induced by vites negundo in albino mice, Indian J Pharmacol, 36(3), 2004, 176-177.
14. Seewaboon Sireeratawong, Nirush Lertprasertsuke, Umarat Srisawat, Amornat Thuppia, Acute and subchronic toxicity study of the water extract from Tiliacora triandra (Colebr.) Diels in rats Songklanakarin, J. Sci. Technol., 30(5), 2008, 611-619.
15. Bailey SA, Zidell RH, Perry RW, Relationship between organ weight and body weight in the rat: what is the best analytical endpoint, Toxicol. Pathol., 32(4), 2004, 448-466.
16. Mustafa abd, almajeed Hussein, Effect of *Vitex agnus castus* extract on some physiological parameters of mice (MUS MUSCULUS L.) the medical journal of Basrah University, MJBU, 25, 2007, 2.
17. Silver M, Langsjoen P, Szabo S, Patil H, Zelinger A, Effect of atorvastatin on left ventricular diastolic function and ability of coenzyme Q10 to reverse that dysfunction, Am J Cardiol, 94, 2004, 10, 1306-1310
18. Nawarskas JJ, HMG-CoA reductase inhibitors and coenzyme Q10, Cardiol Rev, 13, 2005, 2, 76-79.
19. James M, Roza CN, Zheng Xian-Liu, Najla Guthrie effect of citrus falvonoids and tocotrienols on serum cholesterol levels in hypercholesterolemic subject alternatives therapies, 13, 2007, 6.

**Source of Support:** Nil, **Conflict of Interest:** None.

