



The Acute Toxicity of *Commiphora molmol* oleo-gum-resin Methanol Extract

Ghaith A. Jasim^{*1}, Adeb A. Al-Zubaidy², Shallal M. Hussein¹, Hayder B. Sahib², Basim Sh. Ahmed³

¹University of Mustansiriyah, College of Pharmacy, Department of Pharmacology and Toxicology, Baghdad, Iraq.

²Al-Nahrain University, College of Pharmacy, Department of Pharmacology and Toxicology, Baghdad, Iraq.

³University of Mustansiriyah, College of Medicine, Department of Pathology, Baghdad, Iraq.

*Corresponding author's E-mail: gaithali@yahoo.com

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ABSTRACT

The study aimed to investigate the safety of the most biological active extract of *C. molmol* oleo-gum-resin in terms of blood vessel growth inhibition. The active extract was subjected to the toxicity studies; The dose that consider lethal to the 50% of the animal exposed to the extract (LD₅₀) study 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. Acute toxicity study in rats administered with 5000mg/kg extract, the toxicity in the animals was carried out by assessing the effects on body weight, relative organ weight, biochemical parameters and histopathological study for heart, lung, liver, kidneys, spleen, stomach and sex organs for both male and female, following oral administration of methanol extract. the LD₅₀ value of *C. molmol* methanol extract was calculated from the linear regression equation and equaled to 15.504 g/kg. Serum biochemical findings show no significant differences in comparison to control. No significant body weight or relative organ weight changes occurred throughout the study, histopathology of selected organs showed no remarkable pathology. *Commiphora molmol* methanol extract showed No signs of toxicity were observed during the acute toxicity study and the LD₅₀ value indicates that the extract is to be safe.

Keywords: Antiangiogenesis, *Commiphora molmol*, Acute toxicity of Herbs, Lethal dose fifty for *C. molmol*.

INTRODUCTION

C*ommiphora molmol* (Myrrh) is an oleo-gum resin, obtained from the stem of various species of genus *Commiphora* of family Burseraceae, which grow in north-east Africa and Arabia, the part of *C. molmol* used medicinally is the resin, the resinous exudates of the genus *Commiphora* are commonly used as perfume, incense, or embalming ointment, and their medicinal values have been gradually recognized by humankind¹. As a herbal medication, *C. molmol* is used as an effective antimicrobial agent, for sore throats, and gingivitis. Sesquiterpene-rich fractions of *C. molmol* have antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria and *Candida albicans*. *C. molmol* is used both experimentally and clinically as antiparasitic effect against human trematode infections, particularly schistosomiasis and fascioliasis with trials of success and disagreement regarding its efficacy against schistosomes³, a *C. molmol* pharmaceutical preparation known as Mirazid[®], was produced and marketed in Egypt as gelatin capsules of purified *C. molmol* extract for the treatment of schistosomiasis and fascioliasis⁴. Some other studies provide data about *C. molmol* cytoprotective effects on gastric mucosa, In addition, myrrh has anti-inflammatory activity, antipyretic activity⁵.

The aim of this study is to investigate the safety of the most biological active extract of *C. molmol* oleo-gum-resin, and to identify its safety for human administration.

MATERIALS AND METHODS

Experimental animals

Experimental animals were divided into the following test groups according to the type of experiment. All the animals were allowed to free access to food and tap water, kept at 28-30°C.

1. Seventy male and female Swiss albino mice weighing 20 – 25 g each, were used to perform the lethal dose that killed 50% of the animals used in experiment.
2. Twenty Albino rats were used in the acute toxicity study.

The animals were obtained from the Animal House Facility in the Iraqi Center for Cancer and Medical Genetics Research, University of Mustansiriyah, Baghdad, Iraq. The experiments were approved by the Animal Ethical Committee in College of Medicine, Al-Nahrain University, Baghdad, Iraq.

The Lethal Dose Fifty (LD₅₀)

The lethal dose that killed 50% study was carried out using seventy male and female Swiss albino mice weighing 20 – 25 g each. The animals were equally distributed into twelve treatment groups (6 male groups, and 6 female groups) with five animals each, and two control groups (one male and one female group) also five animals each. All animals provided with water and food and were allowed to adapt to the laboratory conditions for seven days before starting the experiment. After deprivation of food overnight, the animals in control



group received 0.3 ml of 2% Tween 80 solution orally. And each treated group received orally the *C. molmol* methanol extract, which was prepared by diffusing 8.0 g in 10 ml volume of 2% Tween 80 in the following doses: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg^{19,20}. The animals were observed for death or change in general behavior and other physiological activities. Animal observation continued for the first 4 hours and then every 24 h for the following 48 h after administering of the extract⁶.

Acute Toxicity Study

The acute oral toxicity was evaluated following the World Health Organization (WHO) guideline (WHO, 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for chemical testing^{7,8}. Twenty rats were selected by stratified randomization and then divided into four groups of five each. The first two group served as treatment (five males and five females) and the other two control groups of ten animals (five males and five females), The treated groups were orally given the aqueous extract of *C. molmol* in a single dose of 5.0 g/kg body weight, while the control groups received only water vehicle (3ml). The first day of dosing was taken as Day 0 whereas the day of sacrifice was designated as Day 14⁹. The following parameters were measured: Mortality, Body weight, Relative organ weight, Serum biochemistry and Microscopic examination^{10,11}.

Mortality

During the 2 week, animals were observed daily for clinical signs and mortality patterns.

Body weight

The body weight of each rat was assessed using a sensitive balance during study period, once on Day 0, 7 and 14 before sacrifice.

Relative Organ Weight

On day 14 of the study period, all the animals were euthanized by exsanguination under chloroform anesthesia. Different organs namely the heart, lungs, liver, kidneys, spleen, stomach and sex organs were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

$$\text{Relative Organ Weight} = \frac{\text{Absolute Organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Preparation of sera samples and Serum biochemistry

On Day 14 of the study period, all the animals were exsanguinated under chloroform anaesthesia and blood samples were drawn from the heart of each sacrificed animal.

The samples were collected in plastic test tubes and allowed to stand for 3 h to ensure complete clotting. Clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen. The following parameters were

determined colorimetrically by employing the standard ready-to-use kits and methods of Human; blood glucose, blood urea, serum creatinine, glutamate oxaloacetate transaminase (GOT, AST), glutamate pyruvate transaminase (GPT, ALT), total bilirubin, conjugated bilirubin.

Microscopic Examination

Macroscopic examination of the organs carried out. Tissue samples from the heart, lungs, liver, kidneys, spleen, stomach and sex organs were fixed in 10% formal saline. These were processed with an automatic tissue processor. Following dehydration and embedding, sections were cut at 4-5 μ with the rotary microtone, stained with hematoxylin and eosin and examined microscopically.

RESULTS

The Lethal Dose Fifty (LD₅₀)

The Lethal dose which killed fifty percent of mice (males and females) receiving the methanol extract is represented in Figure (1).

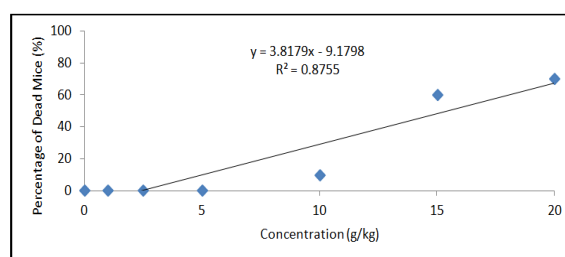


Figure 1: Lethal dose fifty (LD₅₀) of methanol extract of *Commiphora molmol* on mice groups.

LD₅₀ value of *C. molmol* methanol extract was calculated from the linear regression equation ($y = 3.817x - 9.179$), which LD₅₀ equals to 15.504 g/kg.

Where: y = the percentage of inhibition and x = concentration.

After administration of a single dose of the serial concentrations of *C. molmol* methanol extract to each of the six groups of mice (males and females) each group received a concentration and the control group received the vehicle, (Table 1).

Acute toxicity study of *Commiphora molmol* (Myrrh) methanol extract in rats

After the rats were orally given a single dose of 5 g/kg of the methanol extract of *C. molmol* to the tested animals, neither signs of toxicity nor death of rats were observed during the fourteen days of the acute toxicity experiment period. The alterations of body weight at day zero, day seven and day fourteen were recorded and also the relative organ weights were calculated at day fourteen, organ tissue samples and blood samples were also collected at day fourteen from treated groups and their controls.

Table 1: The number of deaths of different groups of mice with different administered doses.

Group	Dose (g/kg)	No. of Mice (Male & Female)	No. of Dead Mice	Accumulative Percentage (%)
Control	0	5 Male	0	0
		5 Female	0	0
1	1	5 Male	0	0
		5 Female	0	0
2	2.5	5 Male	0	0
		5 Female	0	0
3	5	5 Male	0	0
		5 Female	0	0
4	10	5 Male	1	10
		5 Female	1	10
5	15	5 Male	3	60
		5 Female	3	60
6	20	5 Male	4	80
		5 Female	3	60

Table 2: Relative organ weights of rats receiving *C. molmol* methanol extract and their controls after 14 days of acute toxicity experiment.

Organs		Treatment*		
		<i>C. molmol</i> (5 g/kg)	Control	P Value
Male				
	Heart	0.534 ± 0.066	0.560 ± 0.087	0.577
	Lungs	0.883 ± 0.219	0.919 ± 0.180	0.813
	Liver	3.948 ± 0.811	4.116 ± 0.443	0.704
	Kidneys	0.944 ± 0.131	0.892 ± 0.159	0.671
	Spleen	0.598 ± 0.218	0.621 ± 0.132	0.888
	Stomach	0.912 ± 0.231	0.848 ± 0.247	0.747
	Testes	2.617 ± 0.612	1.983 ± 0.515	0.215
Female				
	Heart	0.478 ± 0.054	0.549 ± 0.099	0.258
	Lungs	0.908 ± 0.158	0.909 ± 0.163	0.993
	Liver	3.881 ± 0.462	4.535 ± 0.884	0.176
	Kidneys	0.703 ± 0.128	0.844 ± 0.059	0.162
	Spleen	0.426 ± 0.111	0.447 ± 0.110	0.796
	Stomach	0.735 ± 0.111	0.786 ± 0.088	0.564
	Ovaries	0.151 ± 0.052	0.108 ± 0.038	0.246

* Data are expressed as Mean ± SD, n=5 for each group.

Table 3: The Serum biochemical tests for rats receiving single dose 5g/kg of *C. molmol* methanol extract and their controls.

Biochemical Tests	Laboratory animals (Rat)*					
	Male			Female		
	Treatment group	Control group	P value	Treatment group	Control group	P value
Blood Sugar (mg/dl)	181.62 ± 24.91	169.73 ± 31.86	0.527	180.54 ± 27.63	168.64 ± 23.02	0.452
Blood Urea (mg/dl)	16.47 ± 4.37	15.8 ± 2.67	0.808	13.34 ± 0.98	14.01 ± 1.12	0.580
S. Creatinine (mg/dl)	39.95 ± 1.91	41.54 ± 3.12	0.463	48.79 ± 2.68	46.49 ± 3.56	0.166
ALT (SGPT) U/L	40 ± 7.81	47 ± 10.36	0.385	29.6 ± 12.58	30.6 ± 5.94	0.833
AST (SGOT) U/L	121 ± 37.99	127.6 ± 33.61	0.809	125.6 ± 36.52	120.6 ± 26.29	0.830
T. Bilirubin (mg/dl)	2.73 ± 1.19	2.76 ± 0.65	0.975	3.29 ± 1.53	3.34 ± 0.67	0.950
Conjugated B. (mg/dl)	1.78 ± 0.22	1.82 ± 0.28	0.794	2.56 ± 1.21	2.46 ± 0.61	0.872

* Data are expressed as Mean ± SD, n=5 for each group.



The weight of the animals in the acute toxicity study

Results show the changes in weight between the treatment groups (male and female) in compared to their control groups during the fourteen days of experiment. Weigh for the male and female groups were measured three times, at day zero, seven and fourteen, (Figure 2). There was no significant changes in weight among treatment groups in comparison to their controls ($P>0.05$) for both male and female groups.

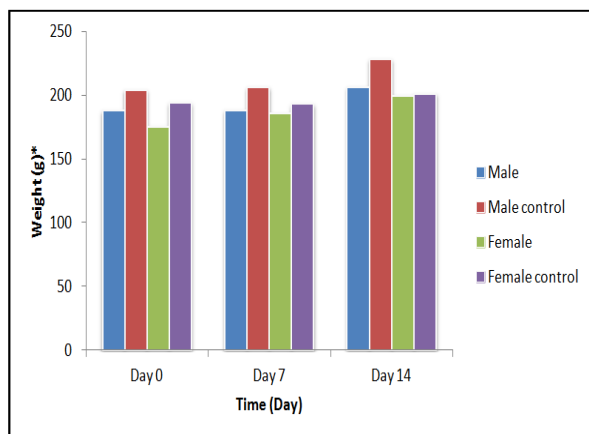


Figure 2: Weight changes in the treated male and female rats and their controls at days zero, seven and fourteen. * Data are expressed as Mean \pm SD, n=5 for each group.

The data showed no significant weight change in animals ($P>0.05$). Day zero P value = (0.189) and (0.170) for male and female rats and their control respectively. At day seven P value = (0.186) and (0.729) for male and female rats and their control respectively. While at day fourteen P value = (0.465) and (0.934) for the male and female rats respectively, in compared with their controls.

Relative organ weights in the acute toxicity study

Results showed the changes in the relative weight of organs for traded male and female rats in compared to their controls, the results showed no significant changes occurred between treated groups and their control ($P>0.05$), (Table 2).

Serum biochemical tests of rats in the acute toxicity study

The rats received 5 g/ml of *C. molmol* methanol extract, results showed some serum biochemical tests for the tested animals compared to their controls. There was no significant difference between treated groups compared to their control groups ($P>0.05$), (Table 3).

Histopathology examination for selected organs in the acute toxicity study

The histopathological study of the selected organs in the acute toxicity study in rats, showed no remarkable pathology by comparing between the treated and control groups in both males and females, Figure (3) and (4). In images "A" & "B" we can see normal cardiac muscle, branched eosinophilic striated muscle fiber with central

located nucleus with no remarkable pathology. In Images "C" & "D" shows normal alveolar walls with no remarkable pathology. "E" & "F" images show normal hepatic lobules and acinus apart from congestion of portal veins with no remarkable pathology. In "G" & "H" the sections show normal glomerular and tubular tissue of kidney without remarkable pathology in both cortex and medulla. (Figure 3).

Images "I" & "J" show normal splenic white pulp and red pulp with normal tissue sinuses and cords without remarkable pathology. "K" & "L" sections show normal gastric tissue without remarkable pathology. "M" & "N" images show normal spermatogenesis within the seminiferous tubules without remarkable pathology, and "O" & "P" images show ovary tissue with normal stages of maturation of vesicular follicles no changes in the ovarian stroma (Figure 4).

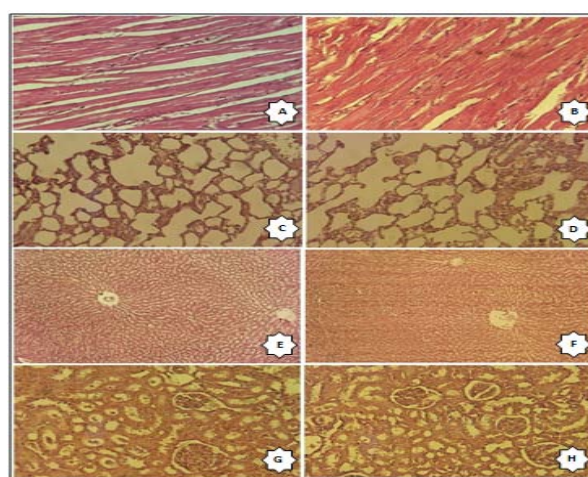


Figure 3: Histopathology images of selected organs in the acute toxicity study of *C. molmol* methanol extract. Where A, B, C, D, E, F, G and H represent heart, control heart, lung, control lung, liver, control liver, kidney and control kidney respectively.

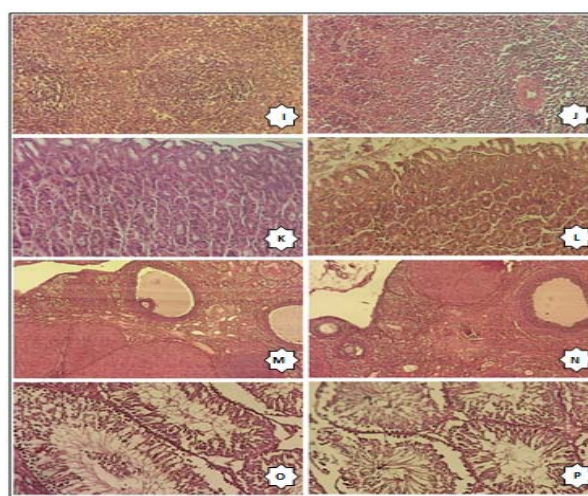


Figure 4: Histopathology images of selected organs in the acute toxicity study of *C. molmol* methanol extract. Where I, J, K, L, M, N, O and P represent spleen, control spleen, stomach, control stomach, ovary, control ovary, testes and control testes respectively.

DISCUSSION

Lethal dose killing 50% of laboratory animals

The acute toxicity lethal dose killing 50% (LD₅₀) was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no lethality at all. Even though the acute toxicity (LD₅₀) test has been widely criticized as a parameter for assessing toxicity¹², useful information could be obtained from such studies as giving a clue on the range of doses that could be used in subsequent toxicity testing, also it could reveal the possible clinical signs elicited by the substance under study. It's also a useful parameter estimating the therapeutic index (ie: LD₅₀ / ED₅₀) of drugs and xenobiotics¹³. LD₅₀ is used also to identify the safe dose that should be tested *In vivo* study and to in the later clinical trial, as the starting concentration is 10% of the LD₅₀¹⁴. The present study has shown a fairly high oral LD₅₀ values more than 15000 mg/kg in mice, in relation to its folkloric therapeutic dose for *C. molmol* (Myrrh) (10 mg/kg/day) or (600 mg/day). Thus, its high oral therapeutic index might be used as a rough indication of a wide margin between the effective and toxic doses¹⁵. Lethal dose kill 50% of animals is a standard measurement of acute toxicity that is stated in milligrams (mg) of plant extract per kilogram (kg) of body weight of tested animal. Because LD₅₀ values are standard measurements, it is possible to compare relative toxicities among same species, the lower the LD₅₀ dose, the more toxic¹⁶. *C. molmol* methanol extract was assigned in category "5" according to Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS) of OECD as test sample in category "5" is of relatively low acute toxicity and the calculated LD₅₀ is more than 5000mg/kg (LD₅₀> 5g/kg). This agrees with Kennedy who stated that test sample with LD₅₀ more than 5000mg/kg is considered as nontoxic¹⁷. The results showed that Myrrh methanol extract has LD₅₀ of (15.504 g/kg). A previous study showed that, the LD₅₀ of the chloroform extract of *Commiphora molmol* (Myrrh) was 7.5g/kg mice body weight which is equivalent to 58.125 g for a 70 kg human body weight¹⁸. However, the differences are not high between the two studies both are still above the 5g/kg, the differences may be due to the different method used for extraction and the different solvent that may have extracted different solutes¹⁹.

Acute toxicity study of *Commiphora molmol* (Myrrh) methanol extract in rats

After the rats were orally given a single dose of the methanol extract of *Commiphora molmol* (Myrrh) at 5 g/kg, no signs of toxicity or death of rats were observed during 14 days of the acute toxicity period. Also no significant change in body weight or organ weight (relative organ weight) of treated rats in compared to that of the control group. The body weight changes serve as a sensitive indication of the general health status of animals^{20,21}. However, weight gains were observed in all

animals administered with *C. molmol* (Myrrh) methanolic extract. It can be stated that the methanol extract did not interfere with the normal metabolism of experimental animals as corroborated by the non-significant difference from animals in the control group. The mean body weight of all treatment groups gradually increased over the observation period.

According to OECD (Organization for Economic Co-operation and Development) guideline 423, the weight variations in rats are acceptable if they did not exceed $\pm 20\%$ SD of the mean weight²². The clinical biochemistry analyses were done to evaluate the possible alterations in hepatic and renal functions influenced by the extract. Liver and kidney function analysis is very important in the toxicity evaluation of and plant extracts as they are both necessary for survival of organism²³. The methanol extract of *C. molmol* (Myrrh) at 5 g/kg showed no sign of toxicity and blood profile showed no significant changes in compared to the control groups for both male and female rats. There are no significant changes for the serum levels of glucose, blood urea, creatinine, liver transaminases ALT and AST, total bilirubin levels and conjugated bilirubin ($P > 0.05$). After the oral administration of *C. molmol* methanolic extract, microscopic examination revealed that none of the organs from the treatment groups showed any alteration in cell structure when viewed under the light camera microscope. The structure or coordination of cells in treated organs more or less similar compared with the control organs. Commonly, damage to the parenchymal liver cells results in elevations of both transaminases in the blood²⁴. Therefore, the non-significant change in ALT, AST activities strongly suggest that the administration of *C. molmol* methanol extract did not alter the hepatocytes as observed in the histopathology of liver tissue. Also, there was no significant change in urea and creatinine in the *C. molmol* methanol extract treatment group when compared to the control group. Any rise in urea and creatinine levels is only observed if there is marked damage to functional nephrons^{25,26}. This finding was further confirmed by histopathological observations of the kidney tissue. Therefore, the results showed in this study aids that the methanolic extract of *C. molmol* did not alter the liver or renal functions and further support the non-toxic nature of the extract.

The study agreed with a previous literature that preformed acute and chronic oral toxicity studies on *Commiphora molmol* oleo-gum-resin in mice. There was no significant difference in mortality in acute or chronic treatment as compared to controls^{27,28}.

CONCLUSION

Commiphora molmol methanol extract showed no signs of toxicity were observed during the acute toxicity study and the LD₅₀ value indicates that the extract is to be safe. This herb may have promising activity against tumor as adjuvant with chemotherapy or in targeting angiogenesis related diseases.



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REFERENCES

- Langenheim J.H. Plants resins: Chemistry, Evolution, Ecology, and Ethnobotany. Timber Press, Portland, Oregon, USA. 2003.
- Dolara P, Corte B, Ghelardini C. Local anaesthetic, antibacterial and antifungal properties of sesquiterpens from myrrh. *Planta Med.* 66(4), 2000, 356-358.
- Abdul-Ghani R, Loutfy N, Hassan A. Myrrh and trematodosis in Egypt: An overview of safety, efficacy and effectiveness profiles. *Parasitol Int.* 58(3), 2009, 210–214.
- Barakat R, Elmorshedy H, Fenwick A. Efficacy of myrrh in the treatment of human *Schistosomiasis mansoni*. *Am J Trop Med Hyg.* 73, 2005, 365-367.
- Qureshi S, Al-Harbi MM, Ahmed MM, Raza MM, Giangerco AB, Shah AH. Evaluation of the genotoxic, cytotoxic and antitumor properties of *Commiphora molmol* using normal and Ehrlich ascites carcinoma cell bearing swiss albino mice. *Cancer ChemotherPharmacol.* 33, 1993, 130-138.
- Shah Ayub MA, Garg SK, Garg KM. Subacute toxicity studies Pendimethalin in rats. *Indian J. Pharm.* 29, 1997, 322-324.
- Organization of Economic Co-operation and Development (OECD), The OECD Guideline for Testing of Chemicals. 420.
- Kwan Yuet Ping, Ibrahim Darah, Yeng Chen, Subramaniam Sreeramanan, and Sreenivasan Sasidharan. Acute and Subchronic Toxicity Study of *Euphorbia hirta* L. Methanol Extract in Rats. *BioMed Research International.* 2013, 14 pages.
- Chunlaratthanaphorn S., Lertprasertsuke N., Srisawat U., Thupia A., Ngamjarriyawat A., Suwanlikhid N. and Jaijoy K. Acute and subchronic toxicity study of the water extract from dried fruits of *Piper nigrum* L. in rats. *J. Sci. Technol.* (1), 2007, 109-124.
- Darout I.A. Cristy, N. Shaug and F. Egeberg. Identification and quantification of some potentially antimicrobial anionic components in Miswake extract. *International Journal of Pharmacology.* 32, 2000, 11-14.
- Stanley O. Aniagu, Florence C. Nwinyi, David D. Akumka, Gloria A. Ajoku, Sunday Dzarma, Kazeem S. Izebe, Matthew Ditse, Patrick E. C. Nwaneri, Charles Wambebe and Karynius Gamanie. Toxicity studies in rats fed nature cure bitters. *African Journal of Biotechnology.* 4(1), 2005, 72-78.
- Renu S. and Badri P. N. New methods for extracting phytoconstituents from plants. *International Journal of Biomedical and Advance Research.* 03(10), 2012, 770-774.
- Klaassen CD. Ed: Casarett and Doull's Toxicology, The basic Sci. of Poisons, 6th Edition McGraw-Hill, New York. 2001.
- Rang HP, Dale M, Ritter J. *Pharmacology*, 4th ed. (USA ed.); New York, Churchill Livingstone. 2001.
- George Francis, Zohar Kerem, Harinder P. S. Makkar and Klaus Becker. "The biological action of saponins in animal systems: a review". *British Journal of Nutrition.* 88(6), 2002, 587–605.
- Abo-Madyan AA, Morsy TA, Motawea SM. Efficacy of Myrrh in the treatment of schistosomiasis (haematobium and mansoni) in Ezbet El-Bakly, TamyiaCenter, El-FayoumGovernate, Egypt. *J Egypt SocParasitol.* 34(2), 2004, 423-446.
- Soliman OE, El-Arman M, Abdul-Samie ER, El-Nemr HI, Massoud A. Evaluation of myrrh (Mirazid) therapy in fascioliasis and intestinal schistosomiasis in children: immunological and parasitological study. *J Egypt SocParasitol.* 34(3), 2004, 941-966.
- Amein Al-Ali, Abdul Aziz Alkhawajah, Mohammad AkramRandhawa, Nisar Ahmed Shaikh. Oral and intra-peritoneal LD₅₀ of thymoquinone, an active principle of *NIGELLA SATIVA*, in mice and rats. *J Ayub Med Coll Abbottabad.* 20(2), 2008.
- Kennedy G. L., Ferenz R. L., and Burgess B. A., "Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD50, *Journal of Applied Toxicology.* 6(3), 1986, 145-148.
- Nagwa M. Ammar, Seham S. El-Hawary, Ahmed A. Mahdy, Rehab A. Hussein, TatsufumiOkino. Phytochemical Study of the Biologically Active Fractions of the Oleo-gum-resins of *Boswellia carteri* and *Commiphora myrrha*. *Advances in Environmental Biology,* 7(9), 2013, 2573-2583.
- Jothivel N., Ponnusamy S.P., Appachi M., Singaravel S., Rasilingam D., Deivasigamani K. and Thangavel S. Anti-diabetic activity of methanol leaf extract of *Costus pictus* D. Don in alloxan-induced diabetic rats. *Journal of Health Science.* 53(6), 2007, 655-663.
- Rani S. Sellers, Daniel Morton, Bindhu Michael, Nigel Roome, Julie K. Johnson, Barry L. Yano, Rick Perry, and Ken Schafer. Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations Toxicologic Pathology. 35, 2007, 751–755.
- El Hilaly J., Israili Z. H., and Lyoussi B., "Acute and chronic toxicological studies of *Ajugavain* experimental animals," *Journal of Ethnopharmacology.* 91(1), 2004, 43–50.
- Organisation for Economic Cooperation and Development, "OECD Guideline for the testing of chemicals. Test guideline 423, acute oral toxicity-acute toxic class method. <http://www.oecdilibrary.org>.
- Olorunnisola O. S., Bradley G., and Afolayan A. J., "Acute and sub-chronic toxicity studies of methanolic extract of *Tulbaghia violacea* rhizomes in Wistar rats," *African Journal of Biotechnology,* 11, 2012, 14934-14940.
- Slichter S. J., "Relationship between platelet count and bleeding risk in thrombocytopenic patients," *Transfusion Medicine Reviews,* 18(3), 2004, 153–167.
- Lameire N., Van Biesen W., and Vanholder R. Acute renal failure. *The Lancet,* 365(9457), 2005, 417–430.
- Rao R.M., Z.A. Khan and A.H. Shah. Toxicity studies in mice of *Commiphora molmol* oleo-gum-resin. *Journal of ethnopharmacology,* 76(2), 2001, 151-154.

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