Effect of various level of dietary *Commiphora myrrha* on rats

Sawsan Ali Omer[1*], Mai A. Elobeid[1], Maha ElAmin[1], Promy Virk[1], Maha Dagestani[1], Nadia AlEisa[1], Reem AlAjami[1], Ebtisam AlOlayan[1], Albandary AlRajeh[1] and Amira AlMahasna[1]

[1]Department of Zoology, Faculty of Science, King Saud University, Women's Students-Medical Studies & Sciences Section, Riyadh 11451, Saudi Arabia

[*]Corresponding Author: **Sawsan A. Omer**, E: mail: sawsanaomer@gmail.com, Phone number: +966502129044

Abstract

Background: The possible effect of *Commiphora myrrha* oleo-gum resin was studied on rats fed different concentrations of the crude plant. The plant is used in different regions for treatment of various ailments but the toxic effect of this plant has not been studied in details.

Methods: *Commiphora myrrha* was fed to rats at 2, 5, 10, and 20% of the basic diet.

Results: Rats on 10% and 20% *Commiphora* food exhibited depression, soft faeces, abdominal pain and dyspnoea prior to death. Lesions were fatty change and necrosis of hepatocytes, catarrhal enteritis, renal tubular cell degeneration, splenic haemosiderosis and lymphocytic infiltration in the hepatic portal area, renal cortex, and intestinal lamina propria and between the cardiac muscle fibers. These changes were correlated with alterations in haematology and clinical chemistry. Two and five percent *C. myrrha* diets were not toxic to rats.

Conclusion: *Commiphora myrrha* oleo-gum resin was found to be lethal to rats at 10% and 20% concentrations of the basal diet. At lower concentrations it was found to be toxic and toxicity was indicated biochemically and histopathologically.

Keywords: *Commiphoram myrrha*, rats, toxicity, biochemistry

Introduction

Nearly all cultures from ancient times to the present day have used plants as a source of medicines. A considerable percentage of the people in both developed and developing countries use medicinal plant remedies and the number is on the increase especially among the younger people. Overall, the trade in botanicals has increased through their use in the health food and cosmetic industries. In the USA, imports in 1980 were valued at $ 44.6 million and China, the production of traditional plant remedies was valued at $ 571 million and the country-wide sales of crude plant drugs at $ 1.4 million annually (Li Chaojin, 1987; WHO, 1987).

Plants continue to provide basic raw materials for some of the most important drugs which may be employed for different medicinal purposes; however, over-dosage of plants containing medicinal compounds may cause toxic reactions when introduced into animals or human beings. It is well known that medicinal plants are most important to daily health particularly in tropical countries where these floras constitute an important component of the diversity of the plant kingdom. Not only do the tropics contain the largest number of species per unit area, but the tropical plants also display an amazing variability in their genetic makeup e.g. in the biochemical compounds which they produce.

About half the world's medicinal compounds are derived or obtained from plants. The active constituents of plants such as glycosides, alkaloids, terpenes, sesquiterpenes and tannins have been isolated and used in the treatment of various ailments. The medicinal products from plants are, in general, more important in developing countries than in industrialized nations. But even in these where the focus is on chemical discovery and synthesis of pharmaceuticals, drug products from plants are major contributors to the human health services sector of the economy each year.

It has been estimated that as many as 75-90% of the world's rural people rely on herbal traditional medicine as their primary health care. Consequently, there is a growing interest in medicinal plants and traditional medicine and the governing bodies of the World Health Organization (WHO) of the United Nations (1987) have within the last

decade called for the intensification of efforts in the development of national traditional medicine activities.

Commiphora myrrha oleo-gum resin selected for this investigation is used in the traditional medicine of many Asian nations and East African countries including Saudi Arabia for the treatment of various disorders but this has not been confirmed by experiments. *Commiphora myrrha*, a member of the family Burseraceae, is known locally as Morr Hijazi or Myrrh and commercially as Arabian Myrrh or Karam (True myrrh).

In the Kingdom of Saudi Arabia, the plant widely grows at Gizan on the Red Sea coast, a distinct so bare and dry that it is called "Tehama" meaning very hot "hell". It is also found in Somaliland and other East African countries (Migahid, 1978). It is used as an astringent, tonic in dyspepsia, antiseptic, anti-inflammatory, antipyretic, expectorant, carminative, emmenagogue (Tariq et al 1985), antidiabetic (Helal et al., 2005) and antimicrobial (Omer et al., 2011, Chandrasekharnath et al., 2013**).** It is also used as a wash for spongy gums, ulcerated throat and aphthous stomatitis and the tincture is applied to indolent ulcers (Satyavati et al., 1969).

Myrrah contain myrrin, volatile oil, gum, terpenes, sesquiterpenes which possess mutagenic activity (Al–Harbi et al., 1994), it also contains aldehyde and eugenols that are oxygen radical scavengers, and have anti mutagenic and antilipemic potentials (Wiendl and Franz,1994) resin acid and protein (Provan and Waterman, 1988). Three new tetraterpenyl esters which may be used as chromatographic markers for quality control of the drugs and two aliphatic esters has been identified (Mohd et al., 2013).

The present study was undertaken to investigate whether *C. myrrha* can induce any toxicity in rats fed the crude plant in their basal. The toxicity was indicated by investigating the clinical, pathological, and hematological and serobiochemical effects on the rats fed the plant in their basal diet.

Material and methods

Rats and diets

A total of thirty male Wistar white rats weighing 150-180 gm were obtained from the Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research, Khartoum and housed at the Laboratory Animal Unit of the department of Veterinary Preventive Medicine and Public Health, University of Khartoum. Rats were fed on pelleted diets consisting of 80 kg of wheat flour, 4 kg of bovine liver, 1 gallon of peanut oil and sodium chloride. Water was provided and *ad libitum.* The rats were assigned at random to 5 groups of 6 rats each. Group 1 rats served as control and were fed on the control basal diet. Finely ground *C. myrrha* was fed at 2 % (group 2), 5 % (group3), 10 % (group 4) and 20 % (group 5) of the basal diet for 2 weeks.

Clinical examination and hematology

Records of clinical abnormalities and mortality rates were recorded. Lots of three rats from each group were anaesthetized with diethyl ether and sacrificed at week 1 and 2 for pathological examination. Specimens of intestines, liver, kidneys, heart and spleen were fixed in 10 % neutral buffered formalin and processed for histopathology. Blood samples collected at slaughter were examined for hematological changes and alterations in serum constituents.

Sera were analyzed for the activities of alkaline phophatase (ALP) and alanine transaminase (AST) and for the concentrations of total protein, albumin, globulin, bilirubin, cholesterol, creatinine and magnesium using commercial kits (Randox laboratories Ltd, UK). Blood samples were examined for hemoglobin (Hb) concentrations, packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular hemoglobin (MCHC) and mean corpuscular volume (MCV). The methods used were described by Schalm et al. (1975).

Results

Clinical signs and mortality rates

Details of the rats fed various levels of *C. myrrha* are shown in Table 1. One rat in group 4 (10 % C. *myrrha)* died on day 7 and 4 rats in group 5 (20 % *C. myrrha)* died between days 5 and 8. Rats in group 4 and 5 exhibited depression, soft feces and dyspnoea. When

touched, some of the rats in group 5 had abdominal pain prior to death. None of the rats in groups 1 (control), 2 (2% *C. myrrha)* and 3 (5 % *C. myrrha)* showed clinical signs or died during the 15-day period of experiment.

Table 1. Details of rats fed various levels of dietary *C. myrrha*

Group No.	No. of rats per group	Concentration of myrrh in diet (%)	No. of rats died	Maln Signs	Mortality (%)
1 (Controls)	6	0	0	Nil	0
2	6	2	0	Nil	0
3	6	5	0	Nil	0
4	6	10	1*	Depressive, soft faces.	16.66
5	6	20	2**	Depressive, soft faces, dyspnoea	33.33

Changes in serum constituents

Table **2** shows the increases ($p<0.05$-0.001) in the activity of serum ALP and ALT; in the concentrations of bilirubin, creatinineand cholesterol and the decreases ($p<0.05 – 0.001$) in the total protein, albumin and globulin levels in group 4 (10 % *C. myrrha)* and 5 (20% *C. myrrha).* Serum magnesium concentrations of serum constituents between the control rats (group 1) and those in group 5 (20 % *C. myrrha)* and 3 (5 % *C. myrrha)* respectively.

Table 2. Changes in the concentration of serum constituents in rats fed *C. myrrha* for 15 days (Mean ± D)

Group No.	Total Protein gm/dl	Albumin gm/dl	Globulin gm/dl	Cholesterol mg/dl	Bilirubin mg/dl	Creatinine mg/dl	Magnesium mg/dl	ALP IU	ALT IU
1 (Controls)	7.85 ± 0.49	3.95 ± 1.77	3.9 ± 1.28	65.45 ± 13.16	0.10 ± 0.07	0.50 ± 0.14	1.25 ± 0.92	117.65 ± 24.96	21.80 ± 0.85
2 (2% *C. myrrha*)	7.17 ± 0.17$^{N.S}$	3.2 ± 0.67$^{N.S}$	3.97 ± 1.5$^{N.S}$	78.53 ± 7.08$^{N.S}$	0.20 ± 0.35$^{N.S}$	0.73 ± 0.23$^{N.S}$	1.43 ± 0.65$^{N.S}$	181.5 ± 17.50$^{N.S}$	27.63 ± 6.21$^{N.S}$
3 (5% *C. myrrha*)	8.90 ± 0.9$^{N.S}$	3.53 ± 1.1$^{N.S}$	5.37 ± 2.4$^{N.S}$	97.33 ± 14.9$^{N.S}$	0.17 ± 0.21$^{N.S}$	1.00 ± 0.36**	1.00 ± 0.36$^{N.S}$	177.37 ± 21.6$^{N.S}$	41.1 ± 12.87$^{N.S}$
4 (10% *C. myrrha*)	5.23 ± 0.08**	2.33 ± 0.67*	2.9 ± 0.21*	130.8 ± 5.75**	0.9 ± 0.04**	0.9 ± 0.03**	0.60 ± 0.44$^{N.S}$	221.47 ± 4.73*	55.64 ± 0.78**
5 (20% *C. myrrha*)	4.20 ± 0.13**	1.80 ± 0.30*	2.4 ± 0.14*	145.5 ± 3.96**	2.85 ± 0.02***	1.5 ± 0.05***	1.30 ± 0.57$^{N.S}$	322.97 ± 2.16***	68.76 ± 0.42***

N.S = Not Significant; *=p<0.05; **=p<0.01; ***=p<0.001

Hematological changes

These are summarized in Table 3. The RBC, Hb, PCV, and WBC values in group 5 (20% *C. myrrha)* were lower (p<0.05-0.001) and thos of WBC in group 4 (10 % *C. myrrha)* were lower (p<0.05) than the controls (group 1). The values of MCHC did not change.

Table 3. Hematological changes in rats fed *C. myrrha* 15 days (Mean ± SD)

Group No.	Hb gm/dl	RBC 10^6/µl	PCV %	MCH pg	MCHC %	MCV fl	WBC 10^3/µl
1 (Controls)	9.83 ± 0.75	5.27 ± 0.38	39.65 ± 6.54	18.65 ± 6.40	24.79 ± 4.90	75.24 ± 7.30	9.40 ± 6.40
2 (2% *C .myrrha*)	9.03 ± 0.57$^{N.S}$	5.10 ± 0.62$^{N.S}$	37.93 ± 3.0$^{N.S}$	17.71 ± 8.40$^{N.S}$	23.81 ± 3.90$^{N.S}$	74.37 ± 6.9$^{N.S}$	9.36 ± 10.76$^{N.S}$
3 (5% *C. myrrha*)	8.60 ± 0.28$^{N.S}$	4.95 ± 0.72$^{N.S}$	36.98 ± 5.4$^{N.S}$	17.37 ± 10.70$^{N.S}$	23.36 ± 7.4$^{N.S}$	74.71 ± 8.20$^{N.S}$	8.97 ± 12.06$^{N.S}$
4 (10% *C. myrrha*)	7.9 ± 0.72$^{N.S}$	4.75 ± 0.53$^{N.S}$	36.15 ± 4.20*	16.63 ± 4.2$^{N.S}$	21.85 ± 5.9$^{N.S}$	76.11 ± 6.30**	6.0 ± 0.32*
5 (20% *C. myrrha*)	6.10 ± 0.05**	2.16 ± 0.04**	27.0 ± 0.8*	28.24 ± 0.8*	22.59 ± 4.90$^{N.S}$	125.0 ± 0.37***	5.21 ± 0.47**

N.S = Not Significant; *=p<0.05; **=p<0.01; ***=p<0.001

Pathological changes

In rats of group 5 (20 % *C. myrrha),* there was diffuse fatty cytoplasmic vacuolation of hepatocytes (Fig 1), catarrhal enteritis (Fig 2), congestion of the blood capillaries in the intestinal lamina propria, lymphocytic infiltration in the renal cortex and between the cardiac muscle fibers, renal tubular cell degeneration (Fig 3) and slight splenic

haemosiderosis . These changes, in group 5, were more marked on day 15 than day 7. In group 4 rats on 10% *C. myrrha* food, changes were less pronounced and were absent in the control rats (group 1) and in the rats on 2 % *C. myrrha* diet (group 2) and in those on 5 % *C. myrrha* diet (group 3).

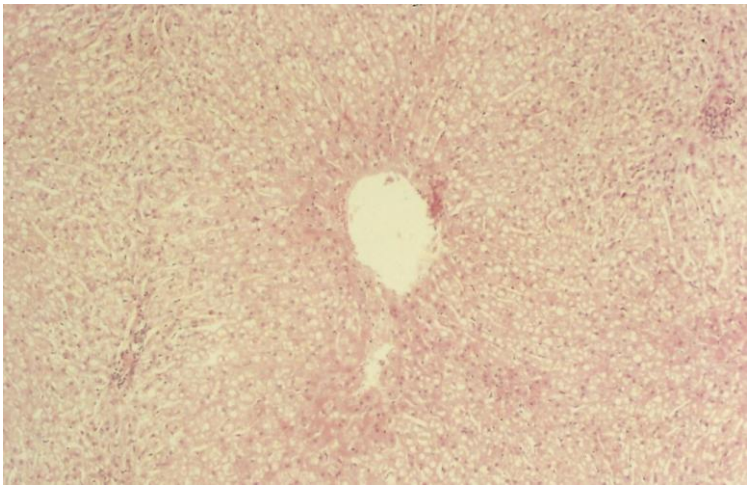

Figure 1. Diffuse fatty cytoplasmic vacuolation of the hepatocytes of rat on 20% *Commiphora myrrha* diet for 15 days. H and E x60.

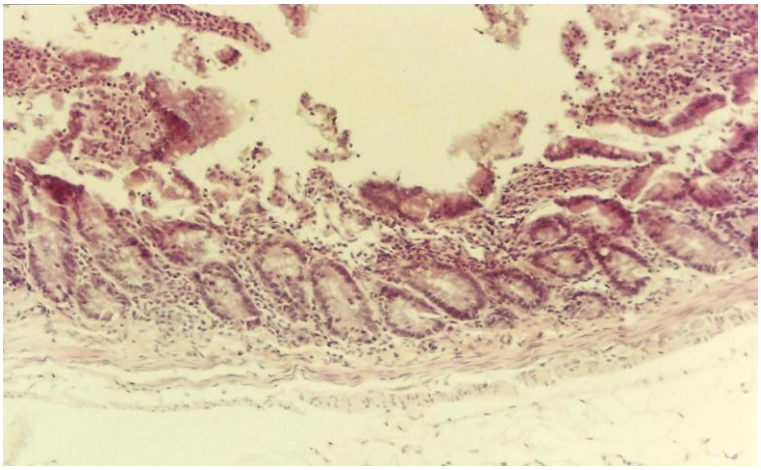

Figure 2. Catarrhal enteritis and lymphocytic infiltration in the intestinal lamina propria in a rat on 20% c my diet for 15 days. H and E X 60.

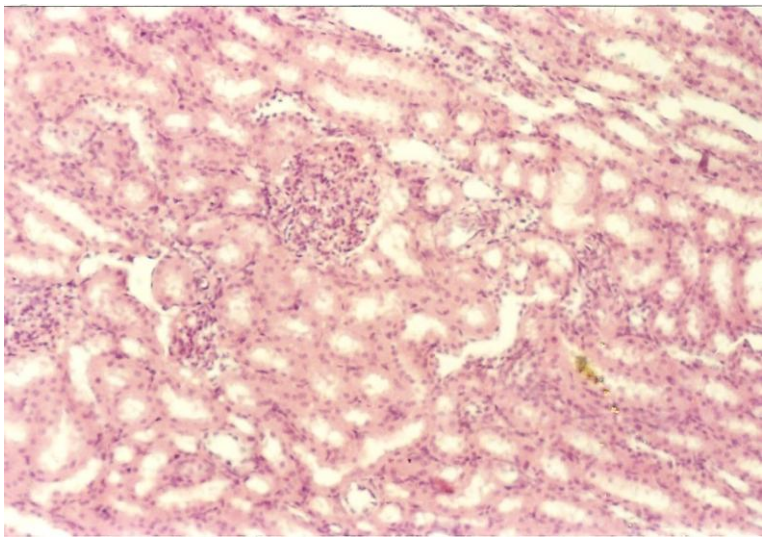

Figure 3. Renal tubular degeneration and lymphocytic in a rat on 20% co m diet for 15 days. H and E x60.

Discussion

Commiphora myrrh oleo-gum resin is widely used in different part of the world especially in Saudi Arabia for treatment of various ailments. Toxicological information on its effect on rats is unavailable**.** A part from the work conducted by Omer et al. (1999) no work is available on the toxicity of *C. myrrha* in other animal species.

In the present study, the effects on rats given different levels of *C. myrrh* were investigated. The result indicated that the plant is toxic and lethal to rats at 10% and 20%. The characteristic features of myrrha toxicity were enterohepatonephropathy, leucopenia and anaemic. These were evidenced by alterations in the serum levels of ALP and ALT and in alterations in the concentrations of total protein, albumin, bilirubin, cholesterol and creatinine. Hematological investigations showed leucopenia and decrease values of erythrocytic series. Poisonous myrrha in rats had significant decreases in serum calcium. This may have been at least in part, due to decreases in total serum protein, as it known that hepatic damage is associated with myrrah toxicity to goats given 5g/kg body weight (Omer et al., 1999).

Hypoproteinaemia was probably due to impaired synthesis of albumin by damaged hepatocytes (Adam, 1972; 1978; Bakhiet and Adam, 1996). Adequate amounts of vitamin

D3 are essential for maintenance of normal plasma calcium and phosphorus. The first stage of vitamin D3 metabolism is hepatic conversion to 25-$(OH)_2$-D3, which is further metabolized in the renal proximal convoluted tubules. Hepatorenal syndromes my cause hypocalcaemia similar to that described in Wister albino rats which had been fed with *Rhazya stricta* and in goats which had been orally dosed with *Acanthspermum hispidum* (Ali and Adam, 1978) and *Pennisetum typhoides* (AbdelGadir and Adam, 1999).

Considerable variations in the toxicity to rodents, poultry and livestock of different plant constituents are well documented. It seems that the susceptibility of rats to administration of plant materials (e.g. leaves, seeds and barks) and extracts (ethanolic, chloroformic, and butanolic) is dependent on the type of active constituents, concentration in the plant material and extract, the route of administration and the rate of their metabolic conversion in the liver to metabolites and consequent excretion.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-033.

References:

1) AbdelGadir, W.S. and Adam S.E.A. 1999. Development of goiter and enterohepatonephropathy in Nubian goats fed with millet (*Pennisetum typhoides*). The Veterinary Journal. 157: 178-185.

2) Al –Harbi MM, Qureshi S, Ahmed MM et al. 1994. Effect of *Commiphora molmol* (oleo gum resin) on the cytological and biochemical changes induced by cyclophosphmide in mice. American Journal of Chinese Medicine, 22: 77-82.

3) Ali, B. and Adam SEA. 1978. Effects of *Acanthospermum hispidum* on goats. Journal of Comparative Pathology. 88: 533.

4) Chandrasekharnath, N., Mahlakshmi, Y.V., Jayalakshmi, L., Venkanna, B. and Uma, A. 2013. Screening and isolation of bioactive factors from *Commiphora myrrha* and evaluation of their antimicrobial activity. International Journal of Engineering Research and Applications, 3: 1291-1294.

5) Chaojin, L. 1987. Management of Chinese traditional drugs. In role of traditional medicine in primary health care in china ,eds .O.Akerele, G.Stott and Lu Welbo. Aamerican Journal of Chinese Medicine, suppl. No.1, 39.

6) Helal G.E., A.M., El-Badawy , E.E. and Kahwash, A.A. 2005. Effect of *Commiphora myrrha* extract on some physiological parameters and histological changes in diabetic albino rats. The Egyptian Journal of Hospital Medicine 20: 148 – 162.

7) Migahid, A. M. 1978. Flora of Saudi Arabia, 2nd ed., Riyadh University press, Riyadh.

8) Omar, S. A., Adam, S.E. I. and Mohammed, O. B. 2011. Antimicrobial activity of *commiphora myrrha* against some bacteria and *Candida albicans* Isolated from Gazelles at King Khalid Wildlife Research Centre. Research Journal of Medicinal Plants. 5: 65-71.

9) Omer S. A. and Adam S.E.I. 1999.Toxicity of *Commiphora myrrha* to goats. Vet Hum Toxicol. 41: 299-301

10) Provan, G.J.,Waterman, P.G. 1988. Major triterpenes from the resins of *Commiphora incia* and *C kua* and their potential chemotaxnomic significance. Phytochem 27:3841-3843.

11) Satyavati, G. V., Dwarakanath, C. and Tripathi, S.N. 1969. Experimental studies on the hypocholeserolaemic effect of *commphora mukul* (guggul). Indian Journal of Medical Research, 57:1950

12) Mohammed S., Alia, M. and Naquvia, K.J. 2013. The phytochemical investigation of the oleo-resin of *Commiphora myrrha* (Nees) Engl. led to the isolation of three new tetraterpenyl esters which may be used as chromatographic markers for quality control of the drugs. Der Pharma Chemica, 5: 133-138

13) World Health Organization (WHO). 1987. Global medium term programme (traditional medicine) covering a specific period 1990-1995. WHO (Document TRM/MTP/87.1).

14) Wiendl, R.M. and Franz, G. 1994. Myrrh: New chemistry of an old crude drug. Dtsch Apoth Ztg, 134: 27-29.