Research Article

Determination of Essential Oil Percentage with Evaluation of Antihyperlipidemic Activity of Three Natural Gums in Rats

Ibrahim S. Abass[1], Muthanna I. Al-Ezzi[2], Inam S. Arif[2], Ghaith A. Jasim*[2]
[1]Al-Mustansiriyah University, College of Pharmacy, Department of Pharmacognosy and Medicinal Plants, Baghdad, Iraq.
[2]Al-Mustansiriyah University, College of Pharmacy, Department of Pharmacology and Toxicology, Baghdad, Iraq.
***Corresponding author's E-mail:** gaithali@yahoo.com

Accepted on: 29-04-2016; **Finalized on:** 30-06-2016.

ABSTRACT

This study was conducted to determine the essential oil percentage of three natural gums of three medicinal plant, *Commiphora molmol* (myrrh), *Boswellia serrata* (Indian frankincense) and *Aloe vera* (true aloe) with evaluation of the antihyperledemic activity of the extracts of the three plants in rats. After converting all resins to powder the essential oil was extracted and percentage of essential oil of all gums was determinate and also evaluation of the antihyperlipedemic activity, the powder samples of all gums were extracted with ethanol by soxhlet apparatus. Animals were divided into six groups that where feed on an atherogenic diet (AD), (Control group, Atorvastatin group, Aloe gum extract group, Frankincense gum extract group, Myrrh gum extract group and Combination of all gum extracts group). The results were referred to percentage of essential oil of myrrh, frankincense and aloe gums were (1.2), (1.5) and (0.8) percentages respectively. Results also showed significant effect of all gums extracts in decreasing the total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) with increasing the high density lipoprotein (HDL) compared with control groups. The results of this study denote that the three extracts have significant antihyperlipidemic effects. These results affirm the traditional use of myrrh, frankincense and aloe gums thereby reducing the risk of obesity and cardiovascular complication like atherosclerosis.

Keywords: Natural Gums, essential oils, myrrh, frankincense, aloe, hyperlipidemia.

INTRODUCTION

Hyperlipidemia considered as one of the risk factors for coronary heart disease, stroke, high blood pressure and type 2 diabetes[1]. Cholesterol is present in the diet of all people, and it can be absorbed slowly from the gastrointestinal tract into the intestinal lymph, it is highly fat soluble and specifically capable of forming esters with fatty acid.[2]

In individuals with type 2 diabetes, metabolic syndrome, and the combined dyslipidemia, cardiovascular risk is increased by a clustering of risk factors such as abdominal obesity, impaired fasting glucose, increased blood pressure, low HDL-cholesterol (HDL-C), increased triglycerides (TGs), and an increase in small, dense LDL particles.

The current increase in the incidence of type 2 diabetes in the population perhaps poses the most urgent cardiovascular risk.[3]

Atorvastatin is a member of the drug class known as statins used primarily for lowering blood cholesterol and for prevention of event associated with cardiovascular diseases.

World health organization (WHO) estimates 80% of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care.

Active compounds of medicinal plant such as polyphenolic compounds, flavonoids, resins, volatile oils glycosides and others, have different pharmacological and biological actions against different diseases.[4-6]

Resin, in the most specific meaning of the term, is a hydrocarbon secretion of many plants, particularly coniferous trees.

It is distinct from other liquid compounds inside plants or exuded by plants, such as sap latex or mucilage. More broadly, the term "resin" is also used for, some artificial polymer bases (synthetic resins), that during normal use, harden into transparent or opaque solids. Myrrh is also an (oleo-gum resin) of thorny tree (*Commiphora molmol*) which is an essential oil termed oleoresin[7].

Myrrh resin is used as a fragrance in cosmetic and as a flavoring agent in food and beverages. It has also been used as an astringent as antiseptic to be applied to inflamed lesions of the throat and mouth as emmenague, antispasmodic, treatment of cancer and for infectious disease[8].

Commiphora 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. *C. molmol* Myrrh consists of water-soluble gum, alcohol-soluble resins and volatile oil. The gum contains polysaccharides and proteins, while the volatile oil is composed of steroids, sterols and terpenes. Myrrh's characteristic odor is derived from furanosesquiterpenes[6]. The part of this plant used medicinally is the resin, the resinous exudates of the genus *Commiphora* are commonly used as perfume,

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

incense, or embalming ointment, and their medicinal values have been gradually recognized by humankind.[7,8]

Boswellia serrata (Indian frankincense), the gum of tree is in the order sapindales, known for their fragrant gum which has many pharmacological uses, particularly as anti-inflammatory effect.

Extracts of *B. serrata* gum have been clinically studied for their osteoarthritis and joint treatment effect, particularly for osteoarthritis of the knee joint, with the studies showing a slight improvement of both pain and function compared to placebo group.[9,10]

Aloe vera (true aloe), the plant gum is used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties[11,12].

There is some preliminary evidence to suggest that oral administration of *A. vera* might be effective in reducing blood glucose in diabetic patients.[13]

This study was conducted to determination the essential oil percentage of all gums used in the study with evaluation the effect of gums ethanol extracts on blood lipid profile parameters (TC, TG, LDL, VLDL and HDL) in rats.

MATERIALS AND METHODS

Distillation of volatile oil

The sample of gums were converted to powder then mixed with distilled water (plant: water, 1:5 w:w) in a two-liter, round bottom.

After complete distillation (2-4h.), the collected oil was dried by passing over an hydrous sodium sulfate on a filter paper (What man No. 1) in a glass funnel the percentage of volatile oil of each sample was determined according the procedure previously described.[14]

Preparation of ethanolic extracts

After converting all gums to powder, one hundred gram (100 g) of each gum powder was extracted with 500 ml of 90% ethanol by soxhlet apparatus for six hours at 40-60 °C, then solution was evaporated to dryness by rotary evaporation apparatuses.

Total phenolic content

Ethanol extracts of all gums in this study were tested for presence or absence of phytoconstituents according to the standard procedure available in the literature.[15]

The total phenolic content of all extracts was determined by Folin-Ciocalteu assay and expressed as milligrams of gallic acid equivalents (GAE) per 100 grams dry mass (Mg GAE/100g w). Gallic acid equivalents (GAE), Absorbance (at 765nm).[16]

Experimental animals

Thirty male rats were randomly divided into 6 groups with 5 animals per group. Hyperlipidemia was induced by daily administration of atherogenic diet (AD) consisting of 2% cholesterol, 1% choline chloride and 2% lard in normal pellet diet over a period of 21 days.

Study groups included: AD+Atorvastatin (positive control), AD+Aloe gum extract, AD+frankincense gum extract, AD+myrrh gum extract, AD+combination of all gums extract and final group AD+Control (negative control).

Assay of lipid profile

On 22nd day, animals were anaesthetized with diethyl either and blood was collected by retro orbital puncture.

The blood was allowed to clot for 30 min. at room temperature and subjected to centrifugation at 2000 rpm for 15 min, to obtain serum.

The resulting upper serum layer was collected in clean, dry labeled micro-centrifuge tubes. This serum was analyzed for serum TG, TC, HDL-C using commercially available kit[17,18].

Serum LDL-C, VLDL-C and atherogenic indexes (AI) were determined by the following calculation:

$$AI = \frac{LDL + VLDL}{HDL}$$

$$LDL\text{-}C\ (mg/dl) = \frac{TC - (HDL\text{-}C + Triglycerides)}{5}$$

$$VLDL\text{-}C\ (mg/dl) = \frac{Triglycerides}{5}$$

Statistical analysis

A one-way analysis of variance was performed to test whether group variance was significant or not, the comparison between groups were used analysis of variance test (ANOVA). *p-value* < 0.05 was considered significant difference; *p-value* > 0.05 was considered no significant difference, the statistic analysis was carried out by using SSPS 16.0 for Windows (SPSS Inc, Chicago, IL).

RESULTS AND DISCUSSION

The results of the current study presented the percentages of essential oil of myrrh, frankincense and aloe 1.2, 1.5 and 0.8% respectively in table (1), results partly resembles the percentages in a previous study.[19]

The preliminary phytochemical investigation of the ethanolic extract of the three natural gums showed the presence of most of the phytoconstituents like steroids terpenoids, flavonoids, glycosides, tannins, saponins and

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

alkaloids in the three extracts used in this study (Table 2), results of the preliminary screening for phytochemicals was in line with many previous studies.[20-24]

The amount of phenolic content of myrrh, frankincense and aloe were 7.5%, 6.4% and 4.8% (W/W) respectively (Table 1).

Table 1: Essential oil percent and total phenolic content for the three medicinal plants.

Medicinal plant	Essential oil (%)	Total phenolic content (%)
Commiphora molmol	1.2	7.5
Boswellia serrata	1.5	6.4
Aloe vera	0.8	4.8

Table 2: General phytochemical screening of the plants.

Medicinal plant	steroids	terpenoids	flavonoids	glycosides	tannins	saponins	alkaloids
Commiphora molmol	+	+	-	+	+	+	+
Boswellia serrata	+	+	+	+	+	+	+
Aloe vera	-	+	+	+	+	+	+

Table 3: Effect of myrrh, frankincense and aloe gums extracts on serum lipids parameter in rats.

Treatment group	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL/HDL ratio
AD + Atorvastatin	69±4.2	62±3.3	44±2.5	12±1.5	13±1.3	3.38
AD + Aloe gum extract	81±4.1	107±3.9	26±2.2	21±1.9	33±2.8	0.79
AD + Frankincense gum extract	86±4.6	109±5.3	28±3.1	22±4.3	36±1.3	0.78
AD + Myrrh gum extract	87±1.1	63±2.4	10±1.6	13±2.1	60±3.2	0.17
AD + All three extracts	71±4.4	91±2.2	33±3.2	18±2.4	20±2.6	1.65
AD control	125±2.9	140±6.4	150±4.5	29±2.1	17±1.1	8.82

LSD: Lest sign differences at 0.05

The results shown in table (3) referred to all treatment groups recording the significant effect in decreasing the following lipid profile parameters: TC, TG, LDL, VLDL and increasing the HDL.

Combination treatment with all extracts showed a higher effect in decreasing total cholesterol level with a mean of 71mg/dl in compared to the other extract treatment groups, whereas atorvastatin the well-known lipid lowering agent had the highest effect in lowering TC with a mean of 69mg/dl. The higher TC level was obtained at placebo group (AD+control) with 125mg/dl. Treatment with myrrh extract gave more effect in decreasing TG with mean value of 63mg/dl in compared to the other plant extracts. Still with highest result in atorvastatin treatment group (62mg/dl). The higher value of TG was obtained from placebo group (140mg/dl). Also the treatment with myrrh gum extract exhibited more effect in decreasing LDL with mean value 10mg/dl, while the higher values were obtained from placebo treatment group 150mg /dl. Atorvastatin stated more effect in decreasing VLDL with mean value of 12mg/dl. But when treating with different gums extracts the best result was obtain by myrrh, combination, aloe then boswellia, the higher value was recorded by placebo treatment group 29mg/dl.[25]

Treatments with myrrh gum extract gave more effect in increasing HDL with mean value of 60mg/dl, while the lower appeared with placebo treatment group 17mg/dl, this result resembled a previous study regarding myrrh extract hypolipidimic ability.[25]

The lipid levels of atherogenic diet control group were significantly higher than other groups which indicate that AD is a potential source of dietary cholesterol and oxidative stress caused by atherogenic diet is associated with peroxidation of cellular lipids.[26,27]

The activity of natural resins in decreasing all lipid parameters may be belong to unsaturated fatty acid content of these gums that prevent the oxidation of LDL and VLDL and lead to increased the HDL percentage.

These results agreed with other studies that referred to lipid peroxidation occur when free radical are generated adjacent to polyunsaturated fatty acids in membrane lipid. The antioxidant and protective effects of the extracts are owed to their content of antioxidant active constituents such as eugenol, cuminic aldehyde and sesquiterpenes.[28,29]

Several studies referred to natural substances that stimulate the pancreatic exocrine secretion of lipase enzyme which analyzes fat and therefore less LDL as well

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

as active the thyroid gland where low activity leads to a high cholesterol, while higher activity leads to a low level in blood.[30]

CONCLUSION

This study demonstrated the hypolipdemic activity of three natural gums (myrrh, boswellia and aloe), which may have role in reducing the risk of cardiovascular complication specially atherosclerosis.

REFERENCES

1. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: A Pathophysiologic approach, 7th ed, Hyperlipidemia, New York, Mc Graw Hill, 2008, 385-408.
2. Tortora GJ, Grabowski SR, Principles of anatomy and physiology, 9th ed, New York, John Wiley and Sons, 2000, 584-897.
3. Gilling L, Suwattee P, DeSouza C, Asnani S, Fonseca V, Effects of the thiazolidinediones on vascular risk factors, Am J Cardiovasc Drugs, 2, 2002, 149-56.
4. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE, Health benefits of herbs and spices: the past, the present, the feature, Med J Aust, 185(4), 2006, 4-24.
5. Lai PK, Roy J. Antimicrobial and chemoprentive properties of herbs and spices, Curr Med Chem, 11(11), 2004, 1451-60.
6. Jabricant DS and Farnsworth NR, The value of plants used in traditional medicine for drug discovery, Environ Health Perspect, 109(1), 2001, 69-75.
7. Landenheim JH, Plant Resins: Chemistry, evaluation, ecology and enthnobotany, 2003, Timber press, Portland Cambridge.
8. Tucker AO, Frankincese and myrrh, Economic Botany, 40(4), 1986, 425-433.
9. Abdel Tawab M, Werz 0, Schubert Zsilavecz M, Boswellia serrata: an overall assessment of *in vitro*, perclinical, pharmacokinetic and clinical data, Clin Pharmacokinet, 1, 50(6), 2011, 349-69.
10. Geetha K and Ganapaty S, Antihyperlipidemic activity of Boswellia ovalifoliolata Bal. Henry in atherogenic diet induced rats, Inter J Phytotherapy Research, 3(3), 2013, 11-17.
11. Zheng G, Yang L, Chen H, Chu J, Mei L, Aloe vera for prevention and treatment of infusion phlebitis, Joh Wiley andsons, 6(6), 2014, 162.
12. Kim K, Kim H, Kwon J, Lee S, Kong H, Im SA, Lee YH, Lee YR, Oh ST, Jo TH, Park YI, Lee CK, Kim K, Hypoglycemic and hypolipidemic effects of processed Aloe vera gel in a mouse model of non-insulin-dependent diabetes mellitus, Phytomedicine, 16(9), 2009, 856-63.
13. Rajasekaran S, Ravia K , Sinvagnanam K, Subramanian S, Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes, Chin Exp Pharmacol Physiol, 33(3), 2006, 232-237.
14. Abbas lS, Hamzah MJ, Majeed AH, Analysis with evaluation of drying temperature on essential oil content of Achillea fragrantissima L. and Artemisia herb-alba L., Int J Pharm Pharm Sci, 5(3), 2013, 913-914.
15. Khandelwal KR, Practical Pharmacognosy: Techniques & Experiments, Nirali Prakashan, 2008, 149-156.
16. Berker KI, Ozdemir Olgun FA, Ozyurt D, Demirata B, Apak R, Modified Folin-Ciocalteu antioxidant capacity assay for measuring lipophilic antioxidants, J Agric Food Chem, 61(20), 2013, 4783-91.
17. Manco M, Calvani M, Mingrone G, Effect of dietary fatty acid on insulin sensitivity and secretion, Diabetes Obes Metab, 6(6), 2004, 402-413.
18. Fossati P, Prencipe L, Serum Triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide, Clin Chem, 28(10), 1982, 2077-80.
19. Chen Y, Zhou C, Ge Z, Liu Y, Liu Y, Feng W, Li S, Chen G, Wei T, Composition and potential anticancer activities of essential oils obtained from myrrh and frankincense, Oncology Letters, 6(4), 2013, 1140-1146.
20. Raphael E, Phytochemical constituents of some leaves extract of Aloe vera and Azadirachta indica plant species, Glo Adv Res J Environ Sci Toxicol, 1(2), 2012, 014-017.
21. Thu K, Mon Yin Y, Khaing Tin A and Tun Ohn M, Study on Phytochemical Properties, Antibacterial Activity and Cytotoxicity of Aloe vera L., International Scholarly and Scientific Research & Innovation, 7(5), 2013, 285-289.
22. Subhashini DP, Satyanarayana B, Tarakeswara NM, Phytochemical Screening for Secondary Metabolites in Boswellia serrata Roxb. And Wrightia tinctoria (Roxb.) R.Br., Not Sci Biol, 6(4), 2014, 474-477.
23. Al-Daihan S, Al-Faham M, Al-shawi N, Almayman R, Brnawi A, Seema zargar, Ramesa shafi Bhat, Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms, Journal of King Saud University - Science, 25, 2013, 115-120.
24. Alhussaini MS, Alghonaim MI, Saadabi AM, Elfakki K, An Evaluation of the Antimicrobial Activity of Commiphora myrrha Nees (Engl.) Oleo-gum Resins from Saudi Arabia, J Med Sci, 15(4), 2015, 198-203.
25. Shalaby MA, Hammouda AA. Analgesic, anti-infl ammatory and anti-hyperlipidemic activities of Commiphora molmol extract (Myrrh), Journal of Intercultural Ethnopharmacology Intercult Ethnopharmacol, 3(2), 2014, 56-62.
26. Spiteller G, The relation of lipid peroxidation processes with atherogenesis: a new theory on atherogenesis, Mol Nutr Food Res, 49(11), 2005, 999-1013.
27. Kodali G and Seru G, Antihyperlipidemic Activity of Commiphora Caudata Leaves in Atherogenic Diet Induced Rats, International Journal of Biological & Pharmaceutical Research, 4(4), 2013, 250-255.
28. Kammoun M, Miladi S, Ben Ali Y, Damak M, Gargouri Y, Bezzine S, *In vitro* study of the PLA2 inhibition and antioxidant activities of Aloe vera leaf skin extracts, Lipids in Health and Disease, 10(30), 2011, 1-7.
29. Ghaith A, Adeeb A, Shallal M, Hayder B, The Antiangiogenic Activity of Commiphora molmol oleo-gum-resin Extracts, Int J Pharm Sci Rev Res, 33(1), 2015, 83-90.
30. Chandrasekaran CV, Vijayalakshmi MA, Prakash K, Bansal VS, Meenakshi J, Amit A, Review Article: Herbal approach for obesity management, American Journal of Plant Sciences, 3, 2012, 1003-1014.

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

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.