academicJournals

Vol. 8(8), pp. 235-239, 28 February, 2014
DOI: 10.5897/AJPP2013.3488
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

*Full Length Research Paper*

# Hypolipidemic and antithrombotic evaluation of *Myrtus communis* L. in cholesterol-fed rabbits

**Rafeeq Alam Khan[1,3]*, Zeeshan Feroz[2], Maria Jamil[3] and Mansoor Ahmed[4]**

[1]College of Medicine-Jeddah, Department of Basic Medical Sciences, King Saud bin Abdulaziz University for Health Sciences, Kingdom of Saudi Arabia.
[2]College of Science and Health Professions, Department of Basic Sciences, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Kingdom of Saudi Arabia.
[3]Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.
[4]Department of Pharmaceutical Chemistry, University of Karachi, Karachi-75270, Pakistan.

Received 14 February, 2013; Accepted 11 February, 2014

**Herbs have been a great source of natural substances used to prevent many cardiovascular diseases (CVD). Blood lipid levels and coagulation parameters are probably the major determinant for producing CVDs. The present study was undertaken to evaluate the hypo-lipidemic and anti-thrombotic activities of aqueous extract of *Myrtus communis* L. fruit (AEM) in cholesterol-fed rabbits. Hyper-lipidemia was induced following administration of cholesterol for 45 days. Animals of treated group were administered AEM daily for 30 and 45 days at dose of 50 mg/kg. Biochemical tests were performed at the end of dosing that is, on 31st day and again on 46th day. The administration of AEM (50 mg/kg/day) revealed reduction in serum triglycerides and low density lipoprotein; there was also an increase in thrombin and fibrinogen time. Results of present study indicate that the extract exhibited hypolipidemic effects and has also an effect on blood coagulation parameters which may be of value in CVDs. However, further studies are necessary to explore the precise mechanism of action of these effects.**

**Key words:** Herbs, lipid profile, blood coagulation, rabbits.

## INTRODUCTION

Traditional medicine based on herbal remedies has always played a vital role in the health care systems of many countries (Verma et al., 2007) owing to their lesser side effects than synthetic drugs (Srivastava et al., 2006). There are many plants which have been listed in the traditional systems of medicine, and the individuals suffering from cardiovascular diseases (CVD), particularly hyperlipidemia and ischemic heart disease, get relief from these medicines (Mahmood et al., 2010). The World Health Organization (WHO) reports that approximately 80% of the world's population are using herbal drugs for their primary health care (Azaizeh et al., 2003; Mahmood et al., 2010).

*Myrtus communis* L. (Myrtaceae) is widely distributed all over the Mediterranean region and the Middle East. It is an evergreen shrub, about 1 to 5 m high, h as small

*Corresponding author. E-mail: rkhan1959@gmail.com. Tel: +966561320123.

deep-green leaves, white flowers and small dark fruits (Asif et al., 2011). In folk medicine, the decoction of leaves and fruits of myrtle are used orally for stomach aches, hypoglycemia, cough, constipation as well as externally for wound healing (Serce at al., 2010). The volatile oil (Myrtii Oleum) obtained from leaves are used to lower blood glucose (Jung et al., 2006). Similarly, in Italian folk medicine, the fruit of this plant is used in the treatment of many types of infectious disease, including diarrhea and dysentery (Gortzi et al., 2008).

Lipoprotein abnormalities are considered as a highly modifiable risk factor for CVD (Allen at al., 1996). Hyperlipidemia constitutes a foremost etiopathological factor for atherosclerosis (Banerjee and Maulik, 2002) and is often associated with myocardial infarction and cerebrovascular disorders (Ross, 1999). Hence, alteration in plasmalipid levels, coagulation proteins, platelets and fibrinolytic factors may reduce the chance of atherosclerosis (Eitzman et al., 2000). The use of herbal drugs is well-established in many countries. However, despite the increasing scientific interest in this field, there is a lack of summarized data on composition of herbal medicines and therapeutic applications (Aleksic and Knezevic, 2013). Hence, present study has been planned to evaluate the effects of aqueous extract *M. communis* (AEM) on lipid profile and blood coagulation in hyperlipidemia induced rabbits.

## MATERIALS AND METHODS

### *Myrtus communis* extract

*Myrtus communis L.* is a medicinal plant endemic to the Mediterranean area and has been used by locals for its food and medicinal properties since ancient times (Atzei, 2003). *Myrtus* species have been reported to be very rich in volatile oils (Satrani et al., 2006; Shikhiev et al., 1978), phenolic acids and flavonoids (Romani et al., 1999), tannins (Diaz and Abeger, 1986), anthocyanin pigments (Martin et al., 1990) and fatty acids (Cakir, 2004). The fruit of *M. communis* was grinded and aqueous extract was prepared by decoction. The grinded fruit was boiled in water (10 parts of water and 1 part of herb) for 3 h followed by filtration. The filtrate was further concentrated by boiling to obtain the aqueous extract. The extract so obtained was kept at -20°C until further use.

### Animals

Rabbits were selected as experimental animals in the present study since biochemical changes produced in rabbits are relatively similar as observed in humans; rabbits are easily obtainable, easy to handle and cost-effective. The study was conducted on 14 healthy white rabbits of both sexes (1100 to 1600 g), housed at the animal house, Department of Pharmacology, University of Karachi, under controlled temperature condition of 22±2°C, and humidity (50 to 60%) in an alternating 12-h of light/dark cycle. The animals were kept in plastic cages and were given green leafy diet and water regularly. The use of animals in this experiment was in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and approved by the Ethical Committee of University of Karachi.

### Animal's treatment protocols

The aqueous extract of *Myrtus communis* L. (AEM) was initially tested in the dose of 20 mg/kg for 30 days in a group of 5 animals, but no significant response was observed. New rabbits were then uniformly divided into 2 groups of 7 animals each that is, control and treated. Animals of either group received high cholesterol diet (HCD) regularly for 45 days, 0.125 g/kg cholesterol supplied by Merck in 0.5% corn oil. After 45 days, animals of treated group were administered AEM at the dose of 50 mg/kg (Jung et al., 2006) for 30 days during the first phase of study. Animals of control group were given saline equal to the volume of respective doses according to their body weight. During the second phase of study, animals of treated group were further administered AEM for more15 days making a total period of 45 days and compared with control for the same period. All substances were administered through oral route. Blood sam-ples were collected thrice from the ear vein of animals, first after 45 days of HCD then again after 30 and 45 days dosing of AEM.

### Estimation of lipid profile

Blood sample of about 5 ml were collected in gel tube. Serum was immediately separated out by centrifuging blood samples on 14K Humax centrifuge at 3000 rpm for 15 min. Lipid profile were analyzed on Humalyzer 3000 (semi-automatic chemistry analyzer, Model #16700) (Human Germany) using standard kits supplied by Human. Total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) was estimated by CHOD-PAP method; triglyceride (TG) by GPO-PAP methods (Trinder, 1969), and high density lipoprotein cholesterol (HDL-C) by the method of Friedwald et al. (1972).

### Estimation of coagulation parameters

Blood sample of about 3 ml were collected in coagulation tubes containing 3.2% sodium citrate. Plasma was separated by centrifuging blood samples on 14K Humax centrifuge at 3000 rpm for 15 min. Thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen were measured by Humaclot duo (Human Germany), using standard reagent kits supplied by Human (Chan et al., 2007).

### Mortality rate

Mortality rates were observed in animals receiving HCD and AEM during the total period of experiment. The number of animals that died during these intervals was also noted.

### Statistical analysis

All values were compared with the control by taking mean and standard error to the mean using t-test, values of $P < 0.05$ were considered as significant and $P < 0.01$ as highly significant. All statistical procedures were performed according to the method of Alcaraz and Jimenez (1989).

## RESULTS

### Mortality rate

No death was observed in any group of animals during

**Table 1.** Effects of *Myrtus communis* on lipid profile after 30 days.

| Animal group | Parameter (mg/dl) | | | |
|---|---|---|---|---|
| | Cholesterol | Triglyceride | HDL-C | LDL-C |
| Control | 132.4±22 | 234.0±17 | 4.49±0.75 | 176.7±7.4 |
| *Myrtus communis* | 76.0±6.5 | **149.9±7.5 | *2.68±0.26 | 132.7±14.0 |

n = 7. Average value±SEM *P < 0.05 significant as compared to control. **P < 0.01highly significant as compared to control

**Table 2.** Effects of *Myrtus communis* on lipid profile after 45 days.

| Animal group | Parameter (mg/dl) | | | |
|---|---|---|---|---|
| | Cholesterol | Triglyceride | HDL-C | LDL-C |
| Control | 85.2±18 | 115.4±17 | 1.743±0.16 | 141.4±12 |
| *Myrtus communis* | 44.4±3.4 | *61.2±9.7 | 1.72±0.08 | **58.11±1.3 |

n = 7. Average value ± SEM. *P < 0.05 significant as compared to control. **P < 0.01highly significant as compared to control

the total period of experiment.

### Lipid profile

Table 1 gives the comparison of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) levels in animals of control and treated groups after 30 days, while a similar comparison between the same groups of animals after 45 days is presented in Table 2. Animals which received AEM for 30 days showed highly significant and significant decrease in the levels of TG and HDL-C that is, 149.9 ± 7.5 mg/dl and 149.9 ± 7.5 mg/dl in comparison to control values that is, 234.0 ± 17.0 mg/dl and 4.49 ± 0.75 mg/dl, respectively. Conversely there was no significant change in the levels of TC and LDL-C levels at the completion of dosing. Animals which received AEM for 45 days showed highly significant and significant decrease in the level of LDL-C and TG that is, 58.11 ± 1.3 mg/dl and 61.2 ± 9.7 mg/dl in comparison to control values that is, 141.4 ± 12.0 mg/dl and 115.4 ± 17 mg/dl. However the other parameters were not altered significantly at the end of dosing.

### Coagulation parameters

Table 3 discloses the comparison of thrombin time (TT), partial thrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen time in animals of control and treated animals, while a similar comparison between the same groups of animals after 45 days is presented in Table 4. Animals given AEM for 30 days showed significant increase in fibrinogen time that is, 57.3 ± 13 s as compared to control that is, 26.01 ± 2.9 s. However there were no significant changes in TT, aPTT and PT at the completion of dosing. Animals given AEM for 45 days showed highly significant increase in TT that is, 24.7 ± 1.9 s as compared to control, that is, 16.56 ± 1.4 s. However the other parameters were not altered significantly.

## DISCUSSION

AEM have been found to reduce LDL-C and increase fibrinogen time that is, decrease fibrinogen level which seems to be of clinical importance since there are studies which shows that increased fibrinogen has been associated with cardiovascular risks. Fibrinogen increases cardiovascular risk by several mechanisms since it specifically binds to activated platelets via glycoprotein IIb/IIIa, contributing to platelet aggregation, promotes fibrin formation and increase plasma viscosity (James et al., 2000).

Hypercholesterolemia is a strong risk factor for producing atherosclerosis. There is a strong relationship between blood lipids and coagulation parameters since acute changes in plasma lipids appear to have significant effects on factors affecting thrombosis (Eitzman et al., 2000). Coagulation abnormalities are usually present in critically ill patients and may contribute to morbidity and mortality; hence requires rapid examination to establish the underlying cause and to initiate corrective and supportive treatment (Marcel and Steven, 2006). Thus in the present study, highly significant decrease in LDL-C after 45 days and significant increase in the fibrinogen time after 30 days seems to be very important from clinical point of view since there has been a connection between augmented LDL-C and atherosclerosis. High plasma LDL-C concentration is one of the major risk factor for

**Table 3.** Effects of *Myrtus communis* on blood coagulation after 30 days.

| Animal group | Parameter (seconds) | | | |
|---|---|---|---|---|
| | TT | PT | aPTT | Fibrinogen |
| Control | 17.84±1.7 | 7.11±0.6 | 30.5±6.2 | 26.01±2.9 |
| *Myrtus communis* | 30.5±4.0 | 6.2±0.26 | 41.0±4.2 | *57.3±13 |

n = 7. Average value ± SEM. *P < 0.05 significant as compared to control.

**Table 4.** Effects of *Myrtus communis* on blood coagulation after 45 days.

| Animal group | Parameter (seconds) | | | |
|---|---|---|---|---|
| | TT | PT | aPTT | Fibrinogen |
| Control | 16.56±1.4 | 5.871±0.22 | 33.04±3.5 | 26.41±2.3 |
| *Myrtus communis* | **24.7±1.9 | 5.60±0.13 | 39.3±3.6 | 38.7±5.9 |

n = 7. Average value±SEM. **P < 0.01 highly significant as compared to control.

atherosclerosis (Ross, 1999).

There are studies which suggest that reducing LDL-C decreases the risk of CVD (Aghasadeghi et al., 2008). The decrease in LDL-C by AEM, may be due to the presence of myrtle oil (Jung et al., 2006), while natural compounds semi myrtucommulone and myrtucommulone A may also be responsible for potential anti-atherogenic effect of *Myrtus communis* (Rosa et al., 2008). Hypercholesterolemia induces oxidative stress, since it increases the formation of reactive oxygen species from membrane phospholipids during prostaglandin synthesis. Thus antioxidants and hypolipidemic agents suppress the development of hypercholesterolemic atherosclerosis and induce regression of atherosclerosis (Paul and Kailash, 2003). Therefore suppression of atherosclerosis is associated with decrease in oxidative stress and serum lipids (Kabiri et al., 2011). Hence it may be concluded that *M. communis* may have some role in the prevention of atherosclerosis due to its hypolipidemic and anti-inflammatory effects.

Present study also revealed significant increase in TT that indicates deficiency of fibrinogen or inhibition of thrombin (Lane et al., 2005). Hence prolonged TT may be the results of reduced activity of coagulation factors because factors IX and X (Di Cera, 2008), XI and XII are essentially required for thrombin generation (Gailani et al., 2007). Present study also revealed significant increase in fibrinogen time that is, decreased fibrinogen level after 30 days at 50 mg/kg dose. There are studies which show that increases in the fibrinogen levels are a strong risk factor for the development of CVD (Barazzoni et al., 2000; Zhao et al., 2011). Hence, AEM reduces the risk of vascular diseases by reducing the fibrinogen level.

## Conclusion

The present study was conducted to explore the effects of AEM on lipid profile and blood coagulation. The overall results of the study reveal AEM to be effective as hypolipidemic agent in the dose of 50 mg/kg and also have an effect on blood coagulation parameters which may be of value in CVD.

## ACKNOWLEDGEMENT

Authors are thankful to Herbion Pakistan Pvt. Limited for providing the extract.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

### REFERENCES

Aghasadeghi K, Zarei-Nezhad M, Keshavarzi A, Mehrabani D (2008). The prevalence of coronary risk factors in Iranian lor migrating tribe. Arch. Iran. Med. 11(3): 322-325.

Alcaraz MJ, Jimenez MJ (1989). Anti-inflammatory compound from *Sideritis javalambrensis* n-hexane extract. J. Nat. Prod. 52:1088-1091.

Aleksic V, Knezevic P (2013). Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiol. Res. http://dx.doi.org/10.1016/j.micres.2013.10.003.

Allen JK, Young DR, Blumenthal RS, Moy TF, Yanek LR, Wilder L, Becker LC, Becker DM (1996). Prevalence of hypercholesterolemia among siblings of persons with premature coronary heart disease. Arch. Intern. Med. 156(15): 1654–1660.

Asif HM, Akram M, Uddin S, Hasan ZU, Sami A, Iqbal A, Tauseef U, Bari A (2011). *Myrtus communis* Linn. (Pharmacological activity). J. Med. Plant Res. 5(26): 6257-6259.

Atzei AD (2003). Le piante nella tradizione popolare della Sardegna. Sassari, Italy: Carlo Delfino Editore. pp. 319–323.

Azaizeh H, Fulder S, Khalil K, Said O (2003). Ethnomedicinal knowledge of local Arab practitioners in the Middle East Region. Fitoterapia 74:98-108.

Banerjee SK, Maulik SK (2002). Effect of garlic on cardiovascular disorders: a review. Nutr. J. 1:4.

Barazzoni R, Zanetti M, Davanzo G, Kiwanuka E, Carraro P, Tiengo A, Tessari P (2000). Increased fibrinogen production in type 2 diabetic

patients without detectable vascular complications: correlation with plasmaglucagon concentrations. J. Clin. Endocrinol. Metab. 85(9):3121-3125.
Cakir A (2004). Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. and *Myrtus communis* L. from Turkey. Biochem. Systemat. Ecol. 32:809–816.
Chan K, Yin M, Chao W (2007). Effect of di-allyltrisulfide-richgarlicoil on blood coagulation and plasma activity of anticoagulation factors in rats. Food Chem. Toxicol. 45:502-507.
Diaz AM, Abeger A (1986). Study of the polyphenolic compounds present in alcoholic extracts of *Myrtus communis* L. seeds. An Real Acad. Farm. 52:541–546.
Di Cera E (2008). Thrombin Review. Mol. Aspects Med. 29:203-254.
Eitzman DT, Westrick RJ, Xu Z, Tyson J, Ginsburg D (2000). Hyperlipidemia promotes thrombosis after injury to atherosclerotic vessels in apolipoproteinE–deficient mice. Arterioscl. Throm. Vasc. 20(7):1831-1834.
Friedwald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of ultra centrifuge. Clin. Chem.18: 499-502.
Gailani D, Renne T (2007). Intrinsic pathway of coagulation and arterial thrombosis. Arteriosclerosis Thrombosis Vasc. Biol., 27: 2507-2513.
Gortzi O, Lalas S, Chinou I, Tsaknis J (2008). Reevaluation of bioactivity and antioxidant activity of *Myrtus communis* extract before and after encapsulation in liposomes. Eur. Food Res. Technol. 226:583-590.
James JS, Halit S, Geoffrey HT, Travis HM, Patrice S, Izabela L, Joseph MM, Peter FW, James EM, D'Agostino RB (2000). Association of Fibrinogen with Cardiovascular Risk Factors and Cardiovascular Disease in the Framingham Offspring Population. Circulation 102:1634-1638.
Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK (2006). Antidiabetic agents from medicinal plants. Curr. Med. Chem. 13:1203-1218.
Kabiri N, Asgary S, Setorki M (2011). Lipid lowering by hydroalcoholic extracts of *Amaranthus caudatus* L. induces regression of rabbit's atherosclerotic lesions. Lipids Health. Dis. 10: 89.
Lane DA, Philippou H, Huntington JA (2005). Directing Thrombin. Blood 6:2605-2612.
Mahmood ZA, Sualeh M, Mahmood SB, Karim MA (2010). Herbal treatment for cardiovascular disease the evidence based therapy. Pak. J. Pharm. Sci. 23(1):119-124.
Marcel L, Steven MO. (2006). Coagulation abnormalities in critically ill patients. Critical Care10:222 DOI: 10.1186/cc4975.
Martin T, Villaescusa L, De Sotto M, Lucia A, Diaz AM (1990). Determination of anthocyanin pigments in *Myrtus communis* berries. Fitoterapia 61:85.
National Research Council (1996). Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press. pp. 1-7.
Paul L, Kailash P (2003). Suppression of Oxidative Stress as a Mechanism of Reduction of Hypercholesterolemic Atherosclerosis by Cyclooxygenase Inhibitors. Int. J. Angiol. 12:13–23. DOI: 10.1007/s00547-003-0976-4.
Romani A, Pinelli P, Mulinacci N, Vincieri FF, Tattini M (1999). Identification and quantification of polyphenols in leaves of *Myrtus communis*. Chromatographia 49:17–20.
Rosa A, Melis MP, Deiana M, Atzeri A, Appendino G, Corona G, Incani A, Loru D, Dessì MA (2008). Protective effect of the oligomeric acylphloroglucinols from *Myrtus communis* on cholesterol and human low density lipoprotein oxidation. Chem. Phys. Lipids 155(1):16-23.
Ross R (1999). Atherosclerosis- An Inflammatory Disease. N. Engl. J. Med. 340(2):115-126.
Satrani B, Farah A, Talbi M. (2006). Fractional distillation effect on the chemical composition and antimicrobial activity of Moroccan Myrtle. Acta Bot. Gallica 153:235–242.
Serce S, Ercisli S, Sengul M, Gunduz K, Orhan E (2010). Antioxidant activities and fatty acid composition of wild grown myrtle (*Myrtus communis* L.) fruits. Pharmacogn. Mag. 6(21): 9-12.
Shikhiev AS, Abbasov RM, Mamedova ZA (1978). Composition of *Myrtus communis* essential oil. Khimiya Prirodnykh Soedinenii 4:529–530.
Srivastava SK, Rai V, Srivastava M, Rawat AKS, Mehrotra S (2006). Estimation of heavy metals in different *Berberis* Species and its market samples. Environ. Monit. Assess.116:315-320.
Trinder P (1969). Enzymatic colorimetric determination of triglycerides by GPO-PAP Method. Ann. Clin. Biochem. 6:24-27.
Verma AK, Kumar M, Bussmann RW (2007). Medicinal plants in an urban environment: the medicinal flora of Banares Hindu University, Varanasi, Uttar Pradesh. J. Ethnobiol. Ethnomed. 3:35.
Zhao Y, Zhang J, ZhangJ, Wu J (2011). Diabetes mellitus is associated with shortened activated partial thromboplastin time and increased fibrinogen values. PLoS One 6(1):e16470.