ICCBMS21., ICFNEAS21
August 19-20, 2021
Conference Book
ISBN 978-600-98459-7-2

Rosa canina L. Fruit Extract Attenuates Neuropathic Hyperalgesia in Diabetic Male Rats

Behrooz Khakpour Taleghani[1]*, Malek Moien Ansar[2]
[1]Department of Physiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran,(Email: khakpour_b@yahoo.com)
[2]Department of Biochemistry, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran,(Email: ansar_moien@yahoo.com)
*** Corresponding author:** Behrooz Khakpour Taleghani**,** Department of Physiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran,
(Email: khakpour_b@yahoo.com)

Abstract
Background and aim: Peripheral neuropathy is nerve damage caused by chronically high blood sugar and diabetes. Pain in feet, legs or in hands is one of the complications of diabetic neuropathy. Previous studies have shown hypoglycemic effect of Rosa canina L. (RC) fruit extract in diabetic rats. Since hyperglycemia plays an important role in diabetic neuropathy, RC extract may reduce neuropathic pain.
The aim of this study was to investigate the effect of RC fruit ethanolic extract on the streptozotocin (STZ)-induced model of diabetic neuropathy hyperalgesia in male rats.
Materials and Methods: In this experimental study, male wistar rats (250-300 gr) were divided into diabetic and non-diabetic groups. Diabetes was induced by STZ (50 mg/kg/i.p.). Both non-diabetic and diabetic groups were subdivided into 3 groups: control (received normal saline), RC fruit extract groups (250 and 500 mg/kg), diabetic control (received normal saline), diabetic groups which received RC extract (250 and 500 mg/kg) and positive control diabetic group (received sodium salicylate). The rats were intraperitoneally injected the extract every other day for 30 days. For confirmation and evaluation of neuropathy, formalin test was used. Data were analyzed by one-way ANOVA and Tukey's post hoc test. $P<0.05$ was considered as significant difference.
Results: Increased nociceptive response of diabetic rats in formalin test confirmed the development of neuropathy. Administration of both doses of RC extract reduced pain in acute ($P<0.001$) and chronic ($P<0.001$) phases of formalin test in the diabetic rats.
Conclusion: The present study findings showed that administration of RC fruit ethanolic extract improves nociceptive pain in diabetic neuropathy, probably mediated by reducing blood glucose level.
***Key words**: Neuropathy, Diabetes Mellitus, Rosa Canina Fruit Extract, Formalin test, Rat.*

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated serum glucose and impaired secretion and/or activity of insulin as its primary causative mechanisms as well as, various downstream pathophysiologic pathways like glycation, increased oxidative stress and inflammation [1, 2]. The number of diabetic patients was estimated 415 million in 2015 and it will reach 642 million by 2040 [3]. Painful diabetic neuropathy (PDN) is a microvascular complication of diabetic patients which can be manifested as hyperalgesia and allodynia by

ISBN 978-600-98459-7-2

increasing the activity of nerve fibers [4]. Like other chronic pain conditions, PDN is associated with substantial declines in quality-of-life including sleep, recreational activities, normal mobility, general activity, social activities, and mood and may account for up to 27% of diabetes costs [5,6].

PDN continues to represent therapeutic challenges; its pathophysiology has not yet been fully understood and its pain relief is still unsatisfactory. Various pharmacological and non-pharmacological interventions have been used to control PDN. Two main strategies to manage the diabetic neuropathy are control of blood glucose level and the treatment of neuropathy symptoms. The pharmacological treatments, with exception to those targeted to glycaemic control, including tricyclic antidepressants, selective serotonin and noradrenaline reuptake inhibitors, anticoagulants and opioid analgesics are symptomatic treatments, not focused on the pathophysiological mechanisms and still unsatisfactory and limited by side effects and the development of tolerance [7,8,9]. Since, PDN is still remains a great challenge for physicians, it is crucial to continue investigations towards influential treatments with more effective compounds and the least adverse effects [8]. Traditional medicinal formulations from plants and their active phytoconstituents are used globally as a therapy for diabetes and its complications. Several traditional medicines as well as herbs are well-known to prevent, treatment and delay the development of related complications of diabetes [10,11]. Experimental evidences suggest that Rosa canina L. (RC) has hypoglycemic, anti-oxidant, anti-inflammatory, analgesic and immune system modulatory properties [12,13,14]. As far as we know, there is no study on RC extract on diabetic neuropathy. The present study aimed to assess the effect of RC fruit extract on diabetic neuropathy-induced hyperalgesia in male rats.

Materials and methods

Preparation of RC hipe extract

The ripe fruits of RC were harvested from the height of 58 meters above sea level of Guilan province, Iran (Herbarium code: 5243 from the Department of Botany, Faculty of Agriculture, Azad University of Karaj). The fruits were dried at 24-26°C under natural conditions for 7 days and then powdered. The fruit powder was soaked in hydroalcoholic solution and extracted by Soxhlet method. The extract was dried by the oven apparatus at 40 to 50°C for 3 days and then used.

Experimental animals

In this study, male Wistar rats (250-300 gr) with blood glucose lower than 200 mg/dL were used. The blood specimens were taken from lateral tail vein. Four rats were placed in each cage under standard conditions with a temperature of 22 ± 2°C, and reverse light-dark cycle (12h/12h) with food and water available ad libitum. Before beginning the experiments, the animals were allowed to adapt to the laboratory environment for three days at least 15 minutes each day. On the test day, all rats were transferred to the laboratory at least 1hour before the experiment to adapt the environmental conditions. The ethics for use and care of experimental animals were followed strictly.

Experimental Design

For diabetes induction, a single dose of streptozotocin (STZ) (Sigma, USA) 50 mg/kg was injected intraperitoneally. The blood glucose was measured three days after STZ injection and the rats with blood glucose above 250 mg/dl were considered diabetic.

Fifty-six rats were divided into 7 groups (8 in each) as follows: 1) C: (non-diabetic control: received normal saline), 2) R250: (non-diabetic rats: received 250 mg/kg/i.p. RC fruit extract), 3) R500: (non-diabetic rats: received 500 mg/kg mg/kg/i.p. RC fruit extract), 4) D: (diabetic

ISBN 978-600-98459-7-2

control: received normal saline), 5) DR250: (Diabetic rats: received 250 mg/kg/i.p. RC fruit extract) and 6) DR500: (diabetic rats: received 500 mg/kg mg/kg/i.p. RC fruit extract). 7) DSS: (positive control diabetic group: received sodium salicylate). After 2 weeks, extract administration was initiated every other day for 30 days. The day after the last injection, formalin test was performed. DSS group received Sodium Salicylate (200 mg/kg/i.p.) (Sigma, Germany), one hour before the pain assessment. All tests were performed during 9:00 and 15:00. During our experimental period, a total of 10 rats (3 in group D, 3 in group DR500 and 4 in group DSS) died and were excluded from the study.

Formalin test

The rat was placed in a plexiglass chamber (35 × 35 × 35 cm). There was a mirror underneath the chamber with a 45° angle to fully determine the position of the foot of the rats. After 15 min adaptation period, 50 µl of 2.5% formalin solution was injected subcutaneously (s.c) in the plantar surface of the right hind paw of the animals. Nociception was evaluated by measuring the duration of licking and biting of the injected foot in the first 5 minutes (neurological or acute pain) and 15-30 minutes (inflammatory or chronic phase) [15].

Statistical analysis

The data normality was evaluated by the Shapiro test. The statistical significance of differences between groups was assessed with one-way ANOVA with Tukey post-test. If the data distribution was not normal, kruskal - wallis test was used. All data were analyzed using SPSS software version 22. $P<0.05$ was considered significant. Data were expressed as mean ±SEM.

Results

Anti-hyperalgesic effect of RC hipe extract in formalin test

The results of acute and chronic phases of formalin test have been shown in figures 1 and 2. The intraplantar formalin injection produced a response in two discrete stages in all groups. The nociceptive threshold in both acute and chronic phases of formalin test showed a significant decrease in diabetic control rats compared to non-diabetic control ($P<0.001$ and $P<0.05$, respectively). Treatment of diabetic rats with both doses of RC hipe extract (250, 500 mg/kg/i.p.) produced a significant decrease in the time of liking and biting extract received groups in both acute (Fig. 1) and chronic (Fig. 2) phases of formalin test compared to non-treated groups ($P<0.001$ and $P<0.001$, respectively). only chronic phase of formalin test was affected by sodium salicylate ($P<0.001$).

ISBN 978-600-98459-7-2

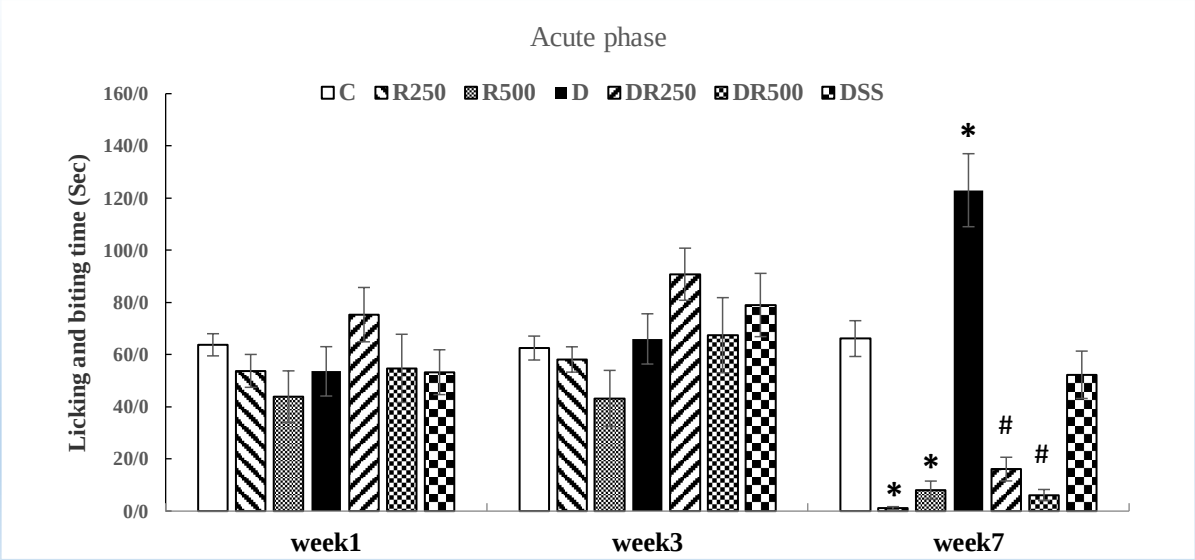

Figure 1. The effect of RC hipe extract administration on diabetic neuropathy-induced hyperalgesia in the first phase of formalin test. Licking and biting times compared between groups. All data were presented as the mean ± SEM. * P<0.001 compared to Group C, # P<0.001 compared to group D. C: Non-diabetic control: received normal saline, R250: Non-diabetic rats: received 250 mg/kg/i.p. RC extract, R500: Non-diabetic rats: received 500 mg/kg mg/kg/i.p. RC extract, D: Diabetic control: Received normal saline, DR250: Diabetic rats: Received 250 mg/kg/i.p. RC extract, DR500: Diabetic rats: Received 500 mg/kg /i.p. RC extract, DSS: Positive control diabetic group: Received sodium salicylate.

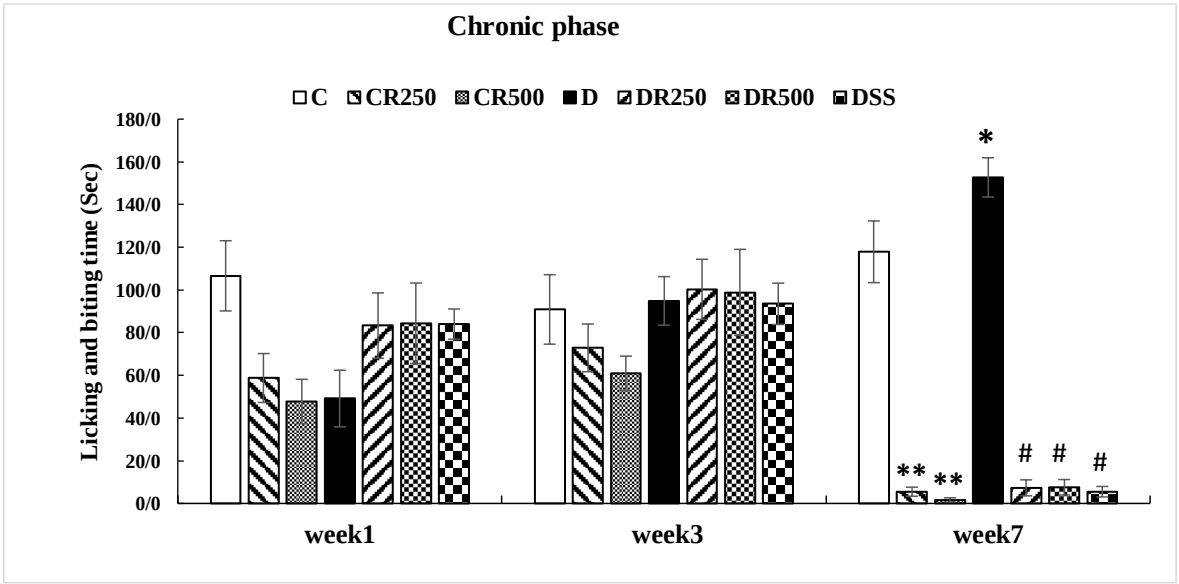

Figure 2. The effect of RC hipe extract administration on diabetic neuropathy-induced hyperalgesia in the second phase of formalin test. Licking and biting times compared between groups. All data were presented as the mean ± SEM. * P<0.05, ** P<0.001 compared to group C, # P<0.001 compared to group D. C: Non-diabetic control: received normal saline, R250: Non-diabetic rats: received 250 mg/kg/i.p. RC extract, R500: Non-diabetic rats: received 500 mg/kg mg/kg/i.p. RC extract, D: Diabetic control: Received normal saline, DR250: Diabetic rats: Received 250 mg/kg/i.p. RC extract, DR500: Diabetic rats: Received 500 mg/kg mg/kg/i.p. RC extract, DSS: Positive control diabetic group: Received sodium salicylate.

ISBN 978-600-98459-7-2

Discusion

The results of this study revealed that STZ reduced nociceptive threshold in diabetic rats and chronic administration of RC fruit extract (250 and 500 mg/kg) to diabetic rats could improve neuropathic hyperalgesia which was evaluated by formalin test. STZ-induced diabetic neuropathy is an accepted animal model of diabetic neuropathy and provides reproducible and reversible diabetes [16]. There are some reports regarding the reducing effect of RC fruit extract on blood glucose of diabetic rats [17]. Also, researchers found that the effect of RC fruit is similar to glibenclamide, a standard blood glucose lowering drug. It has been suggested that RC can be used as a synergist with glucose lowering drugs [18]. On the basis of previous studies, RC fruit extract reduces blood glucose by different mechanisms like increased insulin secretion [17] and inhibition of alpha-glucosidase and alpha amylase enzymes [19,20]. Moreover, RC fruit contains various bioactive substances including vitamin C, flavonoids (quercetin, rutin and kaempferol), ursolic acid, betulinic acid, oleanolic acid, lycopene, linoleic acid, alpha linolenic acid and lutein [21], most of them have anti-diabetic effects [22,23]. Obtained data from the formalin test, revealed hyperalgesia in diabetic rats. Administration of RC extract (250 and 500 mg/kg/i.p.) showed a clear analgesic effect and alleviated both the neuropathic and inflammatory pain in formalin test, while sodium salicylate resulted in antinociception only in the second phase. Previous studies showed that behavioral signs of neuropathy began 4 weeks after STZ injection or by four weeks from the onset of diabetes [24]. In the present study licking and biting time altered after 7 weeks. The formalin test is a biphasic test in which the first phase is modulated by spinal cord and central nervous system but the second phase occurs despite minimal input to the spinal cord and is an inflammatory phase mediated by prostaglandins, bradykinin, histamine, sympathomimetic amines, TNF-α, and interleukins [25,26]. Several studies have confirmed the analgesic effect of RC extract. It has shown that the analgesic effect of RC fruit powder is higher than first-line clinical analgesic drugs such as acetaminophen and paracetamol [27]. One-year use of RC fruit powder caused significant relief in low back pain [28]. RC fruit hydroalcoholic extract has a strong inhibitory effect on the second phase of the hind paw edema in carrageenan-induced mice, and its anti-inflammatory potency is similar to indomethacin [29]. Antinociceptive effect of many components of RC extract have been revealed. Flavonoids such as rutin and quercetin exert analgesic effect in the first phase of the formalin test [30,31,32]. Flavonoids can cross the blood-brain-barrier and control the pain centrally in various ways, including effect on $GABA_A$, opioids and alpha-adrenergic receptors, and inhibition of inflammatory enzymes in the brain [30]. Also, they reduce inflammation and oxidative stress in peripheral nerves and decrease hyperalgesia in diabetic rats by reducing the activity of the complement system and the inflammatory factors such as TNF-α, IL-1β, NF-κB and IL6 [31,33]. It has been reported that the antioxidant capacity of flavonoids even exceeds vitamin E and C [34]. In addition, there is a significant correlation between total phenol content of RC fruits and its inhibitory effect on H_2O_2 [35]. Moreover, many other components of RC extract including triterpene acids (oleanolic acid, ursolic acid and betulinic acid), linoleic and α-linolenic acids, inhibit the activity of NF-kB, Cox2 and lipoxygenase [14,29].

Conclusion

Although the mechanism of diabetic neuropathy is complicated, it is believed that hyperglycemia is the main reason of neuronal damage [36]. Therefore, it seems that in the present study anti-nociceptive effect of RC fruit extract may mainly be due to its hypoglycemic,

ISBN 978-600-98459-7-2

anti-oxidant and anti-inflammatory effects. The obtained results suggest a central and/or peripheral mechanism for its protective effect.

References

[1]. Dilworth, L, Facey, A, Omoruyi, F. Diabetes mellitus and its metabolic complications: The role of adipose tissues. International Journal of Molecular Sciences. 2021; 22: 7644.
[2]. Nigel A, Calcutt. Diabetic neuropathy and neuropathic pain: a (con) fusion of pathogenic mechanisms? Pain. 2020; 161: S65–S86.
[3]. Ogurtsova K, da Rocha Fernandes J, Huang Y, Linnenkamp U, Guariguata L, Cho N, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040Diabetes Res Clin Pract 2017;128:40-50.
[4]. Falo CP, Benitez, R, Caro M, Morell, M, Forte-Lago I, Hernandez-Cortes P, et al. The neuropeptide cortistatin alleviates neuropathic pain in experimental models of peripheral nerve injury. Pharmaceutics. 2021; 13: 947.
[5]. Gupta, M, Knezevic, N N,Abd-Elsayed, A Ray, M Patel K, Chowdhury B. Treatment of painful diabetic neuropathy- A narrative review of pharmacological and interventional approaches. Biomedicines. 2021; 9: 573.
[6]. Gonçalves NP, Vaegter CB, Andersen H, Østergaard L, Calcutt NA, Jensen TS. Schwann cell interactions with axons and microvessels in diabetic neuropathy. Nature Reviews Neurology. 2017;13(3):135-147.
[7]. Shazli Azmi, Uazman Alam, Jamie Burgess, Rayaz a Malik. State-of-the-art pharmacotherapy for diabetic neuropathy. Expert Opinion on Pharmacotherapy. 2021; 22(1): 55-68.
[8]. Naderi Nabi B, Saberi A, Bakhshayesh Eghbali B, Hosseininezhad M, Biazar G, Abbasi Malekabadi A et al . Efficacy and Safety of TENS and Duloxetine in patients with painful diabetic neuropathy: A single blind randomized clinical trial. J Adv Med Biomed Res. 2021; 29 (136) :287-293.
[9]. Nazma Akter. Diabetic peripheral neuropathy: Epidemiology, physiopathology, diagnosis and treatment. Delta Medical College Journal. 2019;7(1):35-48.
[10]. Arun K Jugran, Sandeep Rawat,| Hari P Devkota, Indra D Bhatt, Ranbeer S Rawal. Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. Phytotherapy Research. 2021;35:223–245.
[11]. Gushiken LF, Beserra FP, Rozza AL, Bergamo PL, Bergamo DA, Pellizzon CH. Chemical and biological aspects of extracts from medicinal plants with antidiabetic effects. The review of diabetic studies: RDS. 2016;13(2-3):96-112.
[12]. Orhan N, Aslan M, Hosbas S, Deliorman OD. Antidiabetic effect and antioxidant potential of Rosa canina fruits. Pharmacognosy Magazine. 2009;5(20):309-315.
[13]. Orhan DD, Hartevioğlu A, Küpeli E, Yesilada E. In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from Rosa canina L. fruits. Journal of Ethnopharmacology. 2007;112(2):394-400.
[14]. Saaby L, Jäger AK, Moesby L, Hansen EW, Christensen SB. Isolation of immunomodulatory triterpene acids from a standardized rose hip powder (Rosa canina L.). Phytotherapy Research. 2011;25(2):195-201.
[15]. Chunhong Liu, Mingming Ma, Chaoling Wen, Raihan Uz Zaman, Opeyemi Joshua Olatunji. Antiallodynic and antihyperalgesia effects of Tiliacora triandra against cisplatin-induced peripheral neuropathy. All Life. 2021;14(1), 441-449.

ISBN 978-600-98459-7-2

[16]. Sima AA, Zhang WX, Tze WJ, Tai J. Diabetic neuropathy in STZ-induced diabetic rats and effect of allogenic islet cell transplantation: Morphonometric analysis. Diabetes.1988;37(8):1129-1136.
[17]. Chen SJ, Aikawa C, Yoshida R, Kawaguchi T, Matsui T. Anti-prediabetic effect of rose hip (Rosa canina) extract in spontaneously diabetic Torii rats. Journal of the Science of Food and Agriculture. 2017; 97(12):3923-3928.
[18]. Taghizadeh M, Rashidi AA, Taherian AA, Vakili Z, Sajad Sajadian M, Ghardashi M. Antidiabetic and antihyperlipidemic effects of ethanol extract of Rosa canina L. Fruit on diabetic rats: An experimental study with histopathological evaluations. Journal of Evidence-Based Complementary and Alternative Medicine. 2016;21(4):NP25-NP30.
[19]. Asghari B, Salehi P, Farimani MM, Ebrahimi SN. [alpha]-Glucosidase inhibitors from fruits of Rosa canina L. Records of Natural Products. 2015;9(3):276-283.
[20]. Jemaa HB, Jemia AB, Khlifi S, Ahmed HB, Slama FB, Benzarti A, et al. Antioxidant activity and a-amylase inhibitory potential of Rosa Canina L. African Journal of Traditional Complementary and Alternative Medicines. 2017;14(2):1-8.
[21]. Patel S. Rose hips as complementary and alternative medicine: overview of the present status and prospects. Mediterranean Journal of Nutrition and Metabolism. 2013;6(2):89-97.
[22]. Triggiani V, Resta F, Guastamacchia E, Sabba C, Licchelli B, Ghiyasaldin S, et al. Role of antioxidants, essential fatty acids, carnitine, vitamins, phytochemicals and trace elements in the treatment of diabetes mellitus and its chronic complications. Endocrine, Metabolic and Immune Disorders - Drug Targets. 2006;6(1):77-93.
[23]. Yasunari K, Kohno M, Kano H, Yokokawa K, Minami M, Yoshikawa J. Antioxidants improve impaired insulin-mediated glucose uptake and prevent migration and proliferation of cultured rabbit coronary smooth muscle cells induced by high glucose. Circulation.1999;99(10):1370-1378.
[24]. Luippold G, Bedenik J, Voigt A, Grempler R. Short-and longterm glycemic control of streptozotocin-induced diabetic rats using different insulin preparations. PLoS ONE. 2016;11(6): e0156346.
[25]. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain. 1989; 38:347-352.
[26]. Ferreira AA, Amaral FA, Duarte IDG, Oliveira PM, Alves RB, Silveira D, et al. Antinociceptive effect from Ipomoea cairica extract. Journal of Ethnopharmacology. 2006; 105:148-153.
[27]. Christensen R, Bartels E, Altman R, Astrup A, Bliddal H. Does the hip powder of Rosa canina (rosehip) reduce pain in osteoarthritis patients?–a meta-analysis of randomized controlled trials. Osteoarthritis and Cartilage. 2008;16(9):965-972.
[28]. Chrubasik C, Wiesner L, Black A, Müller-Ladner U, Chrubasik S. A one-year survey on the use of a powder from Rosa canina lito in acute exacerbations of chronic pain. Phytotherapy Research. 2008;22(9):1141-1148.
[29]. Lattanzio F, Greco E, Carretta D, Cervellati R, Govoni P, Speroni E. In vivo anti-inflammatory effect of Rosa canina L. extract. Journal of Ethnopharmacology. 2011;137(1):880-885.
[30]. Akbar S, Subhan F, Karim N, Shahid M, Ahmad N, Ali G, et al. 6-Methoxyflavanone attenuates mechanical allodynia and vulvodynia in the streptozotocin-induced diabetic neuropathic pain. Biomedicine Pharmacotherapy. 2016;84:962-971.
[31]. Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin. Biomedicine Pharmacotherapy. 2017;96:305-312.

ISBN 978-600-98459-7-2

[32]. Anjaneyulu M, Chopra K. Quercetin, a bioflavonoid, attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2003;27(6):1001-1005.
[33]. Shi Wu, Yaobin Pang, Yingjie He, Xiaotong Zhang, Li Peng, Jing Guo, Jinhao Zeng. A comprehensive review of natural products against atopic dermatitis: Flavonoids, alkaloids, terpenes, glycosides and other compounds. Biomedicine and Pharmacotherapy. 2021; 140:111741.
[34]. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82(4):513-523.
[35]. Kiliçgün H, Altiner D. Correlation between antioxidant effect mechanisms and polyphenol content of Rosa canina. Pharmacognosy Magazine. 2010;6(23):238-241.
[36]. Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A.Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. International Journal of Endocrinology. 2014; ID:674987.