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IN VIVO STUDIES OF POLYDATIN IN PERIPHERAL NEUROPATHIC PAIN MODEL ON MALE SPRAGUE DAWLEY RATS

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ABSTRACT

Aim: Polydatin is an active ingredient from polygonum cuspidatum herb, a Chinese plant. Because available allopathic drugs have lot of side effects and unproven therapeutic benefit, polydatin like herbal drug would be a correct safer alternative to treat peripheral neuritis. Peripheral neuropathy (PN) affects peripheral nerves and causes neuropathic pain. Peripheral neuropathy may be caused by diseases like diabetes, drugs like paclitaxel or by nerve injuries. We have done this present study to explore the ameliorative action of Polydatin on peripheral neuropathy induced by paclitaxel on male Sprague dawley rats. **Methods:** Peripheral neuropathy was induced in Sprague dawley rats in group-II by paclitaxel (2mg/kg/2days) intra peritoneal injection (i.p) over the course of one week. Group 4 and 5 administered with Polydatin100mg/kg and 200mg/kg respectively (from 7th day onwards) orally. Group 3 rats were administered with standard drug tramadol orally. Experimental rats from groups 1 to 5 were subjected to in vivo studies like hot plate, cold plate, rorarod performance and locomotor activity and behavioral tests like food eating, water drinking, and weight checking. These values were subjected to one-way ANOVA (Analysis of Variance) statistical method followed by Dunnets't test to assess the significant difference between the groups and diseased (Group II). **Results:** Decrease in jumping and paw licking response in tail flick and cold plate methods, and increase in food intake, water intake and body weight, increased rotarod and locomotor activity in polydatin group animals were observed when compared with the disease group (group II) rats. **Conclusion:** Our study demonstrated the potential of polydatin in ameliorating paclitaxel-induced peripheral neuropathy.

KEYWORDS: Peripheral neuropathy, Polydatin, Paclitaxel induced neuropathy, Neuroprotective, Polygonum cuspidatum, tramadol.

INTRODUCTION

Peripheral neuropathy affects peripheral sensory, motor, and autonomic nerves. Neuropathic pain can be caused by several different diseases like diabetes mellitus, herpes zoster, and human immunodeficiency virus infection and medical interventions like chemotherapy drugs, surgery.[1,2] Drug- induced peripheral neuropathy estimated to be 4% of all neuropathies. 60% patients undergoing chemotherapy will develop peripheral neuropathic pain.[3] Polydatin is an important active ingredient in Polygonum cuspidatum.[5] Literature survey shows polydatin has many pharmacological effects like neuroprotective, anti-inflammatory, antioxidant, hepatoprotective, cardio protective, nephroprotective, and many other pharmacological effects.[6] Polygonum cuspidatum has various biological active ingredients, including polydatin, resveratrol and anthraquinone compounds like emodin and anthraglycoside AB.[7,8] Polygonum cuspidatum also contains flavonoids, tannin and polysaccharides. Polydatin has good oral bioavailability and pharmacokinetic profile. Polydatin scavenge the reactive oxygen species and inhibiting NLRP3 inflammasome (NOD- LRR- and pyrin domain-containing protein 3) and signaling of NK-Kb (Nuclear Factor Kappa B) which have involvement in cancer survival. The incidence of chemotherapy induced peripheral neuropathy (CIPN) from the taxenes group drugs (paclitaxel) may be very high, ranges from 11 to 87 %.[9] Chemotherapy induced neuropathic pain has dose dependent side effect which ultimately lead to either dose reduction or discontinuation of anticancer drug treatment. This reduces the life quality of patients. Therefore, a newer alternative drug is needed. The clinical syndromes of peripheral neuropathy are allodynia, hyperalgesia, and sensations like burning, numbness, spasm and other. Multifactorial pathophysiological processes involved in paclitaxel induced neuropathic pain are oxidative stress, apoptosis, calcium homeostasis alteration, axonal degeneration, immunity processes remodeling of membranes and neuroinflammation due to elevated release of proinflammatory cytokines.[4] Higher mitochondrial reactive oxygen species (ROS) cause mitochondrial and blood brain barrier damage. Taxanes family drugs like paclitaxel act by inhibition of microtubule disaggregation.[10] This study hypothesized that since poldatin has antioxidant and anti-inflammatory effects polydatin would be a good choice for the treatment of peripheral neuropathic pain.[11] Polydatin also exhibit antitumor effect which will be an added advantage in cancer patients.[12]

MATERIALS AND METHODS

Materials used and their source

Polydatin drug was purchased commercially from sigma life sciences. Paclitaxel (Taxeleon) was obtained from nest pharma services. Tramadol capsules were purchased from kasthuri medicals, Chennai.

Ethics: All experimental protocols of our study were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC).

Ethical guidelines given by CPCSEA and IAEC were followed during the animal experimentation of this study. **IAEC with no: 20/321/PO/Re/S/01/CPCSEA/date 14/03/20**

Experimental animals: Male SD rats of 150-200 g weight between 8-10week age group were used for this study. Thirty rats were divided into five groups; each group consisting of six rats. Rats were housed in standard lab conditions at a temperature of 22 ± 20C under 12 hours' dark and light cycle, standard pellets and drinking water were provided.

Induction of peripheral neuropathic pain

Peripheral neuropathic pain was induced in SD rats of group II, III, IV, and V by i.p administration[10] of anti-cancer drug paclitaxel (2mg/kg) injected four times/week. The nociceptive threshold for pain and other parameter was assessed after the 7 th, 14th, and 21 days. Histopathology parameters were assessed on twenty first day.[13]

Polydatin administration: Polydatin was orally administered to group IV and V rats by oral gavage at the dose level of 100mg and 200mg/kg/day from day 7 after Paclitaxel injection was stopped.[14]

Table 1: Treatment schedule for different groups.

Group	Treatment
I	control
Ii	Paclitaxel (2mg/kg/2d) i.p
Iii	Paclitaxel (2mg/kg/2d) + tramadol(20mg/kg/d) (p.o)
Iv	Paclitaxel (2mg/kg/2d) + polydatin 100mg/kg/d (p.o)
V	Paclitaxel (2mg/kg/2d) + polydatin 200mg/kg/d (p.o)

In-Vivo studies

Cold plate method

Place the rats on a cold plate that was cooled by the circulating cold water under it with the temperature set between −5–15 ◦C. (Ma et al, 2010). Maintain the room temperature at 21 ± 1 ◦C. The rats were placed on the cold plate and the time take for jumping or licking was recorded. The latency for cold pain withdrawal of these rats was recorded. A cut-off time 150 seconds was observed during the test.[15,20]

Tail flick method

Tail flick test was done by immersion of rat's tail into a hot water bath. Water temperature was maintained at 48-55◦ c. Twenty second cut off time was observed during the study. Sprague Dawley (SD) rats were commonly used in paclitaxel induced peripheral neuropathic pain model because of consistent induction of thermal and mechanical allodynia and convenient of behavioral readouts.[15,20]

By this method the temperature of hot bath can be maintained at the desired temperature.[16] Application of a heat stimulus to the rat tail and time taken for the tail to "flick" was recorded.

Rota-rod test

The effect of polydatin on motor performance was tested by using an accelerated rota-rod.[17] Rats were placed on drum which is rotating with the speed 4-40 rpm. Rats were forced to walk forward on the drum to avoid falling. Rats were given trial before the experiments. Basal response time was measured and then measure the time of falling after the polydatin drugs administered.

Assessment of locomotor activity

Locomotor activity (horizontal activity) was measured using Actimeter. Inside the cages, projection of infrared beam of light from one side to other. Each time animal moves, it breaks the beam that will be recorded by computer and displayed digitally. Each rat was placed individually in the activity cage floor for 10 min and the locomotion count was observed from the Actimeter.[18]

Behavioral tests

Weight variation test

Rats were weighed during cage changes, once in a week. Three values were taken and average weight was taken.[19, 22]

Food intake test

The amounts of food consumed were determined by weighing the food containers (open cups) before and after each test period. The amounts of food spilt were also measured and appropriate allowances determined by preliminary experiments were made for leakage, and to measure the grams in food and food intake, were determined for 24 hours per week during the study period when the animals were kept in individual metabolic cages.

Measured amounts of food were provided at the beginning when animals were transferred to metabolic cages and at the end of 24 hours the remaining amounts were measured to determine the food intake over 24 hours.[19]

Water intake

Water intake was studied by taking the weight of water bottles before and after each period. Allowances were made any leakage whenever keep the water bottles inside the cage.[21]

Statistical analysis

The statistical analysis was carried out by one-way ANOVA followed by Dunnet's t test. P value < 0.05(95% confidence limit) was considered statistically significant, using software graph pad prism 9.

RESULTS AND DISCUSSION

Cold plate method

When compared with group II, group III (P value<0.004) and group V rats (P value<0.006) showed significant decrease in jumping and paw licking time in cold plate. Group IV rats showed non-significant decrease (Fig.1).

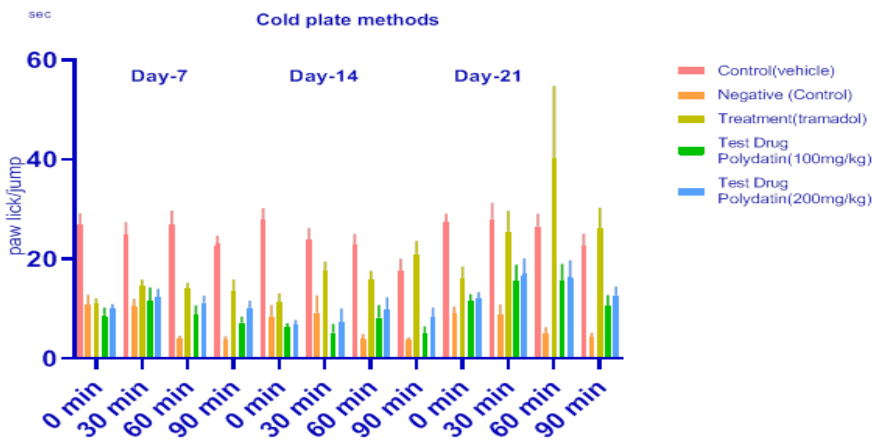

Fig. 1: Effect of polydatin in cold plate method.

Values are represented in mean ± SEM, n=6, Comparison: Group II vs Group III and Group IV and Group V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test, ns-non-significant, *p<0.5, **p<0.01, ***p<0.001, ****p<0.0001.

Tail flick method

The group III (P value<0.0001) and Group V (P value<0.003) showed a significant decrease in time taken for tail withdrawal when compared with group II whereas group IV showed the non-significant decrease in time when compared group II as given Fig.2.

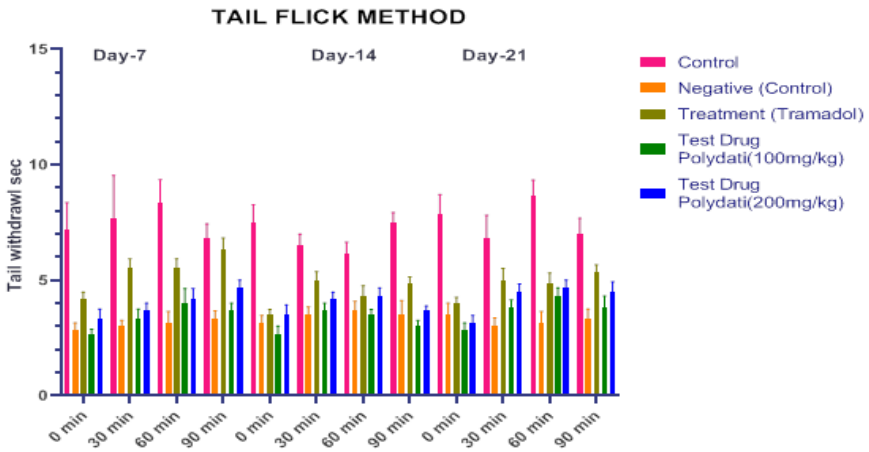

Fig. 2: Effect of polydatin in tail flick method.

Rota-rod test

The Group III (P value < 0.09) rats showed significant increase in average latency of falling time but group IV and V rats showed non-significant increase in falling time when compared with group II (Fig.3).

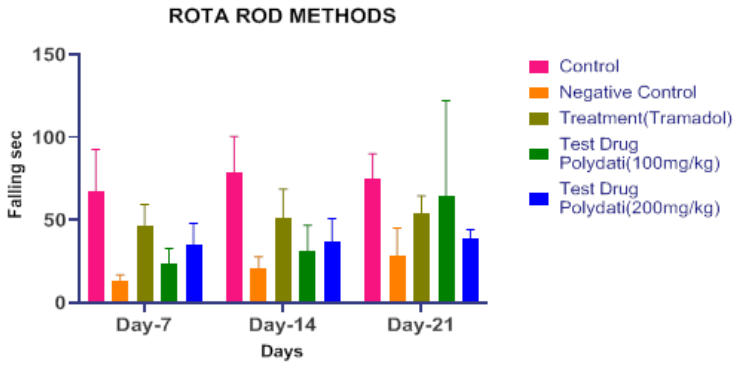

Fig. 3: Effect of polydatin on muscle coordination (Rota rod method).

Assessment of locomotor activity

In the locomotor grooming and rearing were observed and when compared with negative control, group IV (P value <0.0012) showed significant and group III and group V showed non-significant increase in the locomotor activity when compared with group II (Fig.4).

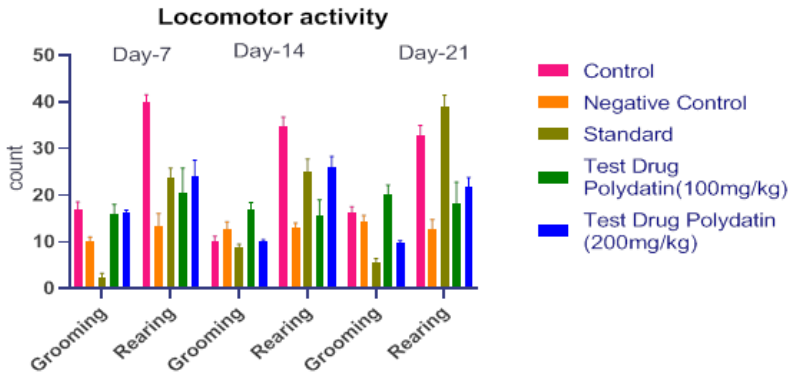

Fig. 4: Effect of polydatin on loco motor activity of rats (Grooming and rearing).

Weight variation test

The group III, IV, V showed non-significant increase the body weight when compared to the Negative control group II (Fig.5).

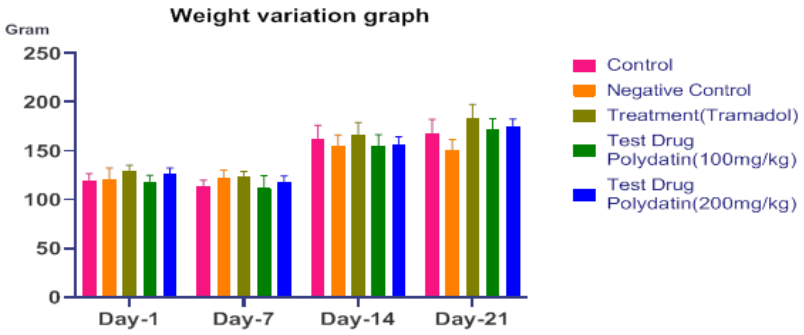

Fig. 5: Effect of polydatin on animal weight.

Food and water intake test

The group III, IV, V showed non-significant increase the food intake when compared to the Negative control group II (Fig.6 and 7).

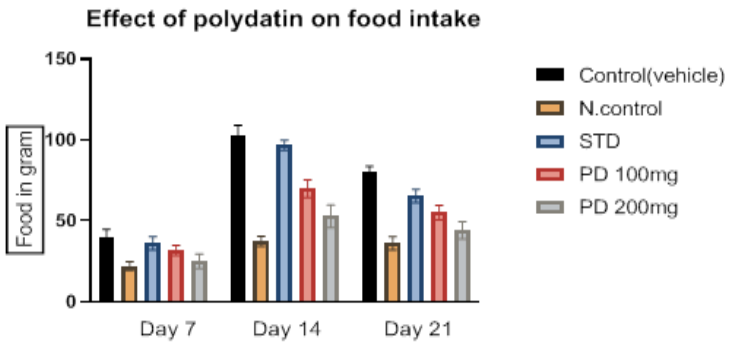

Fig. 6: Effect of polydatin on food intake.

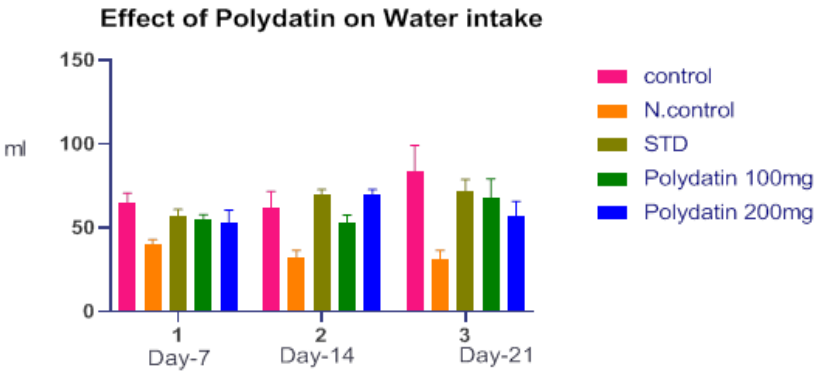

Fig. 7: Effect of polydatin on water intake.

DISCUSSION

Our study is the first study on polydatin for its curative effect on paclitaxel induced neuropathic pain on male Sprague dawley rats. In cold plate and tail flick method polydatin 200mg dose produced significant analgesic effect compared to 100mg dose. Analgesic effect of polydatin has been found out in our study for the first time. Only anti-inflammatory activity had been reported in previous studies.[11,18] In rota-rod test after polydatin treatment rats staying time on the rod was reduced because of decreased muscle grip due to muscle relaxation. Locomotor activity reflects the health status of rat which was depressed after paclitaxel. Grooming and rearing activities are improved after polydatin treatment. Apart from acting as scavenger of reactive oxygen species, polydatin inhibit NLRP3 inflammasome (NOD- LRR- and pyrin domain-containing protein 3) and NF-kB (Nuclear Factor Kappa B) signaling which are cancer survivors.[12] Poldatin is not only attenuate peripheral neuropathic

pain in cancer patients also reducing pro-inflammatory cytokines and act as anticancer agent.[25,26]

Polydatin treatment resulted in weight gain non-significantly. This effect may be due to its antioxidant activity. Water intake of rat also improved after polydatin treatment in rats. The limitation of this study is no effort has been taken to study about mechanism of action of polydatin at molecular level in curing paclitaxel induced peripheral neuropathic pain [24]. Further research is needed in this area.

CONCLUSION

The study reveals the potential of Polydatin in curing peripheral neuropathy induced by paclitaxel apart from analgesic effect. Polydatin can be considered as alternative safer drug for peripheral neuropathic pain to synthetic drugs.

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Conflict of interest

There are no conflicts of interest.

REFERENCES

1. Treede R- D, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, et al. Neuropathic pain: redefinition and a grading system for clinical research purposes. *Neurology*, 2008; 70(18): 1630-35.
2. Attal N, Lanteri-Minet M, Laurent B, Fermanian J and Bouhassira, D. The specific disease burden of neuropathic pain: results of a French nationwide survey. *Pain*, 2011; 152(12): 2836–43. https://europepmc.org/article/med/22019149
3. Hecke OV, Austin SK, Khan RA, Smith BH and Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. *Pain*, 2014; 155(4): 654–662. https://pubmed.ncbi.nlm.nih.gov/24291734/

4. Ma J, Kavelaars A, Dougherty PM and Heijnen CJ. Beyond symptomatic relief for chemotherapy induced peripheral neuropathy: Targeting the source. *Cancer,* 2018; 124(11): 2289- 98. https://pubmed.ncbi.nlm.nih.gov/29461625/
5. Yang F, Zhang T and Ito Y. Large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B from Polygonum cuspidatum Sieb. et Zucc by high-speed counter-current chromatography. *J Chromatogr A,* 2001; 919(2): 443–48. https://pubmed.ncbi.nlm.nih.gov/11442052/
6. Regev-Shoshani G, Shoseyov O, Bilkis I and Kerem Z. Glycosylation of resveratrol protects it from enzymic oxidation. *Biochem J,* 2003; 374: 157–63. https://pubmed.ncbi.nlm.nih.gov/12697026/
7. Wu M, Li X, Wang S, Yang S, Zhao R, et al. Polydatin for treating atherosclerotic diseases: A functional and mechanistic overview. *Biomed Pharmacother,* 2020; 128. https://www.sciencedirect.com/science/article/pii/S075333222030500X
8. ZhangY, Zheng L, Zheng Y, Zhou C, Huang P, et al. Assembly and annotation of a draft genome of the medicinal plant Polygonum cuspidatum. *Front Plant Sci,* 2019; 10(1274): 9. https://www.frontiersin.org/articles/10.3389/fpls.2019.01274/full
9. Banach M, Juranek JK and ZygulskaAL. Chemotherapy-induced neuropathies-a growing problem for patients and health care providers. *Brain Behav,* 2016; 7(1): e00558.
10. Starobova H, Vetter I. Pathophysiology of chemotherapy induced peripheral nuropathy. *Front Mol Nurosci,* 2017; 10: 174. https://pubmed.ncbi.nlm.nih.gov/28620280/
11. 11.PeritoreAF,Amico RD, Cordaro M, Siracusa R, Fusco R, et al. PEA/Polydatin: Anti-Inflammatory and Antioxidant Approach to Counteract DNBS-Induced Colitis. *Antioxidants,* 2021; 10(3): 464.
12. Quagliariello V, Berretta M, Buccolo S, Lovine M, Paccone A, et al. Polydatin reduces cardiotoxicity and enhances the anticancer effects of sunitinib by decreasing pro-oxidative stress, pro-inflammatory cytokines, and NLRP3 inflammasome expression. *Front Oncol,* 2021; 11: 2188.
13. Boyette-Davis J, Xin W, Zhang H and Dougherty PM. Intraepidermal nerve fiber loss corresponds to the development of taxol-induced hyperalgesia and Can be prevented by treatment with minocycline. *Pain,* 2011; 152(2): 308–13. https://pubmed.ncbi.nlm.nih.gov/21145656/
14. Guan S-Y, ZhangK, Wang X-S, Yang L, Feng B, et al. Anxiolytic effect of polydatin through the blockade of neuroinflammation in a chronic pain mouse model. *Molecular Pain,* 2019; 16: 1-13.

15. Huynh PN, Giuvelis D, Christensen S, Tucker KL and McIntosh JM. RgIA4 accelerates recovery from paclitaxel-induced neuropathic pain in rats. *Mar Drugs*, 2019; 18(1): 12.
16. Ma, Chao, Zhang and Jun- Ming. Animal Models of Pain. Totowa, NJ, USA; Humana Press: 2010.
17. Allchorne AJ, Broom DC and Woolf CJ. Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. *Mol Pain*, 2005; 1(36). https://pubmed.ncbi.nlm.nih.gov/16354295/
18. Sheik WME, Alahmar IE, Salem GM, and El-Sheikh MA. Tumor necrosis factor alpha in peripheral neuropathy in type 2 diabetes mellitus. *Egypt J Neurol Psychiatr Neurosurg*, 2019; 55: 37.
19. Deuis, J R, Dvorakova, L S and Vetter I. Methods used to evaluate pain behaviors in rodents. *Front Mol Neurosci*, 2017; 10: 284. https://www.frontiersin.org/articles/10.3389/fnmol.2017.00284/full
20. Chen L, Chen W, Qian X, Fang Y and Zhu N. Liquiritigenin alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model. *Sci Rep*, 2014; 4: 5676.
21. Kumar R, Mitchell E and Stolerman I P. Disturbed patterns of behaviour in morphine tolerant and abstinent rats. *Br J Pharmac*, 1971; 42(3): 473-484.
22. Kasanen IHE, Inhila KJ, Nevalainen JI, et al. A novel dietary restriction method for group-housed rats: weight gain and clinical chemistry characterization. *Lab Anim*, 2009; 43: 138–148.
23. Yasar H, Ersoy A, Yazici GN, Suleyman H, Ozgeris FB, et al. The effect of lutein on paclitaxel-induced neuropathy and neuropathic pain in rats. *Int J Clin Exp Med*, 2020; 13(6): 4316-23.
24. Brzezinski K. chemotherapy-induced polyneuropathy, part 1 pathophysiol. *Contemp Oncol*, 2012; 16: 72-8. https://pubmed.ncbi.nlm.nih.gov/23788859/
25. Li B, Wang X-L. Effective treatment of polydatin weakens the symptoms of collagen-induced arthritis in mice through its anti-oxidative and anti-inflammatory effect and the activation of MMP9. *Mol Med Rep*, 2016; 14: 5357-62. https://pubmed.ncbi.nlm.nih.gov/27840936/
26. Shah FA, Kury LA, Li T, Zeb A, Koh PO, et al. Polydatin attenuates neuronal loss via reducing neuro inflammation and oxidative stress in rat MCAO models. *Front Pharmacol*, 2019; 10(663).