AJBMS AFGHANISTAN Journal of Basic Medical Sciences
ISSN: 3005-6632

Afghanistan Journal of Basic Medical Sciences

2025 Jan 2(1):89-96.

ajbms.knu.edu.af

Effects of Aqueous Extract of Afghan *Hibiscus sabdariffa L.* on Morphine Withdrawal Symptoms in Male Rats

Meysam Sajjadi [1], Adam Khan Alipour [1], Dawood Hossaini [2], *Murtaza Haidary [2,3]

1. 1. Department of Chemistry and Biochemistry, Faculty of Medical Laboratory Technology, Khatam Al- Nabieen University, Kabul, Afghanistan
2. Department of Biology and Microbiology, Faculty of Medical Laboratory Technology, Khatam Al- Nabieen University, Kabul, Afghanistan
3. Medical Research and Technology Center, Khatam Al-Nabieen University, Kabul, Afghanistan

ARTICLE INFO

Type: Original Article
Received: 25 August, 2024
Accepted: 25November, 2024

*Corresponding Author:
E-mail: murtaza.haidary@knu.edu.af

To cite this article: Sajjadi M, Alipour AK, Hossaini D, Haidary M. Effects of Aqueous Extract of Afghan *Hibiscus sabdariffa L.* on Morphine Withdrawal Symptoms in Male Rats. Afghanistan Journal of Basic Medical Sciences. 2025 Jan 2(1):89-96.
https://doi.org/10.62134/khatamuni.63

ABSTRACT

Background: Opioid dependence is a significant and widespread issue in the realm of treatment. The interest in using low-side-effect medications, such as herbal remedies, has grown as a viable option for addressing dependence, making it a critical focus of research globally. We aimed to investigate the effects of the aqueous extract of Afghan *Hibiscus sabdariffa L.* on withdrawal symptoms in morphine dependent rats.
Methods: Thirty male Sprague-Dawley rats were categorized into five groups: Vehicle, Morphine, and three *H. sabdariffa L.* extract groups. Morphine dependence was induced through subcutaneous injections of morphine hydrochloride over seven consecutive days. The *H. sabdariffa L.* extract was administrated orally at the doses of 75, 100, and 150 mg/kg concurrently with morphine. On the seventh day, two hours after the final morphine dose, naloxone was administered, and withdrawal signs were monitored for 30 min.
Results: Administering the aqueous extract of Afghan *H. sabdariffa L.* significantly reduced the occurrence of ptosis and chewing behaviors associated with withdrawal in morphine-dependent rats (P<0.05).
Conclusion: The aqueous extract of Afghan *H. sabdariffa L.* holds promise as an effective treatment for alleviating withdrawal symptoms in morphine-dependent rats.

Keywords: *Hibiscus sabdariffa L.*, Opioid addiction, Morphine dependence, Withdrawal signs

Introduction

Morphine, an opioid derived from opium, is primarily used for its potent analgesic and sedative properties in managing severe pain. It is particularly effective in treating moderate to severe cancer pain. The WHO recognizes it as a strong opioid for cases where non-opioids and weaker opioids fail to provide relief (1). Morphine's effectiveness is attributed to its ability to bind to specific opioid receptors in the central nervous system, leading to pain relief. However, its use is often complicated by the development of tolerance and dependence, which can occur with prolonged administration (2, 3).
Morphine can induce neuroinflammation in the central nervous system (CNS), associated with reduced analgesic efficacy and increased tolerance, dependence, and the rewarding effects linked to substance abuse (4-6). Currently, various chemical agents,

Copyright © 2025 Afghanistan Journal of Basic Medical Sciences, and Ghalib University. All rights reserved.
This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 International License

such as methadone, are used to manage symptoms in individuals with substance use disorders. However, many patients do not respond effectively to these medications and may experience adverse side effects, leading to increased interest in herbal remedies as alternative treatments (7, 8).

This area of research is gaining traction globally due to the potential of plants to serve as effective phytochemical agents for treating various conditions (9). Among these plants, *Hibiscus sabdariffa L.*, a member of the Malvaceae family, is recognized for its numerous health benefits. It contains a variety of bioactive compounds, including flavonoids, alkaloids, and phenolic acids, which contribute to its medicinal properties (10). *H. sabdariffa* exhibits anti-inflammatory, antioxidant, and neuroprotective effects, making it a promising candidate for managing various health issues, including those related to substance dependence (11, 12). Recent pharmacological research has increasingly focused on the potential benefits of utilizing the calyces of *H. sabdariffa* . The primary therapeutic compounds in this plant include polysaccharides, organic acids, and flavonoids, particularly anthocyanins (11, 13). Extracts from the dried calyces are rich in various chemical substances such as organic acids, phytosterols, polyphenols, and antioxidants, which may help reduce the risk of degenerative diseases (10). *H. sabdariffa* is utilized in pharmaceuticals, culinary applications, and cosmetics, and is employed in treating neurological disorders, cardiovascular diseases, obesity, and blood pressure regulation (14). According to the WHO, over 80% of the global population relies on medicinal plants, with Afghanistan being home to over 5,000 plant species, including many with medicinal properties (15).

We aimed to explore the effects of the aqueous extract of Afghan *H. sabdariffa* on morphine withdrawal signs in rats.

Materials and Methods

Animals

In this experimental study, 30 male Sprague-Dawley rats, each weighing between 150 and 200 gr, were randomly selected from the Research and Technology Center at Khatam Al-Nabieen University. The rats were housed in standard Plexiglass cages, ensuring they had unrestricted access to food and water. They were maintained in a controlled environment with a stable room temperature of 23±2 °C and a 12-hour light/dark cycle. To monitor their health, daily weight measurements were taken, allowing for adjustments to the injection solution based on the animals' weight fluctuations.

The study received approval from the Ethics Committee of the Research and Technology Center at Khatam Al-Nabieen University.

Extraction

Calyces of *H. sabdariffa* were collected from the Shigal and Sheltan districts of Kunar Province, Afghanistan. The plant material was then dried in a laboratory setting and ground into a powder at the Research and Technology Center, Khatam Al-Nabieen University. Thirty gr of *H. sabdariffa* calyces were carefully brewed in 200 ml of boiled water to create an infusion. This brewing process allowed the calyces to steep in the hot water for 30 min, facilitating the extraction of the plant's beneficial compounds into the liquid. Following the steeping period, the mixture was filtered to remove the solid remnants of the calyces, resulting in a clear liquid extract. To concentrate the extract further, the filtered solution was subjected to evaporation, which removed the water content and left behind a dark red powder.

The resulting extract was then stored at a temperature of 4 °C (16).

Experimental Groups

The rats were randomly assigned to five distinct groups, with each group containing ten animals (n=10). Group 1 (Treat 1) served as the control group and consisted of saline-treated rats, administered a saline solution to evaluate the baseline behavioral responses without any pharmacological intervention. Group 2 (Treat 2) included rats that were treated with morphine to induce a state of dependence, followed by the administration of naloxone, an opioid antagonist, to assess the signs of morphine withdrawal. Groups 3 to 5 were specifically designed to investigate the effects of *H. sabdariffa* extract on morphine withdrawal. These groups received the extract at three different dosages: 75 mg/kg for Group 3, 100 mg/kg for Group 4, and 150 mg/kg for Group 5 (Treat 3-5). The extract was administered orally in conjunction with morphine to determine whether it could mitigate withdrawal symptoms associated with morphine dependence.

Induction of Morphine Dependence

Morphine dependence was induced in the experimental rats through a carefully controlled regimen of subcutaneous injections. Initially, the rats received morphine at escalating doses of 2.5 mg/kg for the first two injections, followed by 5 mg/kg, 10 mg/kg, 20 mg/kg, and finally 40 mg/kg (17). This dosing schedule was administered twice daily over a period of six consecutive days. The gradual increase in dosage was designed to mimic the process of developing tolerance and dependence, which often occurs in clinical settings with chronic opioid use. On the seventh day, the animals received a single higher dose of 50 mg/kg of morphine (17), a strategy employed to precipitate withdrawal symptoms and confirm the establishment of dependence.

Induction and monitoring of morphine-withdrawal signs

Withdrawal syndrome in the rats was precipitated by administering a single intraperitoneal (IP) injection of naloxone at a dose of 3 mg/kg (17). This injection was timed to occur two hours after the final morphine administration, a critical interval that allowed for the accumulation of morphine in the system and increased the likelihood of observable withdrawal symptoms. Following the naloxone injection, each rat was placed individually in a glass cylinder that measured 30 cm in diameter and 40 cm in height. This environment was chosen to facilitate clear observation of the rats as they exhibited withdrawal signs. The use of a transparent cylinder allowed researchers to closely monitor and record the animals' behaviors without any external distractions that could influence their responses. The observation period lasted for 30 min, during which trained observers meticulously noted the presence and severity of withdrawal signs displayed by each rat. These signs included behaviors such as jumping, rearing, shaking, and other physical manifestations associated with opioid withdrawal. To quantify these observations, the signs were scored using Maldonado's modified method, a standardized approach for assessing withdrawal symptoms in animal models (18). This scoring system assigns specific point values to individual symptoms based on their severity and frequency, enabling a comprehensive assessment of the overall withdrawal experience (Figure 1).

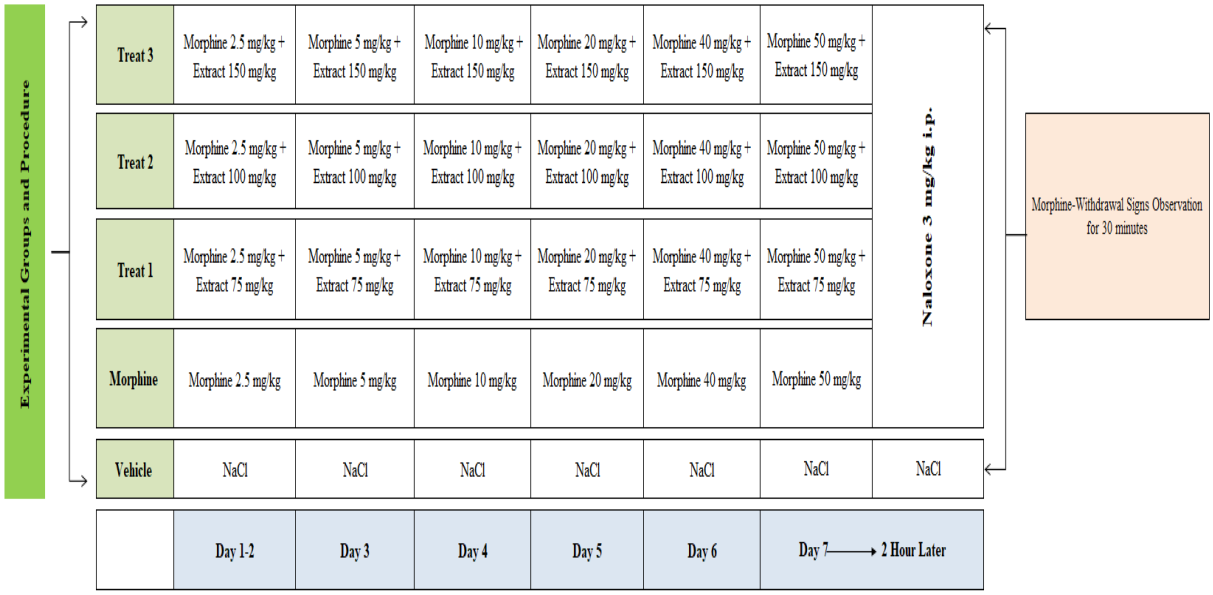

Figure 1: Presents the study timeline that highlights the essential phases and key events throughout the research process.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software. To evaluate the morphine withdrawal signs across the various experimental groups, the Kruskal-Wallis test was employed, as it is well suited for non-normally distributed data. For multiple comparisons among the groups, the One-Way ANOVA test was utilized, allowing for a comprehensive assessment of differences in means. A *P*-value of less than 0.05 was considered statistically significant.

Results

The administration of morphine resulted in the development of significant withdrawal signs in the rats following an IP injection of naloxone hydrochloride at a dosage of 3 mg/kg. Statistical analysis revealed a notable difference in the frequency of withdrawal behaviors when comparing the morphine-treated groups to the control group. Specifically, behaviors such as ptosis, chewing, rearing, teeth chattering, and grooming were significantly more pronounced in the morphine-treated groups ($P<0.001$). Among the treatment groups, the administration of a 150 mg/kg dose of *H. sabdariffa* extract resulted in a significant reduction in the frequency of certain withdrawal signs. Notably, the occurrence of ptosis was decreased ($P<0.01$), as well as chewing behaviors ($P<0.001$), when compared to the morphine-only group. In contrast, the groups that received lower doses of the extract, specifically 75 mg/kg and 100 mg/kg, did not show statistically significant differences in withdrawal signs compared to the morphine-only group (Table 1).

Table 1: Effect of Aqueous Extract of Afghan Hibiscus sabdariffa L. on Naloxone-Induced Withdrawal Signs.

Variable	*Ptosis*	*Chewing*	*Rearing*	*Teeth Chattering*	*Grooming*
Vehicle	1.83±0.40***	0.00±0.00**	1.50±0.22**	0.00±0.00***	0.83±0.3***
Morphine	1.83±0.40***	0.00±0.00**	1.50±0.22**	0.00±0.00***	0.83±0.3***
75mg/kg Extract	3.83±0.40	7.83.±0.60	9.33±1.62	36.33±2.51	48.67±3.56
100 mg/kg Extract	4.16±0.40	4.50±0.34	6.33±0.84	39.00±3.73	38.00±1.73
150 mg/kg Extract	3.66±0.49	1.33±0.80	6.00±0.73	14.33±1.33***	28.83±1.70***

Data are presented as mean ± SEM. Statistical significance is indicated as follows: **$P < 0.01$ and ***$P < 0.001$

Discussion

Medicinal plants have been integral to human health for millennia, serving as a primary source of treatment for various ailments (19). Their role in addressing opioid dependency and alleviating withdrawal symptoms is increasingly recognized (20). In this study, we assessed the impact of the aqueous extract of Afghan *H. sabdariffa* on withdrawal signs in morphine dependent rats. Morphine administration leads to significant withdrawal symptoms in rats, as evidenced by the pronounced behaviors observed following naloxone-induced withdrawal.

The increase in behaviors such as ptosis, chewing, rearing, teeth chattering, and grooming highlights the severity of morphine dependence and the physiological changes that occur during withdrawal. These findings are consistent with existing literature, which indicates that opioid withdrawal syndromes are characterized by a variety of behavioral and physiological manifestations, reflecting the complex nature of dependency and withdrawal mechanisms (21, 22). Morphine significantly contributes to oxidative stress by promoting the formation of free radicals and/or diminishing the activity of various components of the antioxidant system within target cells. Additionally, inducible and neuronal nitric oxide synthase (NOS) isoforms are implicated in morphine dependence and withdrawal symptoms. Signs of morphine dependence may be associated with nitric oxide produced by neuronal nitric oxide synthase (nNOS) (23).

Importantly, the administration of *H. sabdariffa* extract demonstrated a notable therapeutic effect in mitigating withdrawal symptoms, particularly at the highest dose of 150 mg/kg. The observed reductions in ptosis ($P<0.01$) and chewing behaviors ($P<0.001$) suggest that *H. sabdariffa* may possess pharmacological properties that alleviate the severity of withdrawal. This finding is particularly intriguing as it points to the potential of natural extracts as adjunct therapies in the management of opioid withdrawal. The lack of significant effects in the lower doses (75 mg/kg and 100 mg/kg) suggests a dose-dependent response, indicating that higher concentrations of *H. sabdariffa* are necessary to elicit a therapeutic effect. As of now, there is no existing evidence regarding the effects of *H. sabdariffa* . extract on withdrawal symptoms, making it impossible to compare the results of this study with previous research. Nevertheless, *H. sabdariffa* has traditionally been used to treat various conditions, including as a demulcent, sedative (24),

antioxidant (25), anti-inflammatory (26), and neuroprotective agent (27).

The mechanisms by which *H. sabdariffa* may influence withdrawal symptoms are linked to its rich phytochemical composition, particularly flavonoids and anthocyanins (10). These compounds are known for their neuroprotective and anti-inflammatory properties (10), which could modulate neurotransmitter systems z (28, 29) involved in addiction and withdrawal (29). Such properties could explain the observed reduction in withdrawal signs, as they may help stabilize the neurochemical imbalances caused by opioid withdrawal. *H. sabdariffa* exhibits psychoactive properties, particularly sedative effects, as evidenced by various studies. The aqueous extract of *H. sabdariffa* has been shown to prolong significantly the duration of pentobarbital-induced sleep-in rats, comparable to the effects of diazepam. Additionally, it reduces exploratory behavior in mice, indicating a sedative action. Moreover, the extract significantly inhibits apomorphine-induced stereotypic behavior and reduces climbing behavior in mice, further supporting its sedative and potentially antipsychotic effects. The psychoactive substances present in *H. sabdariffa* may modulate neurotransmitter systems involved in addiction and withdrawal, contributing to its traditional use as a sedative in various cultures (27).

As mentioned, inflammatory processes and oxidative stress considerably increase during morphine withdrawal. Various medicinal plants, due to their anti-inflammatory and antioxidant properties, can alleviate withdrawal symptoms (30). Furthermore, *H. sabdariffa* and its constituents exhibit strong anti-inflammatory and antioxidant properties (26, 31). Therefore, the effect of the aqueous extract of *H. sabdariffa* on reducing withdrawal signs in morphine-dependent rats may also be attributed to its influence on inflammatory processes and oxidative stress.

Conclusion

The findings of this study not only reaffirm the significant impact of morphine on withdrawal behaviors but also highlight the potential of *H. sabdariffa* as a therapeutic agent for managing opioid withdrawal symptoms. Future research should focus on elucidating the specific mechanisms of action of *H. sabdariffa* and exploring its efficacy across different dosages and formulations. Additionally, clinical studies are needed to evaluate the translational potential of these findings, aiming to develop effective, natural interventions for individuals struggling with opioid dependence.

Acknowledgments

No financial support was received for this study.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Bujedo BM. Spinal opioid bioavailability in postoperative pain. Pain Practice. 2014;14:350-364.
2. Costantino CM, Gomes I, Stockton SD, Lim MP, Devi LA. Opioid receptor heteromers in analgesia. Expert Rev Mol Med. 2012;14:e9.
3. Rozenfeld R, Abul-Husn NS, Gomez I, Devi LA. An emerging role for the delta opioid receptor in the regulation of mu opioid receptor function. Sci World J. 2007;7:504785.
4. Santoni A, Santoni M, Arcuri E. Chronic cancer pain: Opioids within tumor microenvironment affect neuroinflammation, tumor and pain evolution. Cancers (Basel). 2022;14:2253.

5. Kupnicka P, Listos J, Tarnowski M, Kolasa A, Kapczuk P, Surówka A, et al. The effect of prenatal and neonatal fluoride exposure to morphine-induced neuroinflammation. Int J Molec Sci. 2024;25:826.
6. Liao K, Niu F, Hu G, Buch S. Morphine-mediated release of astrocyte-derived extracellular vesicle mir-23a induces loss of pericyte coverage at the blood-brain barrier: Implications for neuroinflammation. Front Cell Dev Biol. 2022;10:984375.
7. Zhu W, Zhang Y, Huang Y, Lu L. Chapter twelve - chinese herbal medicine for the treatment of drug addiction. In: Zeng B-Y, Zhao K, eds. *Int rev neurobiol*. Academic Press; 2017:279-295.
8. Ward J, Rosenbaum C, Hernon C, McCurdy CR, Boyer EW. Herbal medicines for the management of opioid addiction. CNS Drugs. 2011;25:999-1007.
9. Singh AK, Rai SN, Maurya A, Mishra G, Awasthi R, Shakya A, et al. Therapeutic potential of phytoconstituents in management of Alzheimer's disease. Evid Based Complement Alternat Med. 2021;2021:5578574.
10. Renuka E, Bharti A. *H. sabdariffa* linn: Phytochemical impact on the mechanism of neuroprotective and anti-inflammatory pathways. Recent Advances in Inflammation & Allergy Drug Discovery. 2024;18:1-16.
11. Mahadevan N, Shivali, Kamboj P. *Hibiscus sabdariffa* linn.-an overview. 2009
12. Kumar R, Singh NP, Mukopadayay S, Sah RP. Ethnomedicinal and pharmacological properties of hibiscus sabdariffa: A review. Int J Health Sci. 2022;6:9744-9758.
13. Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, et al. Organic acids from roselle (*Hibiscus sabdariffa l.*)—a brief review of its pharmacological effects. Biomedicines. 2020;8:100.
14. Bedi PS, Bekele M, Gure G. Phyto-chemistry and pharmacological activities of *Hibiscus sabdariffa* linn.-a review. International Research Journal of Pure and Applied Chemistry. 2020;21:41-54.
15. Karmana IW. Artikel review : Bioaktivitas bunga rosella (*Hibiscus sabdariffa l.*) beserta pemanfaatannya. Educatoria : Jurnal Ilmiah Ilmu Pendidikan. 2023;3:208-216.
16. Elmanama AA, Alyazji AA, Abu-Gheneima N. Antibacterial, antifungal and synergistic effect of *Lawsonia inermis, Punica granatum* and *Hibiscus sabdariffa*. 2011
17. Akbari E, Alami K, Hossaini D, Nazari ML, Salehi MH, Sajjadi M, Mousavi SY. Effect of afghan saffron (crocus sativus l.) aqueous extract on withdrawal signs in morphine-dependent rats. Pharmac J. 2020;12
18. Maldonado R. Participation of noradrenergic pathways in the expression of opiate withdrawal: Biochemical and pharmacological evidence. Neurosci Biobehav Rev. 1997;21:91-104.
19. Gulsheen, Kumar A, Sharma A. Antianxiety and antidepressant activity guided isolation and characterization of gossypetin from *Hibiscus sabdariffa* linn. Calyces. Journal of Biologically Active Products from Nature. 2019;9:205-214.
20. Ebrahimie M, Bahmani M, Shirzad H, Rafieian-Kopaei M, Saki K. A review study on the effect of Iranian herbal medicines on opioid withdrawal syndrome. J Evid Based Complementary Altern Med. 2015;20:302-309.
21. Listos J, Listos P, Baranowska-Bosiacka I, Karpiuk A, Filarowska J, Łupina M, et al. Linagliptin, a selective dipeptidyl peptidase-4 inhibitor, reduces physical and behavioral effects of morphine withdrawal. Molecules. 2022;27:2478.
22. Saeedi N, Darvishmolla M, Tavassoli Z, Davoudi S, Heysieattalab S, Hosseinmardi N, Janahmadi M, Behzadi G. The role of hippocampal glial glutamate transporter (glt-1) in morphine-induced behavioral responses. Brain and Behavior. 2021;11:e2323.
23. Skrabalova J, Drastichova Z, Novotny J. Morphine as a potential oxidative stress-causing agent. Mini Rev Org Chem. 2013;10:367-372.
24. Ali SA, Mohamed AH, Mohammed GEE. Fatty acid composition, anti-inflammatory and analgesic activities of hibiscus sabdariffa linn. Seeds. J Adv Vet Anim Res. 2014;1:50-57.
25. Peredo Pozos GI, Ruiz-López MA, Zamora Nátera JF, Álvarez Moya C, Barrientos

Ramírez L, Reynoso Silva M, et al. Antioxidant capacity and antigenotoxic effect of *Hibiscus sabdariffa l.* Extracts obtained with ultrasound-assisted extraction process. Appl Sci. 2020;10:560.

26. Bayani GFE, Marpaung NLE, Simorangkir DAS, Sianipar IR, Ibrahim N, Kartinah NT, et al. Anti-inflammatory effects of *Hibiscus sabdariffa* linn. On the il-1β/il-1ra ratio in plasma and hippocampus of overtrained rats and correlation with spatial memory. Kobe J Med Sci. 2018;64:E73-E83.
27. Amos S, Binda L, Chindo BA, Tseja A, Odutola AA, Wambebe C, Gamaniel K. Neuropharmacological effects of *Hibiscus sabdariffa* aqueous extract. Pharm Biol. 2003;41:325-329.
28. Guardiola S, Mach N. Endocrinología y nutrición therapeutic potential of *Hibiscus sabdariffa* : A Review Of The Scientific Evidence (cid:2). 2022
29. Mojiminiyi F, Aliyu B, Oyeniyi Y, Isezuo S, Alada A. The effect of acute administration of the aqueous calyx extract of *Hibiscus sabdariffa* on blood pressure, heart rate and double product of apparently healthy human subjects during different postures. Niger J Physiol Sci. 2022;37:147-152.
30. de Guglielmo G, Kallupi M, Scuppa G, Stopponi S, Demopulos G, Gaitanaris G, Ciccocioppo R. Analgesic tolerance to morphine is regulated by pparγ. Br J Pharmacol. 2014;171:5407-5416.
31. Bevan P, Pastor MV, Almajano MP, Codina-Torrella I. Antioxidant and antiradical activities of *Hibiscus sabdariffa* l. Extracts encapsulated in calcium alginate spheres. Polymers. 2023;15:1740.