Neurotoxic Assessment of Chronic Abuse of Pregabalin in Wistar Rats

Neveen A. Salem1,2*, Amani M. Alsaedi3, Bedor G. Alasmari3, Razan Z. Almarghalani3, Shahad M. Algobe3 and Nahd H. Alesawy3

1Department of Biochemistry, College of Science, University of Jeddah, Jeddah, Saudi Arabia.
2Department, National Research Centre, Narcotics, Ergogenic Aids and Poisons Cairo, Egypt.
3Department of Medical Laboratory Technology, College of Applied Medical Sciences, University of Jeddah, Jeddah, Saudi Arabia.

Authors’ contributions
This work was carried out in collaboration among all authors. Author NAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMA, BGA and RZA managed the analyses of the study. Authors SMA and NHA managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT
Pregabalin (Lyrica) is an analog of the gamma-aminobutyric acid neurotransmitter, approved for the treatment of epilepsy, generalized anxiety disorder, neuropathic pain, and fibromyalgia. The possibility for abuse and/or dependence on pregabalin has risen recently. Pregabalin is controlled in many countries including Saudi Arabia. However, unofficial use of this substance is also on the increase. The purpose of this study is to assess the potential neurotoxic effects associated with overdose prolonged pregabalin supplementation. Forty male Wistar rats were divided into Group (1) normal control received distilled water, Group (2) received pregabalin (150mg/kg), Group (3) received pregabalin (300 mg/kg), and Group (4) received pregabalin (600 mg/kg). pregabalin consumption in different doses resulted in significant dysregulation in neurotransmitter release, upsurge oxidative stress markers via enhancing lipid peroxidation and depleting antioxidant markers. Also, pregabalin doses evoked brain tissue inflammation through elevating TNF-α, IL-1β.
and MCP-1. Moreover promoted brain tissue apoptosis by activating caspase -3 and suppressed Bcl2. Pregabalin effects on the aforementioned parameters were dose-dependent. These findings could highlight the potential neurotoxic effect of prolonged abuse of pregabalin supplementation through dysregulating brain neurochemical, inflammatory, oxidant/antioxidant, and apoptotic mediators.

Keywords: Pregabalin; abuse; neurotransmitters; oxidative stress; euphoria; apoptosis.

1. INTRODUCTION

Pregabalin([S]-3-(aminomethyl)-5-methyl exanoic acid) is an alkylated analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) designed to diffuse across the blood-brain barrier and act as a central neuromodulating agent [1]. It is one of the newest antiepileptic drugs used to treat partial epilepsy and also manage generalized anxiety disorder; neuropathic pain, fibromyalgia, and post-herpetic neuralgia [2]. A substantial off-label use has been materialized, such as hypnotic-dependent insomnia [3], withdrawal of benzodiazepines [4], and alcohol dependence [5].

Pregabalin (Trade name Lyrica®) exerts its mechanism of action via selective binding to the alpha2-delta subunit of presynaptic voltage-gated calcium channels in the central nervous system [6]. This potent binding of pregabalin at the calcium channel of neurons causes inhibition of calcium-dependent release excitatory neurotransmitters leading to attenuation of post-synaptic excitability [7] related to pain pathway, including glutamate, noradrenaline, and substance P [8] and increases neuronal GABA levels without direct effect on GABAα or GABAB or GABA uptake or degradation [9].

The recommended daily dose of pregabalin is 150-600 mg divided into two or three smaller doses, and the defined daily dose by the World Health Organization is 300 mg [1]. Upon oral administration, Pregabalin has rapidly absorbed and its bioavailability reaches approximately 90%. Although pregabalin is not very lipophilic but able to cross the blood-brain barrier(BBB) and a steady-state is attained within 24-48 hours with repeated administration and less than 2% of pregabalin is metabolized and it is excreted virtually unchanged in the urine and half-life of pregabalin is 6.3 hours [10].

Pregabalin is considered well-tolerated. Consequently, Pregabalin was classified as Schedule V of the Controlled Substances Act [11]. But, similar to other compounds structurally related to neurotransmitter GABA, there were arising concerns regarding pregabalin addictive liability. Several case reports were addressing its recreational misuse [12]. Pregabalin misusers reported entactogenic, euphoric, and dissociative feelings when administered in doses exceeding therapeutic dosages [13]. The routes of abuse of pregabalin include oral, intravenous, nasal insufflation, rectal (“plugging”), smoking, and “parachuting” (emptying the content of the capsule into a pouch) [14]. The WHO report describes diaphoresis, tachycardia, hypertension, tremors, diarrhea, anxiety, auditory hallucinations as symptoms of pregabalin withdrawal [1].

Pregabalin abuse for recreational intention has also been associated with several adverse effects which involve the central nervous system including dizziness, confusion, psychosis somnolence, ataxia, cognitive disorders, CNS depression, and coma [15]. Moreover, those neurotoxic effects were reported to be dose-dependent [13]. However, the magnitude of the abuse potential and the mechanism behind it are not fully known.

The current study aims to investigate the potential neurotoxic effect of chronic abuse of high doses of pregabalin and to explore the underlying biochemical aspects that are related to oxidative stress, inflammation, and apoptosis in brain tissue.

2. MATERIALS AND METHODS

2.1 Animal

A total of forty, four weeks old male albino rats (200 g ± 50 g) were recruited in this study. Rats were obtained from the Animal House Colony of King Fahd Medical Research Center, Jeddah. Rats were kept on a cycle 12:12 light/dark, in a controlled temperature room (25±2°C). Rats had access to food and water ad libitum at King Fahd Medical Research Center Animal Facility Breeding Colony. Rats were housed as one per clean plastic cage to prevent the potential harm
caused by aggressive and violent behavior arising from prolonged pregabalin administration at a high dose.

2.2 Chemicals

Pregabalin 150 mg /capsule was purchased from Pfizer Pharmaceutical Industries (Rabigh, Saudi Arabia).

Three pregabalin doses were used in this study: 150, 300, and 600 mg/kg/day. The doses were given orally by intra-gastric gavage for 90 consecutive days to evaluate chronic abuse of pregabalin [16-17].

These doses are approximately equivalent to 1500 mg, 3000 mg, and 6000 mg in humans which are the most commonly mentioned concentrations used by addicts in different studies and case reports known to produce the euphoric and dissociative effects desired by addicts, and as reported by abusers in case reports for recreational uses [16-17] according to the conversion equation [18]:

\[
\text{Human equivalent dose (mg/kg)} = \frac{\text{animal dose (mg/kg)} \times (\text{animal Km} / \text{human Km})}{\text{Km}}
\]

Km is the average body weight (kg) of species divided by its body surface area (m²).

2.3 Study Design

Rats were randomly divided into four groups (n=10), group 1 received 1ml distilled water, group 2 received pregabalin in distilled water (150 mg/kg/day), group 3 received pregabalin in distilled water (300 mg/kg/day), group 4 received pregabalin in distilled water (600 mg/kg/day) for 90 consecutive days.

At the end of the experimental period, all animals were euthanized and sacrificed by decapitation. Brains were excised, washed with ice-cold saline (0.9%), and homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays.

2.4 Biochemical Analysis

2.4.1 Brain monoamines neurotransmitters

Brain serotonin, dopamine, and norepinephrine were determined using high-performance liquid chromatography (HPLC) system, Agilent technologies 1100 series equipped with a quaternary pump (Quat pump, G131A model).

Separation was achieved on the ODS-reversed phase column (C18, 25 x 0.46 cm i.d. 5 µm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1 ml/min. UV detection was performed at 270 nm, and the injection volume was 20 µl. The concentration of the neurotransmitters was determined by the external standard method by using peak areas. A linear standard curve was constructed where sample concentration was obtained directly from the curve [19].

2.4.2 Oxidative stress markers

Lipid peroxidation malondialdehyde (MDA) was determined in brain tissue according to Ohkawa [20]. Brain reduced glutathione (GSH) was evaluated as per Ellman [21] and superoxide dismutase (SOD) activity was estimated in brain tissue as previously described by Nishikimi [22].

The determination of catalase (CAT) was carried out following Clairborne [23].

2.4.3 Inflammatory mediators and apoptotic markers

Brain inflammatory cytokines: tumor necrosis factor-alpha and interleukin one beta (TNF-α, IL-1β), inflammatory chemokine monocyte chemoattractant protein (MCP-1), and apoptotic markers caspase 3 (Cas3) and B-cell lymphoma (Bcl2) were determined by an enzyme-linked immunosorbent assay using ELISA kits (Invitrogen Corporation Camarillo, CA, USA) and microtiter plate reader (Fisher Biotech, Germany) according to the manufacturer’s instructions.

2.4.4 Statistical analysis

The obtained data were presented as Mean ± SE. The homogeneity of variance for each variable was analyzed using the Levine test. One-way analysis of variance (ANOVA), followed by Duncan’s multiple rank test was performed using the mSTAT-c computer program to determine the statistical significance between the different groups. The difference was considered significant at P =0.05.

3. RESULTS

3.1 Brain Monoamine Neurotransmitters

Rats treated with pregabalin at different doses (150, 300 and 600 mg) showed a significant
decline in both dopamine (-49%, -81% and -91% respectively) and adrenaline (-56%, -80% and -97% respectively) as compared to the control group. whereas, a significant upregulation in serotonin levels was observed following pregabalin dose administration by 1.75, 2.98, and 4.17 folds versus the control group Fig. 1.

3.2 Oxidative Stress Markers

Administration of pregabalin at doses (150, 300 and 600 mg/kg) induced a significant increment in brain tissue MDA level by (4.5, 8.8 and 12.4 folds, respectively) as compared to the control group. While significantly attenuated brain tissue antioxidants level of GSH (-37.7 %, -68.2% and -84.7% , respectively), SOD activity (-37.9%, -58.8% and -79.7% respectively) and CAT level (-38.4%, 61.5% , and -84.7% , respectively) in comparison with the control group Fig. 2.

3.3 Inflammatory Mediators

The present data revealed a significant upsurge in brain tissue inflammatory mediators in a dose-dependent manner as evidenced by a significant elevation in brain tissue TNF-α, IL-1β and MCP-1 levels (109.7%, 125.3%, and 59.1% respectively) after pregabalin (150mg/kg) treatment as compared to the control group. This elevation reached (228.1%,271.5% and 104.1% respectively) by increasing pregabalin dose to 300mg/kg and (378.3%,479.7% and 164.5% respectively) following administration of pregabalin (600 mg/kg) as compared to control group Fig. 3.

3.4 Apoptotic Markers

The data showed a significant elevation in brain tissue Caspase 3 levels (2.4 folds, 4.5 folds, and 6.4 folds) after administration of pregabalin three doses (150mg/kg, 300mg/kg, and 600mg/kg respectively) as compared to the control group. While there was a significant gradual decrease in Bcl2 levels in brain tissues (0.6 folds, 0.3 folds, and 0.08 folds) after administration of pregabalin (150mg/kg, 300mg/kg, and 600mg/kg respectively) versus the control group Fig. 4.

The changes in all the aforementioned parameters were dose-dependent and became more observable by increasing the dose of the drug.

4. DISCUSSION

Pregabalin misuse/abuse represents a growing trend that is causing significant patient harm. Multiple case reports of its abuse potential and dependence, including withdrawal symptoms, have been published [6,24]. Toxicity may occur after an overdose or at prolonged therapeutic doses following accumulation resulting in adverse effects, especially on the CNS. The current study was conducted to highlight the neurotoxic effect of long-term administration of overdoses of pregabalin and signal the biochemical aspects that might trigger these toxic effects.
Fig. 2. Effect of different doses of pregabalin on brain tissue Malondialdehyde level (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), (n=10, Mean ± SEM) * significant from control group, † significant from pregabalin (150 mg/kg), ‡ significant from pregabalin (300 mg/kg).

Fig. 3. Effect of different doses of pregabalin on brain tissue Tumor Necrosis Factor - Alpha (TNF-α), Interleukin-1beta, Monocyte Chemoattractant Protein-1 (MCP-1), (n=10, Mean ± SEM) * significant from the control group, † significant from pregabalin (150 mg/kg), ‡ significant from pregabalin (300 mg/kg).

Fig. 4. Effect of different doses of pregabalin on brain tissue Caspase-3, B-cell lymphoma2 (Bcl2), (n=10, Mean ± SEM) * significant from control group, † significant from pregabalin (150 mg/kg), ‡ significant from pregabalin (300 mg/kg).
The current results showed that prolonged administration of high doses of pregabalin resulted in a significant decline in dopamine and norepinephrine. These results were in the same line with the study of Taha et al. [16] which indicated a significant suppression in dopamine and norepinephrine following pregabalin administration in high doses.

Pregabalin binds potently to the alpha2-delta protein in the brain [25] which is associated with voltage-gated calcium channels. This potent binding has been shown to reduce depolarization-induced calcium influx at nerve terminals, with a consequential reduction in the release of several excitatory neurotransmitters, including dopamine, glutamate, noradrenaline, substance P, and calcitonin gene-related peptide CGRP [26]. On the other hand, our data demonstrated a significant elevation in brain serotonin level after prolonged administration of pregabalin in high doses. Jellestad et al. [27] reported that Pregabalin with its serotonergic action has a liability to cause serotonin syndrome which is caused due to excess serotonin concentration in the central nervous system and/or peripheral nervous system leading to cognitive, autonomic, and somatic effects. Gabapentin, an analog of pregabalin, has been shown to increase serotonin levels in the CNS [28] via inducing alterations in central serotonin metabolism [29]. Gabapentin inhibited the central release of serotonin, and its efflux from blood platelets, thus rendering the transmitter less susceptible to degradation and increasing its availability after stimulation [28].

In the present study, the long-term administration of pregabalin supratherapeutic doses instigated brain oxidative stress and provoked the levels of lipid peroxide. Also, significantly abrogated antioxidant defenses as brain SOD, GSH, and CAT activities which scavenge free radicals and prevent their injurious effects, rendering brain tissues vulnerable to free radicals attack. These results were in agreement with the study of Kamel [30] who reported a significant elevation in brain lipid peroxidation associated with a reduction SOD and CAT following chronic oral pregabalin administration for 90 days. Also, Taha et al [16] demonstrated a significant induction in oxidative stress with a prolonged high dose of pregabalin. The disturbance in oxidants/antioxidant balance could be partially attributed to the inhibition of CGRP induced by pregabalin where CGRP deletion is associated with enhanced oxidative stress and a loss of endogenous antioxidant expression [31]. Also, another study by Pen-Silva et al [32] revealed that increased serotonin increases oxidative stress in heart valves through an MAO-A-dependent mechanism. MAO-dependent generation of reactive oxygen species (ROS) may be important for the understanding of mitogenic actions of serotonin which includes 1-activation and translocation of mitogen-activated protein kinases [33] and the phosphatidylinositol 3-kinase pathway [34].

Inflammation is a defense mechanism that protects the body from the damage caused by endogenous or exogenous stimuli [37]. ROS are reported to be centrally involved in the progression of many inflammatory diseases and present functions in signaling and mediation of the inflammation [38]. In the present study treatment with an overdose of pregabalin for the long term induced an elevation in brain tissue inflammatory mediators (TNF-α, IL-1β and MCP-1). This increase in inflammatory markers may be attributed to the elevation in oxidative stress induced by pregabalin abuse. It is known that oxidative stress increases the gene expression and synthesis of pro-inflammatory cytokines, mediated by activation of the transcription factor nuclear factor-κB, activator protein 1 which translocates to the nucleus augmenting the expression of pro-inflammatory genes such as IL-1β, TNF-α [39]. TNF-α, in turn, stimulates the production of ROS by sensitizing infiltrating leukocytes and MCP-1 [40-41].

The current results indicated a significant elevation in apoptotic markers following high doses of pregabalin as manifested by increased Caspase 3 associated with a decline in Bcl2 these results were per Taha et al. [16]. Prolonged administration of high-dose pregabalin has been reported to enhance the p38-MAPK/JNK/ERK signaling in the cerebral cortex [42]. Mitogen-activated protein kinase (MAPK) signaling pathways organize a great constitution network that regulates several physiological processes, like cell growth, differentiation, and apoptotic cell death. Due to the crucial importance of this signaling pathway, dysregulation of the MAPK signaling cascades is involved with oxidative stress and DNA damage [43]. Activation of p38 MAPK induces stimulation of the mitochondrial apoptosis pathway and regulates the equilibrium between BCL2 and
Phosphorylation of p38 MAPKs promotes the release of cytochrome c from mitochondria through inhibition of BCL2 and shifting of BCL2:BAX ratio, producing successive activation of apoptotic proteases caspase-9, and caspase-3 [16].

5. CONCLUSION

Chronic abuse of high doses of pregabalin could induce neurotoxic effects via deregulating neurotransmitters release, instigating oxidative stress marks, depleting antioxidant defense, inducing inflammatory and apoptotic mediators. The pregabalin-induced neurotoxic effects were dose-dependent. Further studies should address the extent of abuse which increases the liability towards adverse toxic effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the methods used in the present study were approved by the Ethical Committee of King Fahd Medical Research Center. Jeddah, KSA and followed the recommendations of the National Institutes of Health Guide for Care and Use Committee (IACUC) of Laboratory Animals (Publication No. 85-23, revised 1985).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. Papazisis G, Kouvelas D. The abuse liability of pregabalin: A pharmacological approach. Research and Advances in Psychiatry. 2015;2(2):75-80.
2. Li Z, Taylor CP, Weber M, Piechan J, Prior F, Bian F, Cui M, Hoffman D, Donevan S. Pregabalin is a potent and selective ligand for α25-1 and α25-2 calcium channel subunits. Eur J Pharmacol. 2011;667:80–90.
3. Cho YW, Song ML. Effects of pregabalin in patients with hypnotic-dependent insomnia. Journal of Clinical Sleep Medicine. 2014;10(5):545-550.
4. Oulis P, Kalogerakou S, Anyfandi E, Konstantakopoulos G, Papakosta VM, Masdrakis V, et al. Cognitive effects of pregabalin in the treatment of long-term benzodiazepine use and dependence. Human Psychopharmacology: Clinical and Experimental. 2014;29(3):224-229.
5. Guglielmo R, Martinotti G, Clerici M, Janiri L. Pregabalin for alcohol dependence: a critical review of the literature. Advances in therapy. 2012;29(11):947-957.
6. Evoy KE, Morrison MD, Sakiad SR. Abuse and misuse of pregabalin and gabapentin. Drugs. 2017;77:403–426.
7. Fink K, Dooley DJ, Meder WP, Sumant-Chauhan N, Duffy S, Clusmann H, et al. Inhibition of neuronal Ca2+ influx by gabapentin and pregabalin in the human neocortex. Neuropharmacology. 2002;42(2):229-236.
8. Martinotti G, Lupi M, Sarchione F, Santacroce R, Salone A, de Berardis D, Serroni N, Cavuto M, Signorelli M, Aguglia E, Valchera A, Iasevoli F, di Giannantonio M (2013) The potential of pregabalin in neurology, Psychiatry and Addiction: A Qualitative Overview. Curr Pharm Des. 2013;19:6367–6374.
9. Ben-Menachem E. (2004) Pregabalin pharmacology and its relevance to clinical practice. Epilepsia. 2004;45Suppl 6:13-8.
10. Bockbrader HN, Radulovic LL, Posvar EL, Strand JC, Alvey CW, Busch JA, et al. Clinical pharmacokinetics of pregabalin in healthy volunteers. The Journal of Clinical Pharmacology. 2010;50(8):941-950.
11. Al-Husseini A, Wazaify M, Van Hout MC (2018) Pregabalin misuse and abuse in Jordan: a qualitative study of user experiences. Int J Ment Heal Addict. 2018;16:642–654.
12. Ashwini S, Amit D, Ivan N, Alka P. Pregabalin dependence with pregabalin induced intentional self-harm behavior: a case report. Indian J Psychiatry. 2015;57:110–111.
13. Schjerning O, Rosenzweig M, Pottegård A, Damkier P, Nielsen J. Abuse potential of pregabalin. CNS Drugs. 2016;30:9–25.
14. Schifano F. Misuse and abuse of pregabalin and gabapentin: cause for concern? CNS Drugs. 2014;28:491–496.
15. Hindmarch I, Trick L, Ridout F (2005) A double-blind, placebo- and positive-internal-controlled (alprazolam) investigation of the cognitive and psychomotor profile of pregabalin in

64
healthy volunteers. Psychopharmacology. 2005;183(133–143):133–143.

16. Taha SHN, Zaghloul HS, Ali AAER, Gaballah IF, Rashed LA, Aboulhoda BE. The neurotoxic effect of long-term use of high-dose Pregabalin and the role of alpha-tocopherol in amelioration: implication of MAPK signaling with oxidative stress and apoptosis. Naunyn-Schmiedeberg’s archives of pharmacology. 2020;393:1635-1648.

17. Taha SHN, Zaghloul HS, Ali AAER, Rashed LA, Sabry RM, Gaballah IF. Molecular and hormonal changes caused by long-term use of high dose pregabalin on testicular tissue: the role of p38 MAPK, oxidative stress and apoptosis. Molecular Biology Reports. 2020;47(11):8523-8533.

18. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and humans. Journal of basic and clinical pharmacy. 2016;7(2):27.

19. Salem NA, Assaf N, Ismail MF, Khadrawy YA, Samy M. Ozone therapy in ethidium bromide-induced demyelination in rats: possible protective effect. Cellular and molecular neurobiology. 2016;36(6):943-954.

20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. Anal Biochem. 1979;95:351–358.

21. Ellman GL. Tissue sulfhydryl groups. Arch Biochem. 1959;82:70–77.

22. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem J. 1972;126:259–265.

23. Clairborne A. Catalase activity In Greenwald, RA, Ed, CRC Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton. 1985;283-284.

24. Hapangama A, Kuruppuarachchi KALA. Pregabalin misuse: A silent epidemic. Sri Lanka Journal of Psychiatry. 2019;10(2).

25. Taylor CP, Vartanian MG, Po-Wai Y, Bigge C, Suman-Chauhan N, Hill DR. Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to in vitro binding at a novel site labeled by tritiated gabapentin. Epilepsy Research. 1993;14(1):11–15.

26. Fink K, Dooley DJ, Meder WP, Suman-Chauhan N, Duffy S, Clusmann H, et al. Inhibition of neuronal Ca2+ influx by gabapentin and pregabalin in the human neocortex. Neuropharmacology. 2002;42(2):229–236.

27. Jellestad L, Stocker L, Jenewein J, Boetgger S. Serotonin syndrome after initiation of pregabalin on a stable regimen of antidepressant medication. International Journal of Medical and Pharmaceutical Case Reports. 2016;7(5):1-4.

28. Rao ML, Clarenbach P, Vahlensieck M, Krätzschmar S. Gabapentin augments whole blood serotonin in healthy young men. Journal of Neural Transmission. 1988;73(2):129–134.

29. Van Praag HM. Significance of biochemical parameters in the diagnosis, treatments, and prevention of depressive disorders. Biol Psychiatry. 1977;12:101-131.

30. Kamel MA. Study on DNA damage and oxidative stress and some biochemical alterations of long-term administration of alpha-2 delta (Α2-Δ) ligand pregabalin and the possibility of Zingiber officinale in ameliorating these effects in rats. World J Pharm Res. 2016;5:1528–1545.

31. Smillie SJ, King R, Kodji X, Outzen E, Pozsgai G, Fernandes E, et al. An ongoing role of an α-calcitonin gene-related peptide as part of a protective network against hypertension, vascular hypertrophy, and oxidative stress. Hypertension. 2014;63(5):1056-1062.

32. Peña-Silva RA, Miller JD, Chu Y, Heistad DD. Serotonin produces monoamine oxidase-dependent oxidative stress in human heart valves. American Journal of Physiology-Heart and Circulatory Physiology. 2009;297(4):H1354-H1360.

33. Lee SL, Wang WW, Finlay GA, Fanburg BL. Serotonin stimulates mitogen-activated protein kinase activity through the formation of superoxide anion. American Journal of Physiology-Lung Cellular and Molecular Physiology. 1999;277(2):L282–L291.

34. Liu Y, Fanburg BL. Serotonin-Induced Growth of Pulmonary Artery Smooth Muscle Requires Activation of Phosphatidylinositol 3-Kinase/serine-threonine Protein Kinase B/Mammalian Target of Rapamycin/p70 Ribosomal S6 Kinase 1. American Journal of Respiratory Cell and Molecular Biology. 2006;34(2):182–191.

35. Liu Y, Li M, Warburton RR, Hill NS, Fanburg BL. The 5-HT transporter
transactivates the PDGFβ receptor in pulmonary artery smooth muscle cells. The FASEB Journal. 2007;21(11):2725–2734.

36. Simon AR, Severgnini M, Takahashi S, Rozo L, Andrahbi B, Agyeman A, et al. 5-HT Induction of c-fos Gene Expression Requires Reactive Oxygen Species and Rac1 and Ras GTPases. Cell Biochemistry and Biophysics. 2005;42(3):263–276.

37. Moreira L, Da R, Brum E, Da S, Da Silva ARH, De Freitas ML, et al. Antinociceptive and anti-inflammatory effect of the Scutia buxifolia Reissek stem barks extract. Phytomedicine. 2016;23(10):1021–1028.

38. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive Oxygen Species in Inflammation and Tissue Injury. Antioxidants & Redox Signaling. 2014;20(7):1126–1167.

39. Prabhakar NR, Semenza GL. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia-mediated by hypoxia-inducible factors 1 and 2. Physiol Rev. 2012;92:967–1003.

40. Ramesh G, Reeves WB. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. Journal of Clinical Investigation. 2002;110(6):835–842.

41. Funakoshi Y, Ichiki T, Shimokawa H, Egashira K, Takeda K, Kaibuchi K, et al. Rho-Kinase Mediates Angiotensin II-Induced Monocyte Chemoattractant Protein-1 Expression in Rat Vascular Smooth Muscle Cells. Hypertension. 2001;38(1):100–104.

42. Sayin M, Simsek FE. Pregabalin administration induces alterations in neural tube development during early embryonic stages. Turk Neurosurg. 2018;29:269–274.

43. De Chiara G, Marcocci ME, Torcia M, Lucibello M, Rosini P, Bonini P, et al. Bcl-2 phosphorylation by p38 MAPK. J Biol Chem. 2006;281:21353–21361.

© 2021 Salem et al., This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67940