Open Access

Original Article

Effects of Variable Doses of Neurokinin B on Hematological and Coagulation Parameters in Adult Male Rats

Faiqah Ramzan, Tahira Atta*, Muhammad Waqas Luqman*, Muhammad Haris Ramzan*

Department of Animal and Poultry Production, Faculty of Veterinary and Animal Sciences (FVAS), Gomal University, Dera Ismail Khan Pakistan, *Khyber Medical University Institute of Medical Sciences Pakistan

ABSTRACT

Objective: To evaluate the dose-dependent effects of Neurokinin B on hematological and coagulation parameters in adult male Sprague Dawley rats.

Study Design: Laboratory-based experimental study

Place and Duration of Study: Gomal University and KMU Institute of Medical Sciences from February 2019 to April 2021.

Methodology: Adult male Sprague Dawley rats (n=5 per group) were administered intraperitoneally with Neurokinin B at doses of 1 µg, 1 ηg and 1 ρg for 12 days consecutively. Control rats received distilled water injections. After 12 days, rats were sacrificed, and blood samples were collected from the left ventricle for hematological and coagulation analysis.

Results: Bleeding time was significantly prolonged in NKB-µg (114±17.10sec) and Neurokinin B -ηg (72±22.25sec) compared to control (33±12.55sec). Clotting time (CT) was also increased (NKB-µg: 111±17.10sec; NKB-ηg: 69±17.10sec, control: 42±12.55sec), as did prothrombin time (PT) [NKB-µg: 93.60±10.92sec; NKB-ηg: 63.40±12.66sec, control: 15±4.12sec] and activated partial thromboplastin time (APTT) [NKB-µg: 94.80±14.04sec; NKB-ηg:68.60±13.74sec, control: 19.40±2.97sec]. Additionally, mean platelet volume [NKB-µg:8.94±0.36fl, control: 8.04±0.48fl] and leukocyte count (×103/µl) [NKB-µg: 12.14±0.69, control: 8.87±1.32] were significantly increased in NKB-treated animals compared to control. There was a significant increase in international normalized ratio in NKB-µg (6.86±0.80) and NKB-ηg (4.65±0.93) compared to control group (1.10±0.30). Conversely, platelet count (×103/µl) was decreased significantly in NKB-µg group (787.80±48.51) compared to the control group (960.60±58.71).

Conclusion: NKB administration led to leukocytosis, thromboctopenia, and affected coagulation pathways with prolonged BT, CT, PT, APTT and INR dose-dependently.

Keywords: Activated partial thromboplastin time, Blood coagulation, Blood coagulation tests, Neurokinin b, Platelets, prothrombin time, Tachykinins

How to Cite This Article: *Ramzan F, Atta T, Luqman MVV, Ramzan MH. Effects of Variable Doses of Neurokinin B on Hematological and Coagulation Parameters in Adult Male Rats. Pak Armed Forces Med J 2024; 74(5): 1414-1419. DOI: https://doi.org/10.51253/pafmj.v74i5.12328*

INTRODUCTION

Neurokinin B, a decapeptide, belongs to the family of peptide hormones called tachykinins.[1] Biological actions of the tachykinins are mediated through three different receptors namely NK1 (Neurokinin-1), NK2 and NK3 which belongs to the G-protein coupled membrane receptor.[1] Tachykinins (Substance P, Neurokinin A and Neurokinin B) preferentially binds to their receptors NK1, NK2 and NK3 respectively. However, these peptides can bind with a lower affinity to each of these receptors.[1] Neurokinin B is produced from the pre-pro-tachykinin-B gene,1 cleaved enzymatically to form pro-neurokinin B, which is further processed into Neurokinin B.[2] Biological effects of Neurokinin B are primarily mediated through neurokinin receptors, particularly NK3, which are extensively distributed throughout the central nervous system and peripheral tissues.[1]

The critical importance of Neurokinin B was first evidenced by its significant role in reproductive health. Research has demonstrated that mutations in the TAC3 gene, which encodes Neurokinin B, can result in hypogonadotropic hypogonadism.[3,4] The peripheral or central Neurokinin B administration, along with its agonists and antagonists, has produced varying effects on different biological processes. The intracerebral Neurokinin B or its agonist (Senktide) administration, leads to elevated levels of reproductive hormones in male rodents.[5] Antagonists of Neurokinin B exhibit strong suppressive effects on follicular growth and estradiol secretion in females.[6]

Beyond its role in reproduction, Neurokinin B is involved in a spectrum of biological processes. Its activity has been observed in immune responses, inflammation, and even in conditions such as pre-eclampsia, menopause, and cancer.[2-9] Substance P (SP), another tachykinin, can activate immune cells

Correspondence: Dr Muhammad Haris Ramzan , Khyber Medical University Institute of Medical Sciences Pakistan

Received: 06 Jun 2024; revision received: 21 Oct 2024; accepted: 29 Oct 2000

and promote the production of inflammatory molecules.[10] Both Neurokinin B and Substance P are linked to the development of certain cancers, including breast and gastric cancer. Blocking NK1 receptors has been shown to reduce platelet aggregation and thrombus size, highlighting the important role of tachykinins in coagulation.

Bleeding disorders, inherited in an autosomal recessive manner, are common worldwide. Pakistan's high rate of consanguineous marriages likely increases the prevalence of coagulation disorders, posing significant public health challenges.[13] However, these disorders often remain undiagnosed due to a lack of specialized diagnostic facilities, low public awareness, and low clinical suspicion.[10] Insights into how Neurokinin B influences blood cell counts and clotting mechanisms could lead to new therapeutic approaches for managing these conditions. Limited information exists regarding impact of Neurokinin B on hematological and coagulation parameters. Therefore, the study intended to assess the impact of Neurokinin B on hematological parameters and coagulation dynamics in adult male rats following intraperitoneal administration. The study aims to measure changes in leukocyte, red blood cell, and platelet parameters, as well as coagulation parameters after Neurokinin B administration.

METHODOLOGY

This laboratory-based experimental study was conducted at Gomal University, Dera Ismail Khan and KMU Institute of Medical Sciences, Kohat from February-2019 to April-2021. The study was approved by the Ethical Committee of Khyber Medical University (DIR/KMU-EB/EN/000451, dated 19-04-2018).

Inclusion Criteria: Healthy adult male Sprague Dawley rats having an average weight of 250 – 300 grams were calculated.

Exclusion Criteria: Adult male rats that were already on drug therapy, used in any study/experiment within the last month, or on medication for any disease in the past month were excluded.

Healthy adult male Sprague Dawley rats having an average weight of 250 – 300 grams were acquired from the National Institute of Health, Islamabad. The animals were housed in steel mesh cages during the study. Each steel mesh cage contained five rats. The animals were acclimatized for 10 days before the experiment commenced, maintained on a 12-hour light-dark cycle at a temperature of 25±2°C, with access to food and water ad libitum. European Union Regulations for the use of animals in research were followed for animal handling during the experimental period.[11-12]

Neurokinin B (catalogue # 046-26) was acquired in lyophilized form from Phoenix Pharmaceuticals Inc. (Burlingame, CA, USA). Neurokinin B was separately dissolved in dimethyl sulfoxide (1 ml) [VWR, USA] to create a stock-solution. Distilled water was then added to these solutions for intraperitoneal (i.p.) injection. The rats were randomly divided into four groups (n=5 animals per group). Control group received intraperitoneal injections of distilled water, while Neurokinin B was administered to peptide treated groups in three different doses; 1 µg, 1 ηg and 1 ρg. Intraperitoneal injections were administered twice daily, every 12-hours, for 12-days consecutively. After 12-days of peptide treatment, the animals were anesthetized with sodium pentobarbital (60 mg/kg body weight i.p.) three hours after the last peptide dose and then sacrificed.

To determine the effect of Neurokinin B treatment on coagulation parameters, blood samples were collected from the left ventricle and transferred into blue top vacutainers containing 3.2% trisodium citrate [for PT (prothrombin time) and APTT (activated partial thromboplastin time) analysis] and purple top vacutainers with EDTA.K3 (for complete blood count analysis). PT and APTT were measured using Soluplastin and APTTest kits (Wiener Lab, Rosario, Argentina), following the manufacturers' instructions and INR (international normalized ratio) was calculated. Complete blood counts were analyzed using an automated hematology analyzer (RT-7600, China). Bleeding time (BT) was measured using a modified Duke's method, with a 3 mm incision on the rat tail and timing until bleeding ceased.[15] Clotting time (CT) was assessed using the capillary tube method, timing until clot formation upon breaking the tube every 15 seconds.

Statistical Package for Social Sciences (SPSS) version 26.0 was used for the statistical analysis. Data were presented as mean ± SD. Statistical significance was assessed using one-way ANOVA followed by post hoc Tukey's test, with p value of ≤ 0.05 considered statistically significant.

RESULTS

Throughout the experiment, all peptide-treated rats remained healthy as assessed by their vital signs,

overall condition of the body, eating habits, drinking habits, and activity inside the cage. However, a notable exception was observed in the rats treated with Neurokinin B that their incised wounds continued to bleed for a much longer duration post-treatment. In contrast, rats that served as control and received distilled water treatment showed no excessive bleeding tendency.

The study found a significant increase (p<0.001) in the TLC in the Neurokinin B µg group (12.14±0.69 ×103/µl) compared to the control group (8.87±1.32 ×103/µl) [Table].

(72±22.25 sec) animals as compared to control group (33±12.55 sec). PT was significantly prolonged (p<0.001) in the Neurokinin B µg (93.60±10.92 sec) and Neurokinin B ηg (63.40±12.66 sec) as compared to control group (15±4.12 sec). APTT was significantly prolonged (p<0.001) in the Neurokinin B µg (94.80±14.04 sec) and Neurokinin B ηg (68.60±13.74 sec) as compared to control group (19.40±2.97 sec) [Table]. INR exhibited significant (p<0.001) elevation in Neurokinin B µg (6.86±0.80) and Neurokinin B ηg (4.65±0.93) compared to control group (1.10±0.30) (Table).

Table: Hematological and Coagulation Parameters of the Control and Neurokinin B Treated Groups

Variables	Control	Neurokinin B µg	Neurokinin B ηg	Neurokinin B ρg
Total Leukocyte Count (103/µl)	8.87±1.32	12.14±0.69 ***	10.38±0.75 #	8.81±0.78 ###
RBC Count	6.74±0.37	6.69±0.40	6.69±0.40	6.79±0.40
Hemoglobin (g/dl)	11.72±0.45	11.16±0.38	11.16±0.38	11.82±0.44
Hematocrit (%)	38.98±1.39	35.24±1.49	35.24±1.49	38.58±1.27
Mean Corpuscular Volume [MCV] (fl)	54.84±2.78	54.06±1.50	54.06±1.50	55.24±2.15
Mean Corpuscular Hemoglobin [MCH] (ρg)	16.68±0.79	17.12±0.73	17.12±0.73	16.48±0.76
Mean Corpuscular Hemoglobin Concentration [MCHC] (g/dl)	30±0.30	31.66±0.48	31.66±0.48	30.60±0.92
Red Blood Cell Distribution Width [RDW] (%)	38.90±0.78	34.08±1.11	34.08±1.11	34.50±1.10
Platelet Count (103/µl)	960.60±58.71	787.80±48.51 ***	885.60±39.19 #	929.40±52.73 ##
Mean Platelet Volume [MPV] (fl)	8.04±0.48	8.94±0.36 *	8.58±0.47	8.06±0.42 #
Platelet Distribution Width [PDW] (%)	15±0.20	15.18±0.40	15.06±0.30	14.98±0.29
Clotting Time (sec)	42±12.55	111±17.10 ***	69±17.10* ###	48±12.55 ###
Bleeding Time (sec)	33±12.55	114±17.10 ***	72±22.25 * ##	36±17.10 ###
Prothrombin Time (sec)	15±4.12	93.60±10.92 ***	63.40±12.66 *** ###	30.40±9.02 *** ###
Activated Partial Thromboplastin Time (sec)	19.40±2.97	94.80±14.04***	68.60±13.74*** ###	31.60±9.40 ###
International Normalized Ratio (INR)	1.10±0.30	6.86±0.80 ***	4.65±0.93*** ###	2.23±0.66 *** ###

****p<0.001, **p<0.01 and p<0.05 as compared to control; #p<0.05, ##p<0.01 and ###p<0.001*

The red blood cell count (RBC) did not show significant differences among the peptide treated and control groups. Parameters such as RDW, Hb levels, MCH, MCHC, MCV and hematocrit did not exhibit significant variations among the groups. The platelet count showed a significant decrease (p<0.001) in the Neurokinin B µg group (787.80±48.51 ×103/µl) compared to the control group (960.60±58.71 ×103/µl). Mean platelet volume (MPV) exhibited a significant increase in Neurokinin B µg group (8.94±0.36) animals compared to control group (8.04±0.48). CT was significantly prolonged in the Neurokinin B µg (111±17.10 sec) and Neurokinin B ηg (69±17.10 sec) animals as compared to control group (42±12.55 sec) [Table 1]. BT was significantly prolonged in the Neurokinin B µg (114±17.10 sec) and Neurokinin B ηg

DISCUSSION

The present study investigates the anticoagulation effects of intraperitoneally administrated Neurokinin B in adult male rats. We observed a significantly increased CT and BT with increased PT, APTT, INR and TLC in Neurokinin B treated animals as compared to control. Conversely, a decreased platelet count in Neurokinin B treated rats was observed in peptide treated animal as compared to control group. This effect was dose dependent which potentiated as the dose of Neurokinin B increases.

Prolonged BT is linked with the thrombocytopenia, impaired platelet ability to adhere with vascular endothelium, von Willebrand disease

and disseminated intravascular coagulation. Increased clotting time is associated with hemophilia, deficiencies of the factors involved in clotting pathways (II, V, XI, XII) and congenital coagulation disorders. . Elevated PT reflects the activity of the extrinsic coagulation pathway, and is associated with the deficiency of vitamin K or the acute/chronic liver disease. Conversely, APTT evaluates intrinsic coagulation pathway and it is typically performed when there is unexplained bleeding or clotting.[16, 17]

In present study, we observed significantly increased CT and BT, along with prolonged PT and APTT, suggesting an impact on both intrinsic and extrinsic coagulation pathways. We also noted a marked decrease in platelet count, indicating a disruption in the normal coagulation process. It's worth mentioning that prolonged BT is often associated with thrombocytopenia.[16] In spite of the prolonged BT, PT and APTT, coupled with reduced platelet count, the occurrence of disseminated intravascular coagulation (DIC) 16 as a reason for increased bleeding propensity is highly unlikely. This is supported by the fact that the Neurokinin B treated rats remained active, healthy and survived throughout the study period without displaying any signs of internal hemorrhage, sepsis, infection, or liver damage (data not shown). Therefore, our study suggests that Neurokinin B likely blocks the coagulation pathway at its initial stages.

Prolongation of the PT and APTT was observed in Neurokinin B treated rats which suggests interference with the availability of the vitamin K, however it's important to note that vitamin K deficiency typically does not affect BT or platelet count.[16,17] Therefore, while it is speculative at this stage to conclude that Neurokinin B directly impedes the availability of vitamin K to stimulate its anticoagulation effects. However, the possible role Neurokinin B in down-regulating the vitamin K dependent pathways, cannot be entirely overruled.[16]

Neurokinin B has a short plasma half-life and blood samples were collected at least three hours after the last dose of the peptide and anticoagulation profile was assayed. This suggests that the primary anticoagulation effects might occur shortly after peptide administration. This rapid onset of action is similar to anticoagulants like argatroban and heparin, which also induce anticoagulation effects in a short time frame.[18] Despite using citrated blood for the conduction of PT and APTT tests—standard for all coagulation tests.[19]—the observed anticoagulation effect is more likely attributable to Neurokinin B treatment rather than sodium citrate.

The results of the present study demonstrated a negative association between the thrombocytes and MPV. Presence of bleeding disorders or iron deficiency or anticoagulants may present with a similar scenario. Patients having heterozygous thalassemia may have increased MPV. Conversely a decreased MPV has been observed in subjects undergoing chemotherapy. This converse relationship between thrombocyte size and count is valuable in assessing abnormal thrombocyte production.[20]

In present study, marked thrombocytopenia was evident with Neurokinin B treatment and a dose dependent effect was noticed with the higher dose accounting for the more severe Neurokinin B-induced thrombocytopenia. Heparin-induced thrombocytopenia is a serious and often underestimated adverse effect of heparin treatment which occurs in about 2% of individuals when they are exposed to heparin for more than four days..[21] Heparin is commonly prescribed for the treatment of deep vein thrombosis and pulmonary embolism. It is a powerful anticoagulant and it significantly increases the formation of thrombin-antithrombin.[16]

Although Neurokinin B appears to act similarly to heparin, it is a small peptide, and its mechanisms may differ. The exact mechanism behind Neurokinin B -induced thrombocytopenia remains unknown, however the present data suggested a direct action of the peptide on platelets. Discerning reduction in thrombocytes with increased WBC counts, supports the notion of Neurokinin B -induced thrombocytopenia. Additionally, there was no significant change in hemoglobin content and hematocrit, while MCH increased with Neurokinin B treatment.

In vivo, Neurokinin B -induced thrombocytopenia might result from modulation of megakaryocyte production. Thrombocyte count can be used as a prognostic index as the production of the thrombocytes was stimulated by many neoplasms through initiation of megakaryocyte production.[22] Although platelet aggregation tests were not conducted in this study, Neurokinin B -induced disaggregation of thrombocytes might be accountable for the observed anticoagulation effects.

With Neurokinin B treatment, we observed a significant reduced thrombocyte count with prolonged

anticoagulation profiles. These conditions are typically associated with end-stage liver disease, disseminated intravascular coagulation or massive transfusion reactions.[16,17] Although liver function tests were not performed, necropsy of the peptide-treated rats showed no signs of liver damage. Additionally, the animals enrolled in the present study were healthy throughout the experiments and did not show any signs of sepsis as mentioned earlier. Given that the administered peptides are non-toxic, it is likely that the observed anticoagulation effect was attributable to Neurokinin B.

Neurokinin B may exert its effects by down-regulating thromboplastin, thereby deactivating the factors especially VII, X and IX. Another probability is that Neurokinin B directly inhibits thrombin, which can subsequently suppress the subsequent events in the coagulation pathways.[16] Anticoagulatory activity of the Neurokinin B may be activated directly by inhibiting the formation or actions of the thrombin or tissue plasminogen activator. Our results suggests that Neurokinin B can be used as an effective thrombolytic therapy. This potential effect of Neurokinin B warrants additional investigation. It could be highly beneficial for treating venous thromboembolism. Current treatment options for thromboembolism include unfractionated and low molecular weight heparins (LMWHs), warfarin and inferior vena cava filters.[23]

Limited data is available on the effect of tachykinins on the hematological and coagulation profile. It has been demonstrated that SP induces platelet aggregation and degranulation. This effect of SP has been supposed to be mediated through tachykinin receptors, specifically NK1 (the receptor for SP) and NK3 (the receptor for Neurokinin B). 9 Another study highlighted the role of tachykinins (SP and Endokinins A and B) in the activation of platelet function and the formation of thrombi.[24] Furthermore, additional research indicated that the pro-thrombotic effects of tachykinins on platelets are mediated via the neurokinin 1 receptor.[12] In another study, it was found that Substance P and Neurokinin A are crucial for neutrophil priming and the release of superoxide anions from neutrophils.[25]

Anticoagulants often use GPCR to mediate their anticoagulant effects and aggregation of platelets.[16,17] Notably, Neurokinin B, its agonists and antagonists also exerts its actions through a GPCR. 1 Therefore, it is plausible that Neurokinin B promotes its anticoagulation effects by using intracellular second messenger pathways.

To our knowledge, this is the initial report which investigated the effects of continuous administration of Neurokinin B on hematological and coagulation parameters following subcutaneous administration of the peptides. Consequently, direct comparisons with other studies are currently not possible.

LIMITATIONS OF THE STUDY

The study observed significant changes in platelet count but did not analyzed platelet aggregation for understanding the mechanisms behind Neurokinin B-induced thrombocytopenia. Additionally, liver function tests were not performed, though necropsy showed no signs of liver damage. The focus was limited to specific hematological and coagulation parameters, and including additional parameters like fibrinogen levels and D-dimer could provide a more comprehensive understanding. The present study is preliminary and warrants detailed biochemical investigation into the anticoagulation effects of Neurokinin B, as the exact mechanisms behind the observed effects remain unclear and require further exploration of the molecular pathways involved for its potential use as a therapeutic agent.

FUNDING

Authors declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors. However, the Publication charges for this article are fully / partially borne from the Khyber Medical University Publication Fund (DIR/ORIC/Ref/24/00061).

ACKNOWLEDGEMENTS

Authors are indebted to Mrs. Jawaria Khan, Mrs. Hafiza Yumna Shafique and Mr. Uzair Abbas for their support during experiment conduction and data collection.

CONCLUSION

Neurokinin B administration led to increased leukocyte count, altered platelet dynamics with decreased count and increased volume, and affected coagulation pathways with prolonged bleeding and clotting time, PT and APTT. Further research is needed to understand these effects and their clinical significance.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

FR & TA: Conception and study design, acquisition, analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

MWL & MHR: Analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Almeida TA, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martín JD, et al. Tachykinins and tachykinin receptors: structure and activity relationships. Curr Med Chem 2004; 11(15): 2045-2081. https://doi.org/10.2174/0929867043364748
2. Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, et al. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. Nature 2000; 405(6788): 797-800. https://doi.org/10.1038/35015579
3. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 2009; 41(3): 354-358. https://doi.org/10.1038/ng.306
4. Young J, Bouligand J, Francou B, Raffin-Sanson ML, Gaillez S, Jeanpierre M, et al. TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. J Clin Endocrinol Metab 2010; 95(5): 2287-2295. https://doi.org/10.1210/jc.2009-2600
5. Ruiz-Pino F, Garcia-Galiano D, Manfredi-Lozano M, Leon S, Sánchez-Garrido MA, Roa J, et al. Effects and interactions of tachykinins and dynorphin on FSH and LH secretion in developing and adult rats. Endocrinology 2015; 156(2): 576-588. https://doi.org/10.1210/en.2014-1026
6. Skorupskaite K, George J, Anderson RA. Role of a neurokinin B receptor antagonist in the regulation of ovarian function in healthy women. Lancet 2015; 385 (Suppl 1): S92. https://doi.org/10.1016/s0140-6736(15)60407-x
7. Santen RJ, Stuenkel CA, Davis SR, Pinkerton JV, Gompel A, Lumsden MA. Managing Menopausal Symptoms and Associated Clinical Issues in Breast Cancer Survivors. J Clin Endocrinol Metabol 2017; 102(10): 3647-3661. https://doi.org/10.1210/jc.2017-01138
8. Brain SD. Sensory neuropeptides: their role in inflammation and wound healing. Immunopharmacology 1997; 37(2): 133-152. https://doi.org/10.1016/S0162-3109(97)00055-6
9. Graham GJ, Stevens JM, Page NM, Grant AD, Brain SD, Lowry PJ, et al. Tachykinins regulate the function of platelets. Blood 2004; 104(4): 1058-1065. https://doi.org/10.1182/blood-2003-11-3979
10. Bost KL. Tachykinin-mediated modulation of the immune response. FBL 2004;9(6):3331-3332. https://doi.org/10.2741/1484
11. Feng F, Yang J, Tong L, Yuan S, Tian Y, Hong L, et al. Substance P immunoreactive nerve fibres are related to gastric cancer differentiation status and could promote proliferation and migration of gastric cancer cells. Cell Biol Int 2011; 35(6): 623-629. https://doi.org/10.1042/CBI20100229
12. Jones S, Tucker KL, Sage T, Kaiser WJ, Barrett NE, Lowry PJ, et al. Peripheral tachykinins and the neurokinin receptor NK1 are required for platelet thrombus formation. Blood 2008; 111(2): 605-612. https://doi.org/10.1182/blood-2007-07-103424
13. Mahmood R, Mahmood A, Khan M, Ali S, Khan SA, Jaffar SR. Rare bleeding disorders: spectrum of disease and clinical manifestations in the Pakistani population. BR 2020; 55(3): 146-150. https://doi.org/10.5045/br.2020.2020035
14. European Animal Research Association. EU regulations on animal research London, England: European Animal Research Association; Available from: https://www.eara.eu/animal-research-law [Accessed on November 28, 2022]
15. Jy W, Bidot CJ, Jr., Nunez S, Johansen ME, Horstman LL, Ahn YS. Red Cell-Derived Microparticles (RMP) As Hemostatic Agent: Evaluation in a Rabbit Ear Bleeding Model. Blood 2012; 120(21): 2238-2238. https://doi.org/10.1182/blood.V120.21.2238.2238
16. Laffan MA, Manning RA. 18 - Investigation of Haemostasis. In: Bain BJ, Bates I, Laffan MA, editors. Dacie and Lewis Practical Haematology (Twelfth Edition): Elsevier; 2017. p. 366-409. https://doi.org/10.1016/B978-0-7020-6696-2.00018-7
17. Watson H, Culligan D, Manson L. Haematology and transfusion medicine. In: Penman ID, Ralston SH, Strachan MWJ, Hobson R, editors. Davidson's Principles and Practice of Medicine. 24th ed. London, UK: Elsevier; 2023. p. 921-988.
18. Lv B, Guo FF, Lin JC, Jing F. Efficacy and safety of argatroban in treatment of acute ischemic stroke: A meta-analysis. World J Clin Cases 2022; 10(2): 585-593. https://doi.org/10.12998/wjcc.v10.i2.585
19. Adcock DM, Kressin DC, Marlar RA. Effect of 3.2% vs 3.8% Sodium Citrate Concentration on Routine Coagulation Testing. Am J Clin Pathol 1997; 107(1): 105-110. https://doi.org/10.1093/ajcp/107.1.105
20. Levin J, Bessman JD. The inverse relation between platelet volume and platelet number. Abnormalities in hematologic disease and evidence that platelet size does not correlate with platelet age. J Lab Clin Med 1983; 101(2): 295-307.
21. Castelli R, Cassinerio E, Cappellini MD, Porro F, Graziadei G, Fabris F. Heparin induced thrombocytopenia: pathogenetic, clinical, diagnostic and therapeutic aspects. Cardiovasc Hematol Dis Drug Target 2007;7(3): 153-162. https://doi.org/10.2174/187152907781745251
22. Verheul HM, Pinedo HM. The importance of platelet counts and their contents in cancer. Clin Cancer Res 2003; 9(9): 3219-3221.
23. Letai A, Kuter DJ. Cancer, Coagulation, and Anticoagulation. Oncologist 1999; 4(6): 443-449. https://doi.org/10.1634/theoncologist.4-6-443
24. Jones S, Gibbins JM. The neurokinin 1 receptor: a potential new target for anti-platelet therapy? Curr Opin Pharmacol 2008; 8(2): 114-119. https://doi.org/10.1016/j.coph.2008.01.004
25. Brunelleschi S, Tarli S, Giotti A, Fantozzi R. Priming effects of mammalian tachykinins on human neutrophils. Life Sci 1991; 48(2): PL1-PL5. https://doi.org/10.1016/0024-3205(91)90416-9