NEUROPHARMAC JOURNAL 6 (2021) 217-228

NeuroPharmac Journal

Journal Homepage: https://www.neuropharmac.com

Research

Protective effect of *Majoon Muṣaffī-i-Khūn* (MMK) in cyclophosphamide-induced thrombocytopenia in male Sprague Dawley rats

Rukhsar Basheer[1]*, Nasreen Jahan[1], Jamil Ahmad[2]

[1]Department of Ilmul Advia, National Institute of Unani Medicine, Bengaluru, India.

[2]Department of Ilaj Bit Tadbeer, National Institute of Unani Medicine, Bengaluru, India.

ARTICLE INFO

Article history
Received 22 July 2021, Revised 01 August 2021, Accepted 09 August 2021, Published online 30 August 2021

Keywords
Blood purifier, Haematological disorders, Abnormal blood count, *Majoon Muṣaffī-i-Khūn,* Thrombocytopenia

ABSTRACT

Aim of the study: To evaluate the efficacy of *Majoon Muṣaffī-i-Khūn* (MMK) in cyclophosphamide-induced thrombocytopenia in male Sprague Dawley rats.

Material and Methods: Thrombocytopenia was induced by administration of Cyclophosphamide (25 mg/kg, 3 days, SC). Simultaneously, the animals were treated with MMK (300 and 600 mg/kg) orally and continued for three weeks. After completion of treatment, blood samples were collected by retro-orbital puncture and analyzed for WBC, RBC, Hb, and platelet counts, with bleeding and clotting time. MMK insignificantly increased the platelets, WBC, RBC, and Hb with a significant decreased in bleeding ($p<0.001$; $p<0.05$) and clotting times ($P<0.01$, $P<0.001$) which was altered by cyclophosphamide.

Results: The results demonstrated that MMK improved platelet counts, clotting, and bleeding time as well as restored the altered level of WBC, RBC, and Hb.

Conclusion: Based on the above results it can be concluded that the hydroalcoholic extract of MMK may have the potential to normalize the blood composition effectively.

Introduction

Hematological disorders primarily affect the blood and blood-forming organs are being epidemic worldwide. Among all, abnormalities in erythrocytes, leucocytes, and platelets are considered critical and need immediate medication. In which thrombocytopenia is a life-threatening condition which occurs either due to decreased production of platelets from the bone marrow or increased destruction of platelets succumbing to an immune reaction.[1] Thrombocytopenia is defined as reduced platelet count which may arise by one of three mechanisms for instance failure of megakaryocytic maturation and platelet formation, excessive platelet consumption after its release into the circulation, platelet sequestration in an enlarged spleen. Thrombocytopenia may be infection-induced, drug-induced, and heparin-induced.[2] According to Erkurt et al., thrombocytopenia occurs as a result of the falling number of platelet from 150,000/microL, three main reasons of thrombocytopenia are decreasing of formation of platelets, increasing number of platelet destruction and changing pattern of platelet distribution.[3] It is mainly linked to prolonged bleeding and clotting time due to an insufficient number of platelets in the

Corresponding author at: Department of Ilmul Advia, National Institute of Unani Medicine, Begaluru, India
E-mail address: rukhsarbasheer2k11@gmail.com
https://orcid.org/0000-0001-5818-8868
https://doi.org/10.37881/1.619

blood.[4] The decrease in normal platelet count is mainly found in infections like dengue hemorrhagic fever, chickenpox, and bacterial infections.[5] Thrombocytopenia may also result from intake of alcohol, heparin, chloramphenicol, and cancer chemotherapeutic drugs. Thrombocytopenia occurs in both adults and children, with a multimodal incidence with the first peak in childhood and second and third peaks in young adults and the elderly. The incidence of thrombocytopenia in adults is 3.3/100000 adults per year, along with a prevalence of 9.5 per 100000 adults.[6] As a result of accrual incidents many people turn towards medicinal plants for treatment thereby boosting and enhancing human health because professional care is not immediately available, too inconvenient, costly, and time-consuming also.

The Unani system of medicine is a comprehensive medical system that meticulously deals with the various states of health and disease. It provides promotive, preventive, curative, and rehabilitative health care. The signs and symptoms which are found in ghair tabai khun (abnormal blood) co-relate with a modern concept of blood disorders. Many single blood purifier groups of drugs used in the Unani system of medicine have been evaluated for various hematological disorders and found to be very effective like *Ziziphus sativa, Fumaria officinalis, Swertia chiraita, Sphaeranthus indicus, Tephrosia purpurea, Azadirachta indica, Smilax ornata, Smilax china* etc.[7] and various compound formulations such as *Majoon ushba, Majoon chobchini, Arq murakkab musaffie khun, Itreefal shahatra, Joshanda musaffie khun, Sharbat unnab, Majoon mundi, Safoof chobchini* etc.[8] are also used to treat various hematological disorders. Many scientific studies have been reported for their diverse pharmacological properties viz. Ficus has anti-microbial and thrombolytic activity[9] while *Plumbago zeylanica* showed anti-inflammatory, anticancer, and antioxidant activities[10] and *Fumaria officinalis* have antioxidant activity[11]. Based on these properties, it was hypothesized that blood purifier drugs may prove their potential in battling hematological disorders.

Majoon Muṣaffī-i-Khūn is an important pharmacopoeial formulation of Unani medicine. Its ingredients have been listed in table 1. According to Ibn Sina, *Emblica officinalis* is one of the ingredients of MMK is beneficial in decreasing *ghilzate khun* (Viscosity of blood); it helps in the production of blood and prevents premature destruction of blood. *Swertia chiraita* is found helpful in hemorrhage beneath the skin, also used in fever caused by the alteration in phlegm, sanguine and bile, inflammation of liver and stomach[11]. Many scientific studies have been reported for their antioxidant, tonic, and immunomodulatory activities[11]. However, no attention has been paid to elucidate the efficacy of MMK on hematological disorders. The promising therapeutic values of the ingredients of MMK and their pharmacological activity imply that the formulation may be a new source for the treatment of hematological disorders. Therefore, *Majoon Muṣaffī-i-Khūn* was chosen to evaluate its effect in cyclophosphamide-induced thrombocytopenia in male Sprague Dawley rats.

The hydroalcoholic extract (30:70 v/v, water: ethanol) was standardized by extracting the drug in three different drug and solvent ratios i.e. 1:5, 1:10, and 1:15. The extract which showed maximum yield percentage was used for the study. Further, the qualitative phytochemical analysis of the extract of ingredients of *Majoon Muṣaffī-i-Khūn* and GCMS was also carried out.

Material and Methods

a) Experimental animal

The study was carried out on healthy male Sprague Dawley rats, weighing 200-250 gm. They were procured from registered breeders (Biogen Enterprises Bengaluru Karnataka 560091) and allowed to get acclimatized for one week before starting each experiment. They were housed in clean polypropylene cages at room temperature (25±2^{0}C), humidity at 45-55% with 12 hrs light and dark cycle, and provided standard diet and water *ad libitum.* The animal care procedures and experimental protocol were followed

as per the guidelines of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA). The study was conducted after obtaining the ethical clearance from the Institutional Animal Ethics Committee (IAEC) of the National Institute of Unani Medicine (NIUM), Bengaluru with the number IAEC /04/15/IA, dated 5th February 2019.

Table 1: Ingredients of polyherbal formulation *Majoon Muṣaffī-i-Khūn*

S.No	Drugs name	Botanical name	Weight
1.	Neem	Bark of *Azadirachta indica* A. Juss	25 gm
2.	Anjeer	Bark of *Ficus auriculata* Linn.	25 gm
3.	Shahatra	Whole herb of *Fumaria officinalis* Linn.	25 gm
4.	Chiraita	Whole herb of *Swertia chiraita* Roxb. ex Flem. Karst.	25 gm
5.	Sheetraj hindi	Root of *Plumbago zeylanica* Linn.	25 gm
6.	Kishneez khushk	Dried fruit of *Coriandrum sativum* Linn.	25 gm
7.	Poste Halela zard	Fruit of *Terminalia chebula* Retz.	25 gm
8.	Post Halela kabuli	Fruit of *Terminalia chebula* Retz.	25 gm
9.	Post Balela	Fruit of *Terminalia bellirica* Roxb.	25 gm
10.	Amla	Fruit of *Emblica officinalis* Gaertn.	25 gm
11.	Halela siyah	Fruit of *Terminalia chebula* Retz.	25 gm
12.	Badiyan	Fruit of *Foeniculum vulgare* Mill.	25 gm
13.	Gule surkh	Dried flower of *Rosa damascena* Mill.	25 gm
14.	Senna	Dried leaves of *Cassia angustifolia* Vahl.	25 gm

b) Extraction of ingredients of MMK

All the ingredients of *Majoon Muṣaffī-i-Khūn* (table 1), were procured from authentic sources. The test drugs were dried in shade and powdered by the electric grinder, sieved by Sieve no. 80 to get a fine powder. The hydroalcoholic extract (water: ethanol, 30:70) of all the ingredients of *Majoon Muṣaffī-i-Khūn* were obtained by using Soxhlet's apparatus in different drug and solvent ratios i.e., 1:5, 1:10, and 1:15. The extraction was done for 6 to 8 hours at temperature 80ºC and filtered to obtain a clear filtrate then it was dried on a rotatory evaporator to obtain a solid residue of extract. Extract of ratio (1:10) showed the maximum presence of phytoconstituents that is why the same extract was used for the study. The extract was stored in an airtight container in the refrigerator.

c) Dose calculation of MMK

The human therapeutic dose of *Majoon Muṣaffī-i-Khūn* has been mentioned in Unani literature is 5 to 10 gm[8]. The dose for the rat was calculated by taking the higher dose (10 gm) based on body surface area[12]. Since the hydroalcoholic extract of *Majoon Muṣaffī-i-Khūn* was used for the study therefore the dose of the extract for a rat was calculated by its yield percentage with reference to the dose of the crude drug i.e., 10 gm and found to be 300 mg/kg. The study was carried out in two doses to evaluate the dose range effect hence a second dose was taken that is just double of the first dose (600 mg/kg). A fresh solution of extract in a required concentration was prepared in distilled water at the time of the experiment. The extract was administered orally by gastric cannula. Single oral administration of MMK did not produce any toxic signs and behavioral changes in the rats throughout the observation period of 14 days. Therefore, it was concluded that the hydroalcoholic extract of MMK is safe since no morbidity and mortality were observed at 2000 mg/kg[13].

d) Chemicals, solvents, and reagents

All the chemicals and reagents used in this study were of analytical grade and procured from authentic sources. Ethanol was obtained from Changshu Hongsheng Fine chemical Co. Ltd, Jiangsu province, Bengaluru, India. The chemicals used in the phytochemical analysis were procured from Central Drug House (P) Ltd. New Delhi, India. Cyclophosphamide was obtained from Sigma Aldrich, India.

e) Administration of cyclophosphamide to induce thrombocytopenia

The study was carried out by the method of Venkatraman et al. Healthy adult male Sprague Dawley rats were used for the study. The animals were divided into four groups of 6 animals each viz. negative control, positive control, test group A, and test group B. Thrombocytopenia was induced in all the animals except negative control by subcutaneous injection of cyclophosphamide (25mg/kg) once daily for 3 days. On the 7th day, blood samples were collected from the animals of positive control thereafter they were sacrificed while treatment was started in test group A and B with hydroalcoholic extract of *Majoon Muṣaffī-i-Khūn* at the dose of 300 mg/kg; 600 mg/kg respectively daily by oral route from the first day and continued for further 21 days. After completion of treatment, Blood samples were collected by a retro-orbital puncture for the analysis of platelet count; bleeding time, and clotting time; WBC, RBC, and Hb were also analyzed. Thereafter, all the animals including negative control were sacrificed under Isoflurane (3% Isoflurane and 1% air pressure) anesthesia.

Table 2: Experimental protocol of the antithrombocytopenic activity of the polyherbal formulation (MMK)

Treatment	Inference
Distilled water throughout the study	Negative Control (NC)
Disease induction without treatment	Positive control (PC)
Disease induction + hydroalcoholic extract of MMK at low dose	Test group A
Disease induction + hydroalcoholic extract of MMK at high dose	Test group B

f) Determination of the bleeding time

Bleeding time was determined by modified Duke's method. The animal was kept in a restrainer and the tail is exposed out; it was cleaned using hot water, rectified spirit, and its tip was punctured using a sterile needle and blotted on Whatman filter paper until the bleeding was stopped. Bleeding time was recorded in terms of seconds[14].

g) Determination of clotting time

Clotting time was determined by using the capillary tube method. The capillary tube was filled with blood by puncturing the retro-orbital region and the tube was broken for every 30 seconds until a fibrin thread appears. Clotting time was noted in terms of minutes[14].

h) Animal Sacrifice

Animals were sacrificed by overdose of anesthetic agent (thiopentone sodium 150 mg/kg b.w IP). Sacrificed animals were sent in suitable packing for disposal through Karnataka Pollution Control Board (Cynmbranki Pvt Ltd).

i) Statistical Analysis

The statistical analysis was carried out using ANOVA repeated test for intragroup compare and ordinary ANOVA for intergroup comparison and the significant difference between the groups was analyzed using Tukey Kramer multiple comparison test. Statistically software, Graphpad Instat 3 was used for the analysis. $P<0.05$ was considered significant.

Results

Antithrombocytopenic effect of hydroalcoholic extract of ingredients of *Majoon Muṣaffī-i-Khūn* was assessed by hematological parameters viz. WBC, RBC, HB, Platelet counts, Bleeding time, and Clotting time.

i) Platelets counts

In the present study, thrombocytopenia was induced by subcutaneous administration of cyclophosphamide (25 mg/kg) for 3 days. Platelet counts were found to be decreased 39.875 ± 8.825$10^3$/mm^3significantly ($p<0.001$) in the animals of positive control when compared with negative control (288.25 ± 24.5$10^3$/mm^3) which was treated with distilled water only. Administration of hydroalcoholic extract of ingredients of MMK in low dose (300 mg/kg) for twenty-one days, increased platelet production i.e. 82.625 ± 21.76 10^3/mm^3 but it was not found to be significant when compared with the positive control, nevertheless, the extract in high dose (600 mg/kg) did not exhibit any effect on platelets production as the platelet count was noted 39.57 ± 7.40 10^3/mm^3only which was not different from positive control (table3, figure 1).

Table 3: Effect of hydroalcoholic extract of MMK on platelets counts

Groups	Dose/Route	Platelet count (10^3/mm^3)
Negative control (Distilled Water)	1 ml Orally	288.25 ± 24.5
Positive control (Cyclophosphamide)	25 mg/kg Subcutaneously	39.875 ± 8.825***
Test group A (Extract of MMK in low dose)	300 mg/kg Orally	82.625 ± 21.76
Test group B (Extract of MMK in high dose)	600 mg/kg Orally	39.57 ± 7.40

Positive control vs negative control (*** $p<0.001$), n-6

ii) Bleeding time

The bleeding time in negative control was noted 44±2.19 sec. Upon induction of thrombocytopenia with cyclophosphamide, bleeding time increased significantly i.e., 62.85 ± 9.6 sec ($p<0.001$) in comparison to the negative control. When the animals of test groups A and B were treated with hydroalcoholic extract of MMK in low and high doses, a significant decreased of 45.5 ± 2.47sec ($p<0.001$), 51.85 ± 2.71 sec ($p<0.05$) in bleeding time was observed respectively in comparison to a positive control (table 4, figure 2).

iii) Clotting time

Clotting time in the animals of negative control which were treated with distilled water only was noted at 116±4.75sec while in the animals of positive control after treatment with cyclophosphamide, clotting time was found to be significantly increased i.e., 157.7±37.5 sec ($p<0.05$) in comparison to positive control. After treatment with test drug in low dose (300 mg/kg) significant reduction ($P<0.01$) in clotting time i.e., 100.87 ± 8.41 sec was observed in comparison to positive control. Moreover, the test group which was treated with a high dose (600 mg/kg) of MMK took much less time in the clotting of blood i.e., 86.71 ± 3.29 sec ($p<0.001$) in comparison to positive control treated with cyclophosphamide only (table 4, figure 2).

Table 4: Effect of hydroalcoholic extract of MMK on clotting time and bleeding time

Groups	Dose/Route	Bleeding time in seconds (Mean±SEM)	Clotting time in seconds (Mean±SEM)
Negative control (Distilled water)	1ml Orally	44 ± 2.19	116 ± 4.75
Positive control (Cyclophosphamide)	0.3 ml Subcutaneously	62.85 ± 9.6*** a	157.7 ± 37.5* a
Test group A (Extract of MMK in low dose)	300 mg/kg orally	45.5 ± 2.47*** b	100.87 ± 8.41** b
Test group B (Extract of MMK in high dose)	600 mg/kg orally	51.85 ± 2.71* b	86.71 ± 3.29*** b

Figure 1: Effect of hydroalcoholic extracts of MMK on platelets counts

Figure 2: Clotting time and bleeding time of different groups

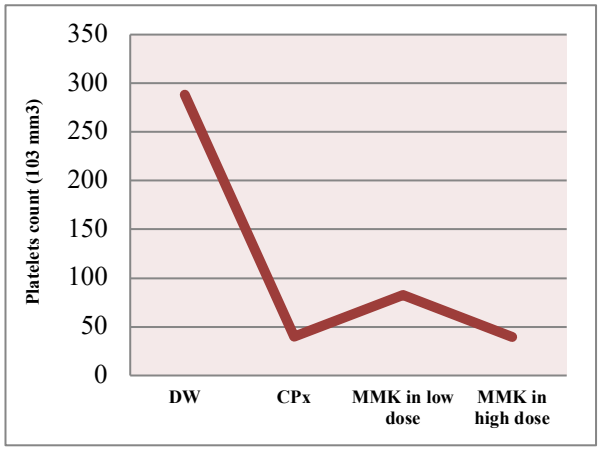


Figure 1

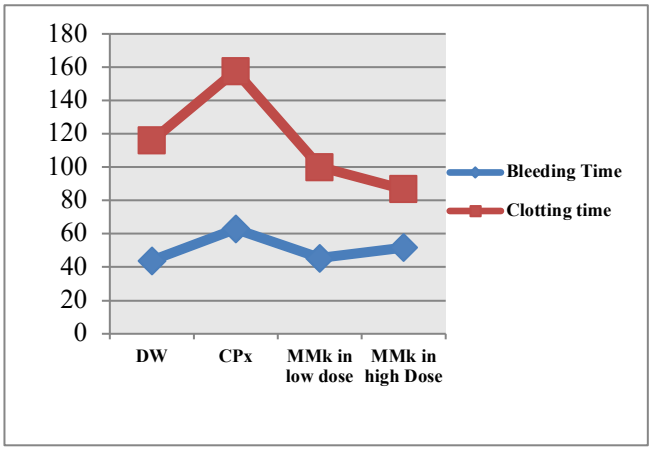


Figure 2

iv) WBC counts

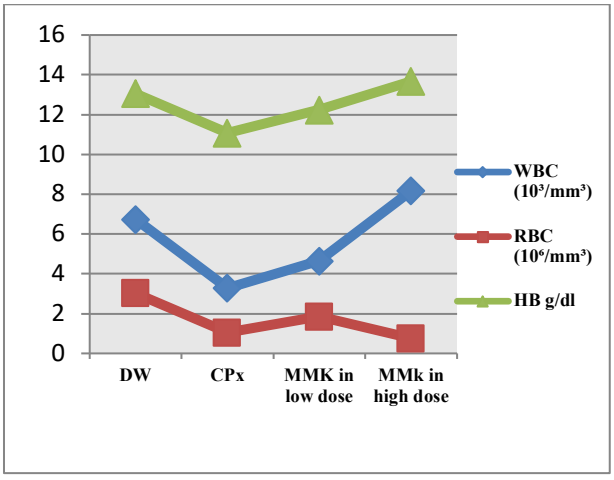
The mean of WBC count in negative control was noted 6.73±2.16 $10^3/mm^3$while in positive control treated with cyclophosphamide only for 3 days was found to be decreased up to 3.28±0.9310$^3/mm^3$. Decreased WBC count in positive control in comparison to negative control indicates the development of thrombocytopenia in rats though it was not significant statistically ($p>0.05$). Administration of hydroalcoholic extract of ingredients of MMK in low dose (300 mg/kg) for twenty-one days, non-

significant increase in WBC count i.e. 4.65 ± 1.02 10^3/$mm^3$was observed in comparison to the positive control, however, the extract in high (600 mg/kg) dose remarkably increased the WBC counts 8.17 ± 1.33 10^3/mm^3(table 5, figure 3).

Table 5: Effect of hydroalcoholic extract of MMK on WBC, RBC, and hemoglobin counts

Groups	Dose/Route	WBC (10^3mm^3)	RBC (10^6mm^3)	Hb (g/dl)
Negative Control (Distilled Water)	1 ml Orally	6.73±2.16	3.02±0.14	13.06±0.68
Positive Control (Cyclophosphamide)	25 mg/kg Subcutaneously	3.28±0.93	1.04±0.17***	11.08±1.47
Test group A (Extract of MMK in low Dose)	300 mg/kg Orally	4.65±1.02	1.84±0.48	12.23±0.46
Test group B (Extract of MMK in high Dose)	600 mg/kg Orally	8.17±1.33	0.76±0.04	13.67±0.79

Figure 3: Effect of hydroalcoholic extract of MMK on WBC, RBC, and Hb counts



V) RBC Counts

The mean of RBC count in negative control was noted 3.02±0.14 10^6/mm^3 while in positive control treated with cyclophosphamide only for 3 days was found to be decreased up to 1.04±0.17 10^6/mm^3. Decreased RBC count in positive control in comparison to the negative control ($p<0.001$) indicates the development of thrombocytopenia in rats. Administration of hydroalcoholic extract of ingredients of MMK in low dose (300 mg/kg) for twenty-one days, increased RBC count i.e., 1.84 ± 0.48 10^6/mm^3 but statistically not significant when compared with the positive control, likewise the extract in high dose (600 mg/kg) did not exhibit any effect on RBC count as the value was noted to 0.76±0.04 10^6/mm^3 found statistically insignificant in comparison to a positive control (table 5, figure 3).

vi) Hemoglobin Counts

The mean of hemoglobin count in negative control was noted 13.06±0.68 g/dl while in positive control treated with cyclophosphamide only for 3 days, decreased up to 11.08±1.47 g/dl. Decreased hemoglobin

count in positive control in comparison to negative control indicates the development of thrombocytopenia in rats, however, the difference was not found to be statistically significant ($p>0.05$). Administration of hydroalcoholic extracts of ingredients of MMK in low dose for twenty-one days increased hemoglobin count i.e., 12.23 ± 0.46 g/dl but it was not found to be significant when compared with positive control. The extract in high dose increased Hb 13.67± 0.79 g/dl in comparison to positive control but not found to be significant statistically (table 5, figure 3).

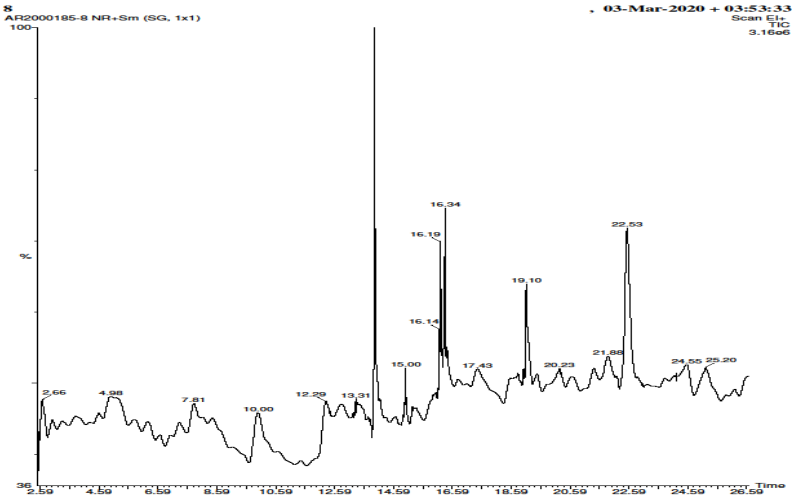
vii) Phytochemical analysis of a hydroalcoholic extract of MMK

The preliminary phytochemical studies of hydroalcoholic extract of MMK showed the presence of flavonoids, saponins, phenols, tannins, carbohydrate, cardiac glycosides, terpenes, fixed oils, coumarins, and quinines while alkaloids, diterpenes, protein, and amino acids, as well as anthraquinones, were found to be absent.

viii) GCMS analysis of MMK

In GCMS analysis of the hydroalcoholic extract of MMK, 39 different compounds were identified. The chromatogram showed 18 peaks in the retention time range 13.95 min to 22.54 min. The predominant volatile compounds found are benzene, tetradecane, chlorophenyl methanol, non-anediol, cyclohexanone, octadecan, cyclopentane methanol, and succinic acid (Figure 4).

Figure 4: GCMS analysis of Ingredients of *Majoon Muṣaffī-i-Khūn*



Discussion

Thrombocytopenia is a life-threatening condition that occurs either due to decreased production of platelets from the bone marrow or increased destruction of platelets succumbing to immune reaction[1]. Platelets are a type of blood cells produced from megakaryocytes by the action of a major hormone thrombopoietin[15]. Platelets are mainly involved in the blood clotting mechanism to control the blood loss during injury along with other blood coagulation factors. Thrombocytopenia is mainly linked to prolonged bleeding and clotting time due to an insufficient number of platelets in blood[4]. The decrease in normal platelet count is mainly found in infections like dengue hemorrhagic fever, chickenpox, rubella, and several bacterial infections. Thrombocytopenia may also result from the intake of drugs such as

alcohol, heparin, chloramphenicol, and cancer chemotherapeutic drugs. Based on these facts, cyclophosphamide, an anti-cancer at 25 mg/kg is used to induce stable thrombocytopenia in rats.[14]

In the view of above, the present study was conducted to evaluate the platelet augmentation activity of hydroalcoholic extract of MMK by using cyclophosphamide. It can induce thrombocytopenia by suppressing the production of megakaryocytes from bone marrow, which is the site of blood cell production[14]. Subcutaneous injection of cyclophosphamide for three days showed a significant reduction ($p<0.001$) in platelet count in the animals of a positive control group. From the 8th day, the animals were treated with the test drug for further 21 days. The platelet count, bleeding, and clotting time along with RBC, WBC, and Hb were analyzed; results demonstrated that hydroalcoholic extract of MMK at low dose increased platelet count though it was not statistically significant whereas at high dose it was ineffective. Bleeding time is a parameter to assess hemostatic function which indicates the capability of the platelets to interact with blood vessel walls to form a blood clot. Thus, this is a useful test that can be applied to detect a qualitative defect of platelets. This directly correlates with the decrease in the number of platelets in circulation[16]. After administration of cyclophosphamide for 3 days subcutaneously, bleeding time increased significantly ($p<0.001$) when compared to the negative control, post-treatment with hydroalcoholic extract of MMK in low and high dose for 21 days a significant decrease ($p<0.001$, $p<0.05$ respectively) in bleeding time was observed. Thus, an improved bleeding time indicated the effectiveness of MMK in thrombocytopenia nonetheless, the effect of test formulation at a high dose was found to be likened to the platelets count.

Clotting time measures the degree of activation of the coagulation pathways, it is the time required for a sample of blood to coagulate under specific conditions. This value reflects the time required for a coagulation cascade to generate thrombin, it promotes platelet activation and ultimately leads to platelet plug formation[16]. In the positive control group clotting time was found to be significantly ($p<0.05$) increased while it decreased expressively ($p<0.01$; $p<0.001$) after administration of MMK in low and high doses respectively. Since the extract of MMK can improve platelet count, clotting time, and bleeding time it is concluded that the extract of MMK has the potential to increase the platelet count, decrease bleeding and clotting time significantly.

The hydroalcoholic extract of MMK did not only increase the platelet count but also restored the altered level of WBC, RBC, and Hb, a greater effect was observed in normalizing the WBC and Hb levels at high doses. These findings are correlating with the results of other studies for instance in a study after administration of cyclophosphamide WBC count gets reduced[17]. Henceforth MMK can be used in the augmentation of platelets in certain viral infections because the mechanism behind cyclophosphamide-induced thrombocytopenia and dengue virus-induced thrombocytopenia are similar i.e., bone marrow suppression[18] thus MMK may also be effective to treat dengue virus-induced thrombocytopenia[14].

In the Unani system of medicine thrombocytopenia as such has not been defined but the term *Riqqate dam* (decreased viscosity of blood) is used which is the main cause of *jiryanuddam* and *qatma (*hemorrhage beneath the skin) [19]. Hemorrhage beneath the skin is a condition in which the viscosity of blood is decreased, and blood becomes liquefied due to an alteration in the equilibrium of humours; disproportionate quantity and quality of sanguine either due to any infection or disease, is specifically accountable for this condition. The ingredients of MMK are tonic to the liver, stomach, and spleen; they restore the function of these organs and normalize the blood composition by their modulator property. *Emblica officinalis* is one of the ingredients of MMK recommended by eminent Unani physicians in the state of hemorrhage. It neutralizes the excessive heat of blood by normalizing the quality and quantity of sanguine[20]. *Swertia chiraita* is another ingredient of MMK potent in treating hemorrhage caused beneath the skin[21]. In a study, *Carica papaya* leaves aqueous extract was investigated against thrombocytopenic

rat model where a significant increase in platelets count was discovered which may be due to the presence of tannins and alkaloids that act on the bone marrow preventing the destruction of the platelets and enhancing its ability to produce platelets. It can also increase the life of the platelet in blood circulation[22]. It has also been reported that some flavonoids possess anti-platelet aggregation effects through different pathways, being the inhibition of the arachidonic acid-based pathway the most representative mechanism of action[23]. Saponins, tannins, and alkaloids act on the bone marrow, preventing its destruction and enhancing its ability to produce platelets. Moreover, it can also prevent platelet destruction in the blood and thereby increase its life span[9] fortunately MMK is abundant with these phytoconstituents viz. flavonoids, saponins, and alkaloids which have strong antioxidant properties[24]. Hence the anti-thrombocytopenic effect of MMK may be due to the inhibition of platelet oxidation.

Furthermore, the ingredients of MMK reported for anti-inflammatory[25], hematopoietic[26,27] tonic[28], anti cancer[29], anti-oxidant, immunomodulatory activities[11]. Manifestation of various effects along with its research reports gives rise to total effects in amelioration of various hematological disorders. Since the ingredients of MMK own a wide range of pharmacological effects it can be said that it may prove its effectiveness by evolving a holistic mechanism and involving its numerous actions.

Conclusion

This study demonstrated that hydroalcoholic extract of MMK can improve platelet count, clotting time, and bleeding time. This preliminary study may serve as a tool for screening the blood purifier activity of Unani drugs. Moreover, this action might qualify the MMK as a novel drug for hematological disorder and lead to further exploration to its full potentials. These drugs could be the best candidate for anti-thrombocytopenic effects in the future with minimal adverse effects, easier availability, and better acceptability.

Acknowledgment

The authors are sincerely thankful to Prof. Abdul Wadud, Director NIUM, and Prof. G. Sofi, HOD, Department of Pharmacology, NIUM, Bengaluru for providing the best possible facilities for the smooth carriage of this work.

Conflicts of Interest

The authors declare that there are no conflicts of interest relevant to this article.

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Cite this article

Basheer R, Jahan N, Ahmad J. Protective effect of *Majoon Muṣaffī-i-Khūn* (MMK) in cyclophosphamide induced thrombocytopenia in male Sprague Dawley rats. NeuroPharmac J. 2021; 6(2): 217-228. DOI: 10.37881/1.619