BioTechnologia vol. 100 (1) • pp. 57–67 • 2019
Journal of Biotechnology, Computational Biology and Bionanotechnology RESEARCH PAPERS
http://doi.org/10.5114/bta.2019.83212

Evaluation of antidote potential of methanol leaf extract of *Bauhinia monandra* on heparin-induced thrombocytopenia in mice

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Abstract

Thrombocytopenia is closely associated with heparin therapy and hematological disorders and other common diseases in patients admitted in the hospital. Globally, thrombocytopenic cases in hospitals leading to 80% of deaths are mainly due to refractory bleeding and lack of availability of platelet concentrates. Currently, chemical compounds for managing thrombocytopenia are needed. The aim of the study was to determine the effect of methanolic leaf extract of *Bauhinia monandra* as a potential antidote for treating heparin-induced thrombocytopenia (HIT). A total of 30 mice were used for the experimental study. The mice were divided into five Groups (I–V) of six mice each. Group I (normal control) was given distilled water only, and Group II (positive control) was given heparin only. Groups III, IV, and V (treatment groups) were given heparin and methanolic leaf extract of *B. monandra* at a dose of 100, 200, and 400 mg/kg body weight (b. wt), respectively. Furthermore, blood samples were collected to determine the blood platelet count. Bleeding time and clotting time were also determined. This study showed that the mice in the group treated with methanolic leaf extract of *B. monandra* at a dose of 400 mg/kg b. wt had significantly ($P < 0.05$) higher platelet counts (225.10 ± 6.41) than the control groups: normal control (181.90 ± 11.38) and positive control (127.65 ± 5.79). It also significantly decreased the bleeding and clotting time. Acute toxicity test showed no significant physical and behavioral changes. The results from this study show that leaf methanolic extract of *B. monandra* is effective as an antidote for treating HIT.

Key words: phytochemicals, *Bauhinia monandra*, thrombocytopenia, antidote

Introduction

Thrombocytopenia is a medical condition characterized by an abnormally low platelet count in the blood. Platelets are a component of the blood whose function is to contribute to hemostasis, the process of stopping bleeding by clumping and clotting blood vessel injuries (Balduini and Melazzini, 2017). In thrombocytopenia, the blood has lower levels of platelets, and hence mild to severe bleeding can occur. Bleeding can be internal or occur on the surface of the skin or just beneath it. The reported incidence of hemorrhagic complications in the literature ranged from 3% to 53%, and the frequency of hemorrhagic deaths ranged from 14% to 24% (Kantarjian et al., 2007). Thrombocytopenia is caused by the bone marrow that cannot make enough platelets, when the body rapidly destroys blood platelets, or medication side effects (Kamthe and Kulkami, 2017). This may occur as a result of a separate disorder, such as leukemia, aplastic anemia, viral infections, genetic conditions, immune system disorders, liver failure, and dehydration. Drugs are also a common cause of acute thrombocytopenia in adults (Visentin and Liu, 2007), and most cases of drug-induced thrombocytopenia (DITP) are caused by drug-dependent antibodies (Warkentin et al., 2006; Ahmed et al., 2007; Warkentin et al., 2008). These antibodies are particularly specific to the drug structure and bind

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tightly to platelets via their Fab regions in the presence of the drug (Warkentin et al., 2006; Ahmed et al., 2007; Peterson et al., 2008). DITP occurs 1–2 weeks after commencing a new drug therapy or suddenly after a single dose, when the drug has previously been administered intermittently (George and Aster, 2009). However, in severe cases, thrombocytopenia can occur immediately after administration of the first dose of an antithrombotic agent such as tirofiban, abciximab, and eptifibatide (George and Aster, 2009). Recovery from DITP usually starts within 1–3 days after stopping the drug administration and completes within a week (Peterson et al., 2008). DITPs often occur suddenly, and in severe cases may lead to major bleeding problems and death (George et al., 1998; Aster and Bougie, 2007; Aster et al., 2009).

HIT is a medical complication commonly associated with heparin therapy. It rarely occurs outside the hospital. Heparin is used in medicine for both the prevention and the treatment of arterial and venous thrombosis. In HIT, the blood platelet count falls below the normal range, which may lead to an increased risk of bleeding. Since it does not help to discontinue the anticoagulant agent in the case of thrombosis, the treatment of HIT requires stopping the heparin treatment entirely for the platelet count to return to normal within days (Watson et al., 2012) and choosing of an agent that will not reduce platelet count any further, such as danaparoid, fondaparinux, argatroban, and bivalirudin (Ortel, 2009). Although DITP is cured after several days in most cases, severe cases result in major bleeding (problems) and occasionally to death. Hence, a rapid recovery from this medical condition is a major concern for researchers. Therefore, any pharmacological agent that can rapidly and effectively reverse the blood platelet count back to the normal range in the case of HIT will be useful in the medical field. Natural plant extracts rich in phytoconstituents such as alkaloids, tannins, polyphenols, flavonoids, and other related compounds are known to possess several health benefits and to reduce the incidence of diseases (Evans and Halliwel, 2001). Therefore, a research focused on natural and herbal drugs as a potential remedy may result in significant health benefits while minimizing the adverse effects (Gowri et al., 2011).

Bauhinia monandra is a species of leguminous trees that belong to the Caesalpiniaceae family (Keay, 1989). It is an evergreen shrub or tree with a rounded crown, which can grow up to a height of 3–15 m. *B. monandra* is commonly known as a cow's foot, orchid tree, Napoleon plume, Flamboyant, and St Thomas tree (Starr and Starr, 2011). In the Yoruba tribe of South Western Nigeria, it is called "Abafe" (Ajiboye et al., 2015), and its decoction is taken to improve blood function, which is one of its health benefits. The tree is also fairly widely distributed throughout the natural grassland of Northern and South Eastern Nigeria (Agbugui et al., 2010). The leaf extract is bitter and traditionally used for the treatment of diabetes in Nigeria, Brazil (McCune and Johns, 2002), and Asia (Macedo, 2008). Its antidiabetic activity has been linked to the presence of antioxidant compounds (McCune and Johns, 2002), which have been studied (Argolo et al., 2004). Traditionally, the leaf extract has been used for treating postnatal bleeding (Onyije et al., 2012) and as the antidote for stonefish stings (Hansworth, 1990). Leaf decoction of *B. monandra* is one of the active ingredients of a blood tonic preparation in Nigeria. Moreover, the methanolic extract of *B. monandra* leaves (MEBmL) has been reported to possess hypoglycemic (Pepato et al., 2002), antioxidant (Argolo et al., 2004; Aderogba et al., 2006), antimicrobial (Ajiboye et al., 2015), anti-inflammatory (Campos et al. 2016; Solomon et al., 2016), and antinociceptive (Campos et al., 2016) activities. Hemagglutinating, trypsin inhibiting, and low disaccharide activities of the seed extracts have been reported (Abreu et al., 1990). In addition, several *Bauhinia* species are utilized as folk medicines worldwide, and studies have reported antimalarial, antiviral, antibacterial, antifungal, antidiarrheal, and antispasmodic activities (Onyije et al., 2012). Studies on the chemical composition of the leaves have led to the isolation of a wide range of bioactive compounds including quercetin-3-O-rutinoside and quercetin and β-carotene (Aderogba et al., 2006). Early phytochemical studies indicated that the species of this genus are rich sources of lactones, flavonoids, terpenoids, glycolipids, steroids, steroids, and tannins (Albuquerque and Silva, 2000; Da Silva et al., 2000; Mendes et al., 2006).

The folkloric claims made by tribal communities and abundant phytochemical contents inspired the present study. However, not many reports on the anti-thrombocytopenic activity of *B. monandra* are available in the literature. Therefore, the purpose of the present study was to determine the effect of the MEBmL as a potential antidote for thrombocytopenic disorder.

Materials and methods

Chemicals and experimental drug

Heparin was purchased from Vangel Pharmaceuticals (Enugu, Nigeria). Methanol, acetic acid, lead acetate, sodium hydroxide, ethyl acetate, hydrochloric acid, tetraoxosulfate (VI) acid, chloroform, ethanol, and ferric chloride were obtained from the chemical store of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria. Solvents were redistilled before use while reagents were used without further purification. All chemicals and reagents were of analytical reagent grade.

Collection of plant materials

The aerial parts of *B. monandra* were collected from the Botanical Garden, University of Nigeria, Nsukka, Nigeria. The plant parts were taken to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria, where they were appropriately identified and authenticated with specimen voucher number, UNH 1559.

Preparation of plant extract

Fresh aerial leaves of *B. monandra* were washed with running tap water to remove dust and dirt. The leaves were then air-dried under shade for a period of 3 weeks and pulverized into powder and stored in plastic airtight containers for further processing. The pulverized leaves (200 g) were macerated in 1 l of methanol and shaken properly. The mixture was allowed to stand for 72 hrs, after which it was filtered using Whatman filter paper. The filtrate obtained was concentrated in a rotary evaporator and the resultant extract was preserved in airtight glass containers and stored at 4 °C in a refrigerator for subsequent use.

Experimental animals

Albino mice of both sexes, weighing between 25 and 32 g, were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria, and housed at 25 °C ± 5 °C in a well-ventilated animal house under 12 : 12 h light : dark cycle. The animals were maintained under standard conditions in the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria. All the animals were provided with a normal pellet diet and water ad libitum, and allowed to acclimatize to environmental conditions prior to commencement of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee of the University of Nigeria, Nsukka, Nigeria.

Laboratory animal ethics

The protocols governing the use and handling of laboratory animals were strictly followed as approved by the Animal Handling Ethics Committee, of University of Nigeria, Nsukka, Nigeria. These principles are also in accordance with the National Research Council Guide for Care and Use of Laboratory Animals (Gannon and Sikes, 2007).

Preliminary phytochemical screening of MEBmL

Phytochemical analyses of reducing sugars, proteins, carbohydrates, flavonoids, saponins, resins, steroids, oils, terpenoids, alkaloids, tannins, cardiac glycosides, and acidic compounds were carried out on the MEBmL using standard procedures (Vaghasiya et al., 2011; Ramamurthy and Sathiyadevi, 2017).

Acute toxicity study

The acute toxicity study of MEBmL was evaluated in mice according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD, 2001). Following the fasting period, a single dose of 5000 mg/kg of crude extract was administered orally to three male and three female mice in the treatment groups. Remaining three male and three female mice were administered distilled water and were regarded as control groups. Food was allotted to the mice exactly 1 h after treatment. The mice were closely and carefully observed within the first 6 hrs for any indication of toxicity effect, daily for a duration of 14 days. The animals were visually observed daily for mortality, behavioral pattern, changes in physical appearance, injury, pain, and signs of illness during the duration of the study.

Induction of thrombocytopenia

Heparin was used to induce thrombocytopenia in the experimental animals. This was achieved by a subcutaneous injection of heparin at the dose of 2500 IU/kg body weight (b. wt) for 2 consecutive days.

Experimental protocol of mice treated with the MEBmL

A total of 30 albino mice were used for the experimental study. The animals were divided into five groups. Group I served as a normal control and received only

distilled water for the duration of the study. Group II served as a positive control and was injected with heparin (2500 IU/kg b. wt, SC) daily for 2 days. Groups III, IV, and V (treatment groups) were injected with heparin (2500 IU/kg b. wt, SC) daily for 2 consecutive days and afterwards received MEBmL (100, 200, and 400 mg/kg b. wt, PO, respectively) for 5 consecutive days. Blood samples were collected on day 0, day 3, day 6, and day 8 from mice in all groups for blood platelet count evaluation.

Examination of the platelet count in mice

On day 0, day 3, day 6, and day 8 of the experiment, blood samples were collected from the veins of mice with a 1 ml syringe and put into heparinized specimen vials. The platelet count was determined using hemocytometer. The mean platelet counts ($\times 10^3/mm^3$) of mice in the five experimental groups were determined.

Evaluation of the bleeding time in mice

The bleeding time in the mice tested was determined by Duke's method (Lewis et al., 2002) with slight modifications. The tail of each mouse was carefully cut with scissors 1 mm from the end, and the time when bleeding started was noted down. The blood oozing from the tail was mopped with a filter paper at intervals of 30 s, until the bleeding stopped. The time between the beginning and the end of bleeding was noted down as the bleeding time.

Evaluation of the blood clotting time in mice

The blood clotting time in the mice tested was estimated by the capillary glass tube method (Provan and Krentz, 2002). Blood samples were collected via the retro-orbital venous sinus with a glass capillary tube. The capillary tube was broken at one end every 30 s until a fibrin thread was formed. The total time taken for the fibrin thread to form was noted down as the clotting time.

Statistical analysis

The recorded values were expressed as mean (± SEM) and subjected to an independent t-test analysis. The values of $P < 0.05$ were considered significant.

Results

Preliminary phytochemical screening of MEBmL

The rough phytochemical analysis of *B. monandra* extracts revealed the presence of important medicinal constituents. The medicinal phytochemicals found were as follows: reducing sugars, proteins, carbohydrates, flavonoids, saponins, resins, steroids, oils, terpenoids, alkaloids, tannins, and cardiac glycosides. The results of the preliminary phytochemical screening showed that the MEBmL is rich in proteins, saponin, oils, alkaloids, and tannins (Table 1).

Table 1. Preliminary phytochemical analysis of methanolic extract of *Bauhinia monandra* leaves

Plant constituents	Inference
Reducing sugar	+
Proteins	+++
Carbohydrate	+
Flavonoid	++
Saponin	++
Acidic compounds	+
Resin	+
Steroids	++
Oils	++
Terpenoid	+
Alkaloid	++
Tannins	+++
Cardiac glycosides	+

+ – present; ++ – moderately present; +++ – highly present; – – absent

Acute toxicity (LD_{50}) of the MEBmL

In acute toxicity, no early or late (when observed for 14 days) mortality was observed in mice administered with single doses of the leaf methanolic extract at 5000 mg/kg b. wt. No animal showed any significant alteration in behavioral, physiological, and physical activities. The indication is that the oral administration of *B. monandra* extract did not produce any visible toxic effects.

Effect of HIT on the physical activity of mice

After the induction of thrombocytopenia, weakness and no aggressive behavior/lack of aggression were observed in animals when they were handled or pricked with the needle. General toxic signs that were observed following the subcutaneous injection of heparin included depression, suppressed movement, anorexia, emacia-

Table 2. Effect of methanolic extract of *Bauhinia monandra* leaves on mean platelet counts in tested mice

Groups		Mean platelet counts [10^3/mm^3]			
		day 0 (baseline)	day 3 (after induction)	day 6 (3 days post treatment)	day 8 (5 days post treatment)
G I	normal control (vehicle only)	172.25 ± 7.93	180.46 ± 9.75	188.35 ± 5.95 (4.37)	181.90 ± 11.38 (0.80)
G II	positive control (vehicle + inducer)	190.30 ± 4.60	94.90 ± 3.77***	104.95 ± 3.67***[a] (10.59)	127.65 ± 5.79** (34.51)
G III	*B. monandra* extract ((100 mg/kg, b. wt) + inducer)	197.10 ± 1.87	93.70 ± 1.48***	180.15 ± 0.88[a] (92.26)	200.50 ± 13.58[b] (113.98)
G IV	*B. monandra* extract ((200 mg/kg, b. wt) + inducer)	178.80 ± 9.47	93.15 ± 6.59***	173.70 ± 2.01*[a] (86.47)	186.55 ± 5.13[a] (100.27)
G V	*B. monandra* extract ((400 mg/kg, b. wt) + inducer)	192.75 ± 5.52	98.75 ± 1.92***	192.60 ± 2.42[a] (95.04)	225.10 ± 6.41*[a] (127.95)

All values are represented as mean ± SEM; n = 5; ***P < 0.001; **P < 0.01; *P < 0.05, when all groups compared normal control group; [a]P < 0.001; [b]P < 0.01; [c]P < 0.05, when all groups compared to positive control group; all values in parenthesis are percentage increase of platelet count calculated relative to day 3 of groups

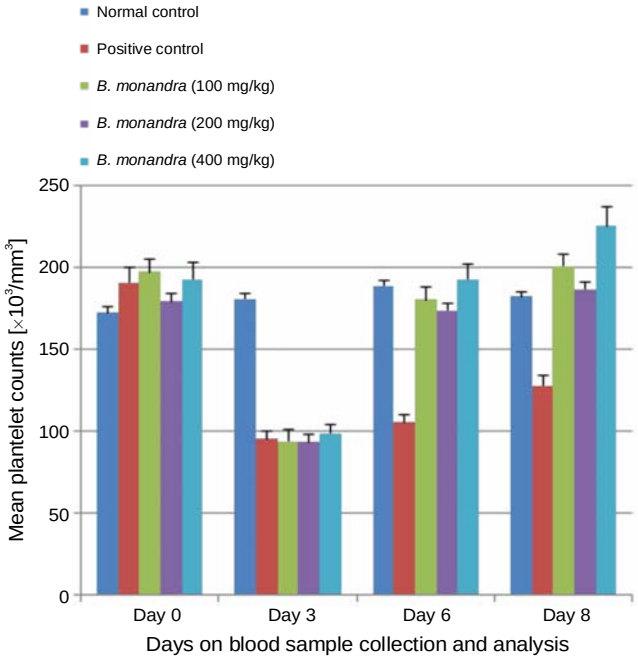

Fig. 1. Effect of methanolic extract of *Bauhinia monandra* leaves on mean platelet counts

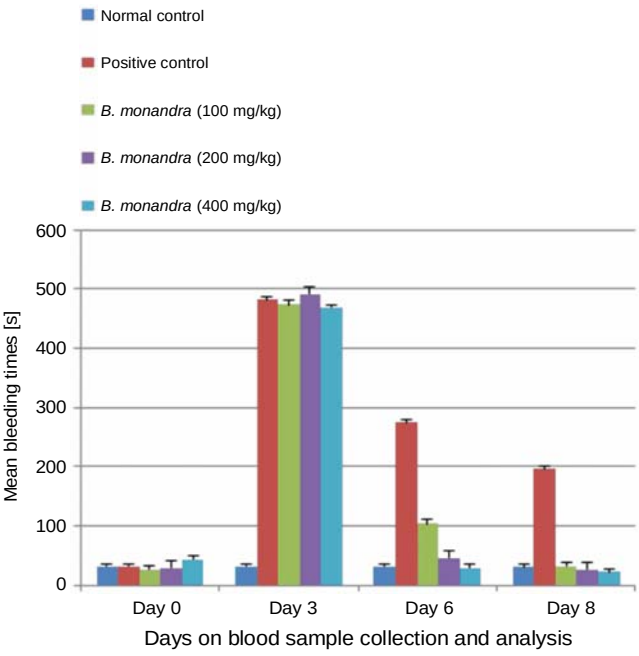

Fig. 2. Effect of methanolic extract of *Bauhinia monandra* leaves on mean bleeding times

tion, stupor, and/or coma. This may indicate a deteriorating health condition of animals due to thrombocytopenia. However, these physical conditions improved when treatment with the MEBmL was complete.

Effect of MEBmL on the platelet count in tested mice

An injection of 2500 IU/kg (subcutaneous) heparin for 2 consecutive days reduced the platelet count in mice, followed by its increase over the subsequent days (Table 2). There was no statistical difference in the baseline mean platelet count among all groups. The change in the mean platelet counts of the mice in all tested groups indicates a similar trend: a reduction in the platelet count after heparin administration and a significant (P < 0.05) increase over the subsequent days after treatment with MEBmL as was observed in Groups III–V,

Table 3. Effect of methanolic extract of *Bauhinia monandra* leaves on mean bleeding times in tested mice

Groups		Mean bleeding times [s]			
		day 0	day 3	day 6	day 8
G I	normal control (only vehicle)	31.25 ± 2.75	31.21 ± 2.44	31.98 ± 2.85	30.83 ± 2.35
G II	positive control (vehicle + inducer)	31.59 ± 2.01	481.60 ± 3.80***	274.97 ± 2.27*** (42.91)	196.30 ± 2.37***[a] (59.24)
G III	*B. monandra* extract ((100 mg/kg, b. wt) + inducer)	26.07 ± 2.50	473.93 ± 13.31***	103.31 ± 3.83***[a] (78.20)	31.34 ± 1.53[a] (93.39)
G IV	*B. monandra* extract ((200 mg/kg, b. wt) + inducer)	28.48 ± 6.41	491.03 ± 4.00***	45.24 ± 4.44*[a] (90.79)	26.06 ± 1.89[a] (94.69)
G V	*B. monandra* extract ((400 mg/kg, b. wt) + inducer)	42.83 ± 13.38	469.13 ± 19.16***	29.51 ± 1.57[a] (93.71)	21.41 ± 1.66*[a] (95.44)

All values are represented as mean ± SEM; $n = 5$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$, when all groups compared normal control group; [a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$, when all groups compared to positive control group; all values in parenthesis are percentage reduction of bleeding times calculated relative to day 3 of groups

compared to control groups (Fig. 1). The administration of MEBmL at different concentrations (100, 200, and 400 mg/kg) showed a significant increase (92.26, 86.47, and 95.04%, respectively) in the mean platelet count after 3 days, compared to the normal control group (4.37%) treated with distilled water only (Table 2). The positive control group showed 10.59% increase in mean platelet counts after 3 days of treatment with distilled water. Treatment with MEBmL (100, 200, and 400 mg/kg) also showed a significant ($P < 0.05$) increase in mean platelet counts (113.98, 100.27, and 225.10%, respectively) after 5 days in a dose-dependent manner, compared to the normal control (0.80%) and positive control (34.51%) groups (Table 2).

Effect of MEBmL on the bleeding time in tested mice

The trend in the influence of the *B. monandra* extract on the bleeding time was similar for all mice; the mean bleeding times significantly increased after heparin administration and decreased after a concurrent treatment with MEBmL for animals from Groups III–V (Fig. 2). There was no significant difference in the initial mean bleeding times in mice from all groups. The mean bleeding times in mice increased after administration of heparin to animals in all groups and reduced significantly after 3 and 5 days treatment with MEBmL to mice from Groups III–V. Administration of MEBmL at different concentrations (100, 200 and 400 mg/kg) showed a significant ($P < 0.05$) reduction in the mean bleeding times after the 3rd (78.20, 90.79 and 93.71 respectively) and 5th (59.24, 93.39 and 95.44% respectively) day of treatment, compared to the positive control (59.24%) group, in a dose-related manner (Table 3).

Effect of MEBmL on the blood clotting time in tested mice

The effect of *B. monandra* on the blood clotting time in mice also presented a similar trend. A significant ($P < 0.05$) increase in the blood clotting time after the administration of heparin and a simultaneous reduction after a concurrent treatment of mice with MEBmL were observed (Fig. 3). There was no statistical significance in the difference between the initial mean blood clotting times in mice in all tested groups. After 3 days of treatment with MEBmL (100, 200, and 400 mg/kg), a reduction in mean clotting times (43.40, 72.41, and 78.47%, respectively) was observed in mice, compared to the positive control (9.02%) group (Table 4). After 5 days, a significant reduction in mean clotting times (64.38, 76.13, and 78.92%) was observed in mice treated with MEBmL (100, 200, and 400 mg/kg, respectively), compared to the positive control group (40.16%).

Discussion

Researches on herbal medicinal products for platelet augmentation are rapidly increasing in numbers due to limited supportive treatments available for thrombocytopenic disorders (Arollado et al., 2013). On the basis of the traditional use of *B. monandra* leaves for blood function improvement, and in the absence of a report vali-

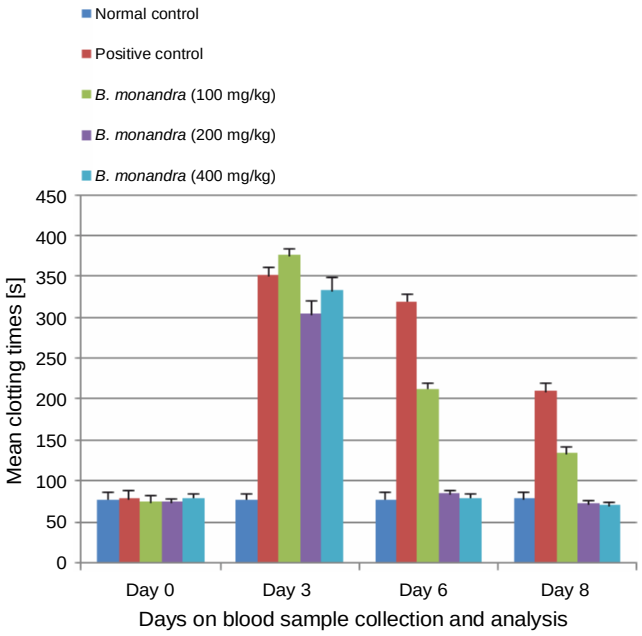

Fig. 3. Effect of methanolic extract of *Bauhinia monandra* leaves on mean clotting times

dating this claim, we evaluated its platelet augmentation activity in HIT through well-established experimental methods. First, MEBmL was subjected to phytochemical screening and the results showed the presence of alkaloids, tannins, flavonoids, terpenoids, saponin, glycosides, and steroids. The methanolic extract of *B. monandra* was evaluated for acute toxicity in mice, and no significant physical and behavioral changes were observed at higher concentrations of 5000 mg/kg b. wt, and no mortality was observed after 14 days of administration of the extract. This result indicates that an oral intake of the leaf extract of *B. monandra* may be deemed safe.

Thrombocytopenia is a pathological condition, often characterized by an abnormally low blood platelet count, which results in an impaired process of stopping bleeding by clumping and clotting blood vessel injuries (Balduini and Melazzini, 2017). In the present study, heparin was used as an agent for an experimental induction of thrombocytopenia. This method was also validated and used in other studies worldwide (Bhavya et al., 2015; Roihatul et al., 2016). In HIT, the immune system produces antibodies (IgG class) against heparin, which bind to platelet factor-4 (PF4). This 70-amino acid long protein neutralizes heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting local antithrombin III and promoting coagulation (Warkentin, 2006; Ahmed et al., 2007; Visentin and Liu, 2007; Warkentin et al., 2008). The IgG antibodies form a complex with heparin and PF4 in the blood, resulting in platelet activation and formation of platelet microparticles that rapidly initiate the formation of blood clots; as a consequence a reduction in the platelet count leads to thrombocytopenia.

In the present study, a heparin injection induced thrombocytopenia in mice and caused a significant ($P < 0.05$) reduction in blood platelet counts (Fig. 1). It also resulted in a significant ($P < 0.05$) increase in the bleeding time (Fig. 2) and the clotting time (Fig. 3) of mice, as compared to the control group. Since the recovery from HIT usually begins within 1–3 days after stopping the drug intake and typically completes within 7–10 days (George et al., 1998; Aster et al., 2009; George and Aster, 2009), the analysis of blood samples was done 3 and 5 days post-treatment. As observed in the present study, treating mice with different concentrations of the MEBmL (100, 200, and 400 mg/kg) showed a significant ($P < 0.05$) increase in mean platelet counts (Table 2), and also showed a significant ($P < 0.05$) reduction in the mean bleeding times (Table 3) and the mean blood clotting times (Table 4) of mice in both groups during the 5-day treatment. Among the different concentrations of *B. monandra* administered, 400 mg/kg b. wt showed the highest potential, followed by 100 mg/kg b. wt and 200 mg/kg b. wt. This result indicates that the potential of the *B. monandra* extract for improving the health condition in HIT is not dose-related (Fig. 1); however, its activity in reducing the bleeding and clotting times is dose-related (Fig. 2 and Fig. 3). It is noticeable that the extract exhibited complete restoration of the platelet count back to the normal levels within 3 days of thrombocytopenic induction (Table 2). Therefore, it is worth noting that since there is no current antidote for heparin overdose or poisoning, the leaf extract of *B. monandra* provides the logical basis for consideration as a potential antidote for HIT.

The likely mode of action of the extract may include inhibiting the activity of antithrombin III or stopping the inhibition of the clotting factor X and IXa (Di Micco et al. 2000) or even the inhibition of IgG antibodies. However, since thrombocytopenia was established in experimental animals before the treatments began, and considering the significant effect presented by the extract to substantially increase the platelet count (Fig. 1), we can assume that this extract has a stimulatory action on the

Table 4. Effect of methanolic extract of *Bauhinia monandra* leaves on mean clotting times in tested mice

Groups		Mean clotting times [s]			
		day 0	day 3	day 6	day 8
G I	positive control (vehicle + inducer)	77.57 ± 7.56	77.32 ± 6.94	77.35 ± 6.23	77.84 ± 8.27
G II	positive control (vehicle + inducer)	78.04 ± 7.98	350.99 ± 14.38***	319.34 ± 9.92*** (9.02)	210.02 ± 16.43*** (40.16)
G III	*B. monandra* extract ((100 mg/kg, b. wt) + inducer)	73.98 ± 3.80	375.89 ± 7.01***	212.75 ± 3.95***[a] (43.40)	133.88 ± 4.73***[b] (64.38)
G IV	*B. monandra* extract ((200 mg/kg, b. wt) + inducer)	73.78 ± 2.89	304.22 ± 6.48***	83.94 ± 4.29[a] (72.41)	72.62 ± 2.30[a] (76.13)
G V	*B. monandra* extract ((400 mg/kg, b. wt) + inducer)	79.20 ± 8.66	332.63 ± 19.97***	79.45 ± 10.51[a] (78.47)	70.13 ± 6.71[a] (78.92)

All values are represented as mean ± SEM; $n = 5$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$, when all groups compared normal control group; [a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$, when all groups compared to positive control group; all values in parenthesis are percentage reduction of bleeding times calculated relative to day 3 of groups

activation of megakaryocytes, leading to an increased platelet production (Muhury et al., 2009). However, the actual mode of action is yet to be determined. In order to elucidate the possible mode of action of the antithrombocytopenic activity of the extract of *B. monandra*, we performed a complete investigation of literature, which revealed the presence of two galactose-specific lecithins, which are carbohydrate-binding proteins macromolecules that are highly specific for sugar moieties of other molecules (Coelho and Silva, 2000; Souza et al., 2011). The first was purified from the leaves of *B. monandra* (Coelho and Silva, 2000), and the other one was purified from *B. monandra* roots and showed termiticidal activity on *Nasutitermes corniger* and antifungal activity on pathogenic *Fusarium* species (Souza et al., 2011). Research has also shown that lecithin possesses a wide range of properties such as antifungal (Costa et al., 2010), antibacterial (Oliveira et al. 2008; Costa et al., 2010), antiproliferative (Aranda-Souza et al., 2014), antidiabetic (Rocha et al., 2013), antiplatelet aggregation (Granguly and Fossett, 1981), or analgesic (Leite et al., 2012).

Preliminary phytochemical studies of MEBmL revealed the presence of alkaloids, tannins, flavonoids, terpenoids, saponin, glycosides, steroids, and acidic compounds (Table 1). Although based on the results of the study we were unable to associate any of these constituents with the activity observed, some of the bioactive phytoconstituents have been reported in previous studies to evoke various influence on hematopoiesis. For instance, two ellagic acid compounds have been reported to improve platelet production by inducing megakaryocyte differentiation in human erythroleukemia cells (Goa et al., 2014). Plant tannins have also been reported by Xiong et al. (2014) to significantly increase red blood cell, white blood cell, and platelet counts in mice with myelosuppression. Chen et al. (2017) reported that plant saponins and their component glycosides possess hematopoietic activities in *in vitro* and *in vivo* mouse models, which involve promoting focal adhesion kinase and extracellular signal-regulated kinase 1/2 activation and modulating cytokine production in the bone marrow. Similarly, Zhang et al. (2017) reported that a combination of four isolated plant flavonoids possesses a potent hematopoietic activity by a possible activation of a regulator of erythropoietin transcription. Another study by Sun et al. (2012) also revealed that terpene glycosides possess hemostatic properties.

Several studies have been conducted on the pharmacological activities of *B. monandra* extracts (Argolo et al., 2004; Macedo, 2008; Agbugui et al., 2010; Starr and Starr, 2011; Ajiboye et al., 2015), but no study was conducted on the effect of the *B. monandra* extract on platelet augmentation or to analyze its hematopoietic potential. However, a previous report revealed that a high content of iron in the extract may assist blood formation (Agbugui et al., 2010). Moreover, previous studies have also reported the use of other plants in platelet augmentation (Osime et al., 2008; Arollado and Osi, 2010; Apostol et al., 2012; Arollado et al., 2013). In this

context, the extracts from *Carica papaya*, *Ipomea batatas*, and *Euphorbia hirta* leaves promoted an increase in platelet counts initially in rats, which was reduced by oral administration of anagrelide (synthetic quinazoline derivative that reduces platelet production) (Arlollado et al., 2013). Further research is also needed to isolate and characterize the compounds responsible for the platelet augmentation activity to further elucidate the most possible mode of action.

Conclusions

B. monandra is a very promising medicinal plant used in folk medicine for treating, curing, and managing many ailments. The methanolic extract of *B. monandra* leaves showed rapid and promising results in the management of HIT. This result provided experimental evidence for the effective use of *B. monandra* in traditional medicine to improve blood function. Based on the evidence from this study, we can conclude that the MEBmL can be used as an antidote in the management of thrombocytopenia disorders.

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