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Platelet count enhancing and hepatoprotective activity of acetogenin isolated from the stem bark of *Miliusa velutina* (DC.) Hook.f. & Thomson

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Miliusa velutina (DC.) Hook.f. & Thomson, commonly called velvety miliusa, is deciduous tree belonging to the family Annonaceae (sugar apple or custard apple family). The stem bark of *Miliusa velutina* is rich in a distinctive phytochemical acetogenin (also present in *Carica papaya*) known to enhance the platelet count. In this study, we analysed the ethanolic extract of the stem bark of *Miliusa velutina* (MVSBE) , isolated the secondary metabolite acetogenin and investigated its Hepatoprotective activity as well as platelet count enhancing ability. The experiment was conducted using cyclophosphamide induced thrombocytopenia model, acetaminophen induced hepatotoxicity and chloroform induced hepatotoxicity model. Cyclophosphamide (25 mg/kg i.p.) used to reduce the platelet count in albino Wistar rat whereas acetaminophen (150 mg/kg i.p.) and chloroform (0.5 mL/kg i.p.) were used to develop hepatotoxicity in treated animals. The levels of haematological parameters viz. ALP, LDH, AST, ALT, and bilirubin levels in blood serum were estimated with improved efficiency. In all the treated animals' acetaminophen induced a rise in blood sugar levels. There was a decrease in liver enzymes in blood serum levels of ALT, AST, LDH and ALP. Bilirubin level was also significantly decreased in chloroform induced hepatotoxicity. The increased total platelet count and also marked hepatoprotective activity demonstrated the ability of MVSBE which could be attributed to the presence of acetogenin in abundance.

Keywords: Hepatoprotective, Immune thrombocytopenia (ITP), Platelet count enhancer, Velvety miliusa

The velvety miliusa, *Miliusa velutina* (DC.) Hook.f. & Thomson is known for its bioactive ingredients with significant therapeutic benefits. It belongs to Annonaceae family and goes by the Bengali name of Gandhagajari. It is an 8-11 m in height deciduous tree found in India, Bangladesh, China, Malaysia, and is also commonly found throughout mainland Asia, Australia and New Guinea. The edible medicinal herb *M. velutina* is used to cure a variety of illnesses in traditional medicine. Locals use it as a food vegetable aas well as a herb to cure a variety of ailments, such as bacterial infections and inflammation. Because several species in the *Miliusa* genus are severely endangered or endangered, there is restricted utilisation of the plant. *Miliusa velutina* has been shown to be cytotoxic. Its Homogenised bioactive extracts of its leaves, flowers, fruits and stem barks, have been shown to possess strong antioxidant, antibacterial, antidiabetic and hepatoprotective properties[1].

Miliusa velutina is reported to be a rich source of phenols, steroids, acetogenins, alkaloids, and sesquiterpenes, among other chemical constituents. These chemicals have a wide range of bioactivities, including the ability to cause haemorrhage, uterine irritation, stomach ache, and sinusitis. Miliutine C -a methyl ester, new drimane sesquiterpene and bioactive alkaloids isolated from the stems of *Miliusa velutina*[2]. The essential oil of *Miliusa velutina* exhibited analgesic properties[3]. DNA repair was harmed by bioactive alkaloids from *M. cf. banacea* such as 10-methoxyliriodenine and 10-hydroxy-liriodenine. The *Miliusa velutina* plant also contains cytotoxic mivelutina A acid, mivelutina B acid, mivelutina B methyl ester, canangalia G, canangalia

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Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate transaminase; CLL, Lymphocytic leukaemia; CMC, carboxymethyl cellulose; DLC, Differential leucocyte count; LDH-5, Lactate dehydrogenase; HCV, Hepatitis C infections; ITP, Immune thrombocytopenia; MVSBE, *Miliusa velutina* stem bark extract; PCM, Paracetamol; SLE, Systemic lupus erythematosus; TLC, Total leukocyte count; TPC, Total platelet count.

H, vanillyl glycol and epoxyconiferyl alcohol, among them epoxyconiferyl alcohol had the greatest potential for cytotoxicity against cancer cell lines $HepG_2$[4]. The recently found Milusanones and Milusanal, this plant can often have antimalarial activity and cytotoxicity against cancerous cells[5,6].

In this study, we tried to characterise the secondary metabolites extracted from the stem bark of *Miliusa velutina*. Additionally, we evaluated the justification for its usage in conventional treatments.

Material and Methods

Collection and drying

The collected plant material of *Miliusa velutina* stem bark was sun-dried, the powdered form of the dried plant part was independently extracted using Soxhlet apparatus. For up to 96 h, ethanol solvents were used to extract the dried and powdered materials of *M. velutina*. Hot water bath (60°C) was used to concentrate the extract. After that, the extract was weighed and the % yield was calculated.

Extraction of plant materials

The plant materials were shade dried at normal room temperature (25°C) for 15 days till constant weight was attained. After that, the plant materials (500 g) were crushed using an electric grinder, and the powdered material was kept in an airtight container before the extraction. The Soxhlet apparatus was used to extract the dried stem bark part (100 g each) of *M. velutina* using ethanol (500 mL) for 70°C for 72 h. Finally, the solvent of the extract was evaporated by a rotary evaporator. The extract was filtered, dried, and then put in a water bath at 60°C for solidify.

Phytochemicals analysis of *Miliusa velutina*

The identification of various photochemical constituents was done by various chemical tests and evaluated for the primary and secondary compounds like terpenoids, alkaloids, carbohydrates, flavonoids, steroids and glycosides, among other distinguishing vital metabolites discovered in the stem bark part of *Miliusa velutina.* This plant extract has physiological as well as beneficial effects[7]. When the phytochemicals test was identified then the colour changes revealed the presence of phytoconstituents in *M. velutina* (stem bark). The qualitative testing of extracts revealed a strong signal of metabolite presence. Preliminary phytochemical identification was important for isolating the plant's active constituents and pharmacologically active components (for e.g. friedelin, lupeol, β-testosterone and caffeic acid)[8].

Experimental animals

Albino Wistar rats weighing 180-200 g of each sex was utilised in this study. They were procured from the NIET Pharmacy Institute's Central Animal House in Greater Noida. (CPCSEA/IAEC/NIET/2022/01/01). Under normal circumstances, the animals were cared after. It was acquired from the Institutional Animal Ethics Committee (IAEC) of the NIET, Pharmacy Institute in Greater Noida, India. Water was supplied to the rats *ad libitum*, and the rodents were kept at 22°C and at relative humidity of 50-70%. An authorized vendor of the CPCSEA supplied the animals' food, UP State Agro Industrial Corporation Ltd., Lucknow, Uttar Pradesh. CPCSEA rules, available at the following site, were strictly followed throughout this investigation to guarantee that all experiments on animals were completely safe (1121\ac\CPCSEA\22).

Chemicals and equipments used

Acetaminophen (Glenmark Pharmaceuticals Ltd.), cyclophosphamide (Sun Pharma Industries Ltd.), chloroform (Mankind Pharmaceuticals Ltd.), *Carica papaya* (Curemax Pharma Ltd.), IR-FTIR (Raj Analytical Sol, Mumbai), Clevenger apparatus (Ikonic LabwaresE-2/87, Delhi), Electronic Balance (AG 135 Mettler Toledo), UV Spectrophotometer (Globetrek Eng. Corporation, Navi Mumbai).

Determination of acute toxicity

To study the acute toxicity potential of MVSBE solution under the OECD guidelines 423, albino Wistar rats were separated into four groups (Gr. I-IV) of n=6 animals of both sex (3 males and 3 females) each administered orally with the test drug of MVSBE at different doses of 5, 50, 300 mg/kg and 2000 mg/kg body weight dissolved in carboxymethyl cellulose (CMC), respectively. Animals were observed for two days after test drug administration for any adverse indications and general behavioural changes, such as convulsant effects, locomotor activity, shivering, difficulty in moving, sluggishness and any other sign of severe toxicity, mortality, etc.

Hepatoprotective activity

Acetaminophen induced hepatotoxicity model

The acetaminophen-augmented liver marker bilirubin, cholesterol, glucose and triglycerides were evaluated and the haematological parameters levels of alkaline phosphatase (ALP), alanine transaminase (ALT), lactate dehydrogenase (LDH-5) and aspartate

transaminase (AST) that have been increased by acetaminophen. Despite being usually regarded as safe, the analgesic-antipyretic medication acetaminophen can have adverse effects on liver function, ranging from severe hepatic damage to total liver failure. It may potentially cause hepatoxicity and harm other organs.

Five groups of six animals in each weighing 180-200 g were taken. The control group had normal saline and acetaminophen (150 mg/kg i.p.) while Grf. II had *Carica papaya* (2 g/kg p.o.); Gr. II & IV were given acetogenin at low and high doses (5 and 10mg/kg, respectively); and Group V received MVSBE (300 mg/kg) for 14 days in addition to their regular diet. The acetaminophen-augmented liver marker enzymes AST, ALP, LDH and ALT, as well as triglycerides, bilirubin and glucose were all decreased by oral administration of MVSBE solution. Although acetaminophen, an analgesic-antipyretic medicine, is usually regarded as safe, an excessive amount of it might have negative effects on liver function, leading to serious hepatic necrosis or even liver failure. It may potentially cause nephrotoxicity and harm other organs.

Hematological parameters for assessment of hepatic damage

The blood serum levels were separated from the blood sample to estimate the serum biochemical parameters of AST, ALP, LDH and ALT, along with bilirubin, triglycerides, cholesterol and glucose. All reports showed decreased levels after oral administration of MVSBE. In liver tissue, glutathione and lipid per oxidation (MDA) were also measured[10].

Chloroform ($CHCl_3$) induced hepatotoxicity model

The chloroform induced hepatotoxicity in Wistar albino rats was determined by measuring the levels of haematological parameters such as ALT, AST, ALP and bilirubin in blood serum[10]. Because of its hepatotoxicity action, acetaminophen was used to induce a blood test to check the blood sugar level. Although $CHCl_3$ is utilised as a solvent, it is dangerous for humans to be exposed to it[9].

Five groups comprising six male/female adult albino Wistar rats weighing about 180-220 g were utilized in this study. Rats in the control group (Gr. I) were given either saline or undiluted 0.5 mL/kg $CHCl_3$ intraperitoneally at 9:00, 13:00, 17:00, 21:00 or 03:00 h. Four hours after the enzyme activity was determined, the animals were beheaded. Group I animals received normal saline (NS); Group II received *Carica papaya* (2 g/kg) as a standard drug administered orally; Group III & IV received acetogenin at low and high doses (5 and 10 mg/kg, respectively); and Group V received MVSBE (300 mg/kg) as test drug to evaluate hepatoprotective activity.

Platelet count enhancing model by cyclophosphamide induced thrombocytopenia

The management of immune thrombocytopenia (ITP), an autoimmune illness that develops over time and is defined by an isolated low platelet count of fewer than 100 109/L2, is important and utilized to check the platelet formation[10]. Although the precise aetiology is uncertain, it is thought to be caused by a combination of reduced platelet production brought on by faulty megakaryopoiesis and autoimmune destruction of platelets brought on by the development of antiplatelet autoantibodies. The aetiology of ITP is significantly influenced by the B- and T-cell immune responses. ITP may be divided into two kinds depending on the underlying reason: primary ITP, which occurs when there is no secondary cause, and secondary ITP, also when there is a condition that is associated to the autoimmune destruction of platelets. The latter comprises lymphoproliferative disorders such chronic lymphocytic leukaemia (CLL), autoimmune diseases including Hepatitis C (HCV) infections and systemic lupus erythematosus (SLE)[6].

Platelet counting

Rat peripheral blood samples of platelets were manually counted in a Neubauer's chamber using a binocular optical microscope with a 40X objective lens. Each blood sample was divided, diluted, and homogenized using Brecher liquid. Additionally, fast panoptic was used to conduct and stain peripheral blood serum[8].

Hematological parameters (*Neubauer's chamber*)

Adult albino Wistar rats weighing 180-200 g each were divided into five groups with 6 animals each. Group I (control) received NS + cyclophosphamide @ 25 mg/kg i.p. for three consecutive days to induce thrombocytopenia experimentally in rats. Group II received *Carica papaya* (2 g/kg) as a standard drug that was administered orally. Gr. II & IV were given acetogenin at low and high doses (5 and 10 mg/kg, respectively). Group V had test drug *Miliusa velutina* stem bark (MVSBE) as a solution (0.1% CMC) orally. The therapeutic dose of test drug (300 mg/day)

was used to determine the effect of MVSBE in platelet count checking.

Blood samples were taken after 1 h in the experiment's start time, as well as on days 7 and 14 after MVSBE administration. Rat blood samples were obtained from the retro-orbital plexus under a mild anaesthetic using glass capillaries, and biochemical parameters were estimated by preserving the blood with disodium ethylene diamine tetraacetate.

Total leukocyte count (TLC), total platelet count (PC), and differential leucocyte count (DLC), which included neutrophils, lymphocytes, monocytes, and eosinophils, were all assessed. The rats' regular behaviors, hair, eating and drinking habits, changes in weight, and death rate were all carefully monitored. For 14 days, the animals were weighed regularly each day.

Statistical analysis

Collected data were analyzed using one-way ANOVA and Dunnett's Multiple Comparison Test using Graph Pad Prism. For group comparisons, Duncan's post hoc test was used. Findings were presented as mean ± SEM. Value of P <0.05 was accepted as statistically significant.

Results

Estimation of extracted plant material

The dried *Miliusa velutina* (stembark) were extracted with ethanol as solvent by using the soxhlet apparatus. The plant extract dried with a hot water bath and the % yield was calculated by the following equation was found to be 7.2%w/w.

$$\% \text{ Yield} = \frac{Weight\ of\ plant\ extract\ -\ Weight\ of\ empty\ china\ dish}{Weight\ of\ powder\ drug} \times 100$$

% Yield = 7.2 % w/w

Preliminary phytochemicals constituent's studies

The identification test of different constituents of *Miliusa velutina* (stem bark) was performed. It consists of numerous alkaloids, steroids, flavonoids, tannins, lignin, insulin and cardiac glycosides (ethanol extract of *Miliusa velutina* (stem bark).

There is a possibility that these identification tests of phytochemicals were important for isolating the plant's bioactive constituents for pharmacologically active components like lupeol, β-testosterone, and caffeic acid. *Miliusa* plant-derived phytoconstituents have pharmacological effects, including acetyl cholinesterase suppression, antibacterial, anticancer, antifungal, antiherpes, anti-inflammation, antimalaria, antioxidant activity, cardiac myosin ATPase activation and cytotoxic action[3].

Determination of acute toxicity

To study the acute toxicity potential of MVSBE solution under the OECD guidelines 423, there were no adverse reactions or mortality observed in experimental animals of both genders were dose levels MVSBE solution of doses 5, 50, 300 and 2000 mg/kg during the entire period of experimentation. There were no clinical signs of toxicity, abnormal behaviour and death in animals were seen. The LD_{50} is a popular dose-response indicator for acute toxicity (Table 1). This dose was determined statistically as the point at which 50% of people should expect to pass away. LD_{50} was typically obtained from acute toxicity studied selected that the MVSBE solution 300 mg/kg.

Hepatoprotective activity

Hematological parameters of acetaminophen induced hepatotoxicity model

The effect of various hematological parameters in acetaminophen-induced hepatotoxicity was estimated in experimental rats at the end of 14 days of drug treatment. The estimation of hematological parameters ALT, AST, LDH, ALP and bilirubin levels in blood serum were increased. The blood glucose level by induced acetaminophen. The test group of MVSBE solution showed that the improvement of hepatoprotective agent normalized biochemical parameters was decreased in the treated rats as shown in Table 2 (*P <0.05 and ***P <0.001).

The effect of MVSBE solution was compared with that of the control group (acetaminophen) and the standard group (*Carica papaya*) treated animals. The possible mechanism responsible for the protection of paracetamol-induced liver damage by MVSBE solution may be that it could act as a free radical scavenger intercepting the radicals

Table 1 — Effect of *Miliusa velutina* stem and bark extract (MVSBE) on acute toxicity)

Groups	0 Day (g)	1 Day (g)	2 Day (g)
I	160.73±3.57	163.91±2.13	172.97±2.90
II	164.47±4.10	171.38±5.16*	189.70±5.94*
III	170.37±5.10	181.46±6.36**	195.70±6.94**
IV	175.47±5.70	189.97±6.59***	194.47±7.61***

[Groups I to IV had MVSBE @5, 50, 300 and 2000 mg/kg, respectively. Mean values are estimated as mean ±SEM of 6 rats in each group. Each value represents the mean±SEM, n=6. Non-significant (NS) and significant dose indicated at*P <0.05, **P <0.01, and ***P <0.001]

Table 2 — Effect of *Miliusa velutina* stem and bark extract (MVSBE) on acetaminophen induced hepatotoxicity model

Hematological parameters of hepatoxicity	Group I [Control]	Group II [Std. *Carica papaya*, 2g/kg]	Group III [Test drug: acetogenin, 5mg/kg]	Group IV [Test drug – acetogenin, 10mg/kg]	Group V [Test drug: MVSBE, 300mg/kg]
ALT (U/L)	29±6.8	21.3±3.5***	27.3±10.9*	25.3±10.9**	24.3±6.8**
AST (U/L)	25.06±6.8	17.75±0.07***	20.3±8.9*	19.3±10.9**	18.3±1.8**
ALP (U/L)	73.75±1.21	64.75±1.21***	71.3±1.9*	74.3±6.9**	65.3±4.8**
LDH-5 (U/L)	316.3±13.05	144.5±12.5***	251.3±18.9*	221.3±13.7**	167.2±16.8**
Bilirubin (mg/dL)	0.86±0.05	0.70±0.07***	0.78±0.05*	0.76±0.06**	0.73±0.05**
TG (mg/dL)	154.3±5.1	83.75±0.2***	91.3±1.9*	89.3±17.7**	84.3±6.8**
Glucose(mg/dL)	143.6±4.7	89.3±2.1***	100.3±10.7*	92.3±10.9**	90.3±6.7**
Cholesterol (mg/dL)	70.6±3.0	80.3±2.0	89.1±2.1*	91.3±1.9**	84.3±1.6**

[Effect of MVSBE solution was compared with that of the control group (acetaminophen) and the standard group (*Carica papaya*) treated animals. All the values are expressed in the mean ±SEM of six rats in each group. Non-significant (NS) and significant at value presented at **P* <0.05, ***P* <0.01 and ****P* <0.001]

Table 3 — Effect of *Miliusa velutina* stem and bark extract (MVSBE) by chloroform induced hepatotoxicity model

Groups	Haematological parameters of hepatoxicity				
	SGT (U/L)	LDH-5 (U/L)	ALP (U/L)	AST (U/L)	Bilirubin (mg/dL)
Gr. I NS+Chloroform (Control)	35.94±0.97	5.44±0.95	88.85±0.23	6.26±0.61	0.86±0.01
Gr. II Standard *Carica papaya* (2 g/kg)	21.35±0.37***	7.22±0.11***	73.04±0.49***	13.3±0.77***	0.69±0.04***
Gr. III Test drug acetogenin (5 mg/kg)	25.32±0.85*	6.47±0.31*	78.50±0.25	18.3±0.39*	.0.75±0.05*
Gr. IV Test drug acetogenin (10 mg/kg)	23.37±0.73**	6.65±0.34**	75.45±0.53**	17.3±0.27**	0.74±0.09**
Gr. V Test drug MVSBE (300 mg/kg)	22.23±0.89**	7.99±0.81**	74.87±0.61**	14.76±0.16**	0.72±0.06**

[Impact of MVSBE solution was compared to that of the animals treated with the standard group (*Carica papaya*) and the control group (chloroform). All the values are expressed in the mean ±SEM of six rats in each group. Non-significant (NS) and significant at value presented at **P* <0.05, ***P* <0.01 and ****P* <0.001]

involved in paracetamol metabolism (acetaminophen) by microsomal enzymes. By trapping oxygen-related free radicals, the extract could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid peroxidative processes.

Statistical comparison

All the values were expressed in the mean ±SEM of six rats in each group. For a given portion, Non-significant (NS) and significant at value presented at **P* <0.05, ***P* <0.01 and ****P* <0.001. ALT, AST, ALP, and LDH-5, bilirubin (mg/dL), TG (mg/dL), glucose(mg/dL), cholesterol (mg/dL).

In hepatotoxicity induced by $CHCl_3$ model, the effect of MVSBE solution in various haematological parameters was determined by the decreased liver enzymes in blood serum levels of ALT, AST, LDH, ALP and bilirubin along with reduction in hepatotoxicity in the liver. They revealed that rats are more vulnerable to liver damage from hepatotoxicity following delivery of drug (Table 3).

The impact of MVSBE solution was compared to that of the animals treated with the standard group (*Carica papaya*) and the control group (chloroform). All the values were expressed in the mean ±SEM of 06 rats in each one group. For a given portion, Non-significant (NS) and significant at value presented at **P* <0.05, ***P* <0.01 and ****P* <0.001.

Platelet count enhancing (Neubauer's Chamber)

The studied platelet counts presented their normal morphology by using them manually in a Neubauer's chamber. The results showed that at the end of the experiment on 14 days, a significant increase in platelet count was observed in experimental groups, the standard drug of *Carica papaya* exhibited a greater platelet amount in the peripheral blood serum level. Mean platelet counts within rats' peripheral blood were found to be 683,680 ± 186,229×10^3 platelets/µL. as compared to ethanol extract of MVSBE solution showed significant value. Therefore, mean the platelet amount was about four times greater than that observed within peripheral blood samples within total WBCs and RBCs (Table 4).

Platelet count enhancing activity effect of ethanol extract of MVSBE solution (300 mg/kg p.o.) as compared with the standard drug of *Carica papaya* (2 g/kg p.o.). Total WBC significantly (****P* <0.001, ***P* <0.01 and **P* <0.05) decreased in the control group on days 7 and 14. Treatment with ethanol extract of MVSBE solution (300 mg/kg) caused no significant differences in the total WBC count as compared to the control group of cyclophosphamide-treated rats.

Table 4 — Effect of *Miliusa velutina* stem and bark extract (MVSBE) on Haematological parameters (Platelet count)

Group	Platelet count (no./µL)
Gr. I (Control)	250,094±397,214
Gr. II Standard *Carica papaya* (2 g/kg)	683,680±586,229***
Gr. III Test drug acetogenin (5 mg/kg)	358,102±285,227*
Gr. IV Test drug acetogenin (10 mg/kg)	439,037±273,276**
Gr. V Test drug MVSBE (300 mg/kg)	599,823±489,259**

Platelet count enhancing activity effect of ethanol extract of MVSBE solution (300 mg/kg p.o.) as compared with the standard drug of *Carica papaya* (2 g/kg p.o.). Control had NS+ cyclophosphamide @25 mg/kg i.p.). Total WBC significantly (****P* <0.001, ***P* <0.01 and **P* <0.05) decreased in the control group on days 7 and 14. Treatment with ethanol extract of MVSBE solution (300 mg/kg) caused no significant differences in the total WBC 14 as compared to the control group of cyclophosphamide-treated rats

Discussion

The presence of phytoconstituents in higher concentration in the ethanol extract of *Miliusa velutina* of stem bark for example alkaloids, glycosides and flavonoids assumes an essential part by considering as an antioxidant, anti-inflammatory, hepatoprotective and platelet count enhancing activity. The preliminary qualitative phytoconstituents test determined the existence of alkaloids, carbohydrates, amino acids, proteins, glycosides and flavonoids, in the ethanol extract of *M. velutina* of stem bark. It is well documented that flavonoids and glycosides are strong antioxidant capacity. Antioxidant principles from herbal resources are multifaceted in their therapeutic effects and provide enormous scope in correcting the imbalance through regular intake of a proper diet. They also show protective effects on biomarkers of oxidative stress in erythrocytes too[11].

In the present study of *in vivo* acute toxicity potential of MVSBE solution under the OECD guidelines 423, there were no adverse reactions or mortality was observed in all experimental animals of both the genders. MVSBE solution at varying doses of 5, 50, 300 and 2000 mg/kg p.o. was given during the entire period of experimentation process. After 14-days of medication treatment period, the impact of different haematological parameters on acetaminophen-induced hepatotoxicity in experimental rats were calculated. ALP, LDH, AST, ALT and bilirubin levels in blood serum were estimated with improved efficiency. In all the treated animals' acetaminophen induced a rise in blood sugar levels. The effect of MVSBE in preventing liver damage induced by acetaminophen was clearly observed from the table. The hepatotoxicity induced by $CHCl_3$ was determined by MVSBE solution by various haematological parameters. There was a decrease in liver enzymes in blood serum levels of ALT, AST, LDH and ALP. Bilirubin level was also significantly decreased in $CHCl_3$ induced hepatotoxicity.

From the results, it is evident that ethanol extract of *Miliusa velutina* stem bark has liver-protective properties which is equivalent to standard drugs which are used as conventional medication. Bioactive substances of stembark of *Miliusa velutina* and their vital chemical constituents are responsible for the selected pharmacological activity. Thus, from the foregoing findings, it was observed that MVSBE is a promising hepatoprotective agent and this hepatoprotective activity of MVSBE may be due to the antioxidant chemicals present in it[12]. The induction of oxidative stress due to PCM was determined by increased generation of MDA and reduced activity of antioxidant enzymes[13]. These studies are associated with our results, including oxidative stress and hepatoprotective role of MVSBE in acetaminophen induced liver damage in rats[14]. PCM induced hepatoxicity is quite severe and has very much severe clinical significances, hence there is an urgent need to develop new hepatoprotective substances obtained from bioactive phytoconstituents so that they may produce less toxicity and lesser cost as compared to that of the commercially available marketed products. Antioxidant substances are mentioned as potential elements in the suppression of chemicals induced hepatotoxicity[15].

Conclusion

The stem bark of *Miliusa velutina* is rich in a distinctive phytochemical name as acetogenin which is validated to enhance the count of platelets. Results of the present study has demonstrated the hepatoprotective and platelet count enhancing activity of the ethanolic extract of *Miliusa velutina* stem bark. There was a decrease in liver enzymes in blood serum levels of ALT, AST, LDH, ALP. Bilirubin level was also significantly decreased in $CHCl_3$ induced hepatotoxicity. The hepatoprotective property of the MVSBE ha sbeen equivalent to the standard drugs used in conventional medication. The protective response and the platelet count enhancing nature of *Miliusa velutina* stem bark could be attributed to the antioxidant bioactive phytochemicals present in the stem bark.

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