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PLATELET ENHANCING EFFECT OF *ECLIPTA ALBA* IN ALCOHOL INDUCED THROMBOCYTOPENIC THROMBOCYTOPENIC RATS

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Key words:

Thrombocytopenia, *Eclipta alba*, Platelets, Megakaryocytes, Bleeding Time, Polyphenols

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ABSTRACT: Thrombocytopenia is a common hematological finding with variable clinical expression. Low platelet count may be the initial manifestation of infections such as HIV, hepatitis, and also associated with some viral infection like dengue hemorrhagic fever, malaria, etc. In the present study, Methanol extract of *Eclipta alba* was evaluated at a dose of 200 mg/kg body weight for 7 days in alcohol induced thrombocytopenic model in rats. Treatment with methanol extract of *Eclipta alba* significantly increased the platelet count within 3-5 days of treatment and also decreased the bleeding and clotting time when compared to alcohol treated groups. Hematological studies revealed that there is an increased in Megakaryocytes number in the bone marrow and histopathalogical examination indicated that significant decrease in liver sinusoidal dilation in the extract treated rats when compared to alcohol control. Therefore, the present study demonstrates potential use of *Eclipta alba* as an anti-thrombocytopenic and the effect on platelet distribution might be due to the presence of antioxidant and polyphenolic constituents.

INTRODUCTION: Thrombocytopenia is a condition where there is an abnormal low platelet count in the blood stream (less than 150×10^9cells/L). There are 3 main reasons for thrombocytopenia a). decreased production of platelets in the bone marrow, b) increased destruction of platelets, c) changing of distribution of platelets or sequestration in spleen. Thrombocytopenic condition is in in 3 stages, Mild:100.000-150.000/micro L, Moderate:50.000-100.000/micro L, Severe< 50.000 micro L. 2.5% of the normal population have got thrombocytopenia Which may be of hereditary, or acquired [1].

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Platelets produced in the bone marrow cells help in blood coagulation hence low platelet count may leads to risk factors like increase in bleeding and clotting time. The risk of bleeding decreases with increasing platelet count. There can be a bleeding after mild trauma in patients with thrombocytopenia depends on the severity [2]. This is because of the deficiency of platelet or functional out of order of platelets [3]. Thrombocytopenia can occur alone or it can develop as a complication with other diseases, such as cancer and viral infection or may result from intake of drugs such as heparin, chloramphenicol and anticancer drugs [4, 5].

Various disease statuses, such as chronic alcoholism, chronic liver diseases and dengue hemorrhagic fever also predispose to these conditions. Thrombocytopenia is a frequent complication of alcoholism, affecting 3 to 43% of non-acutely ill and well-nourished alcoholics and

14 to 81% of acutely ill, hospitalized alcoholics. [4] The only treatment available is the use of expensive recombinant thrombopoeitin and interleukin -11 injections to stimulate the platelet production [6]. Platelet transfusion has also been used but with limited efficiency[7]. These treatments are expensive and not affordable by all classes of the society. So there is a need to identify and develop the agent from either herbal source or synthetic source for an affordable effective therapy. *Eclipta alba* plant belongs to the family Astreaceae and is locally available edible plant with less toxicity [8].

In the present study *Eclipta alba* (EA) was selected owing to its various biological properties [9] including immune modulation and presence of high amount of polyphenols is responsible for its potent antioxidant activity and polyphenols have the property to interact with the receptor present on the surface of the platelets and activate the sequestered platelets in the spleen. *Eclipta alba* an annual herb commonly called as false daisy. It is an erect; much branched, roughly hairy, leaves are opposite, sessile and lanceolate. There are three kinds of *Eclipta alba*, white flowering, yellow flowering, black fruiting but all grow in open wastelands, trunks of river or on the foothills of Himalaya [10]. The whole plant contain ecliptin, reducing sugar, nicotin, stigmasterol, triterpenoids, ecliptabatin, urrolic acid, oleanolic acid, wedelolactone, desmethyl,stigmasterol, stigmasterol-3-*O*-glucoside etc. The plant shows trypsin inhibitor, anti-venom, antibacterial [13], anti-fungal[14], anti-hemorrhage and hepatoprotechtive activity[11, 12] Anti-viral activity [15].

In the present study chronic alcohol induced thrombocytopenic model was chosen because alcoholism is one of the risk factor for thrombocytopenia and chronic alcoholism may damage the liver sinusoids which in turn decreases the production of thrombopoeitin in the liver. Thus megakaryocytes receive no signal for platelets production, and platelet distribution gets altered leading sequestration of platelets in spleen. So availability of platelet to the blood stream is decreased and enhances the bleeding and clotting time. So in the present study the platelet enhancing effect of methanolic extract of *Eclipta alba* (EA) is determined in alcohol induced thrombocytopenic model in rats.

MATERIALS AND METHODS:

Animals:

Animal use was done in accordance with institutional animal ethical committee guidelines. Approval of animal usage was obtained by the Institutional animal ethics committee of the college (approval number 147/2014) and the animal experiment carried out as per the guidelines of CPCSEA. 36 male *Wister albino* rats, weighing 180-200 grams were procured from Central Animal House facility of JSS University, maintained by JSS Medical College, Mysuru. Animals were housed in the animal quarantine, JSS College of Pharmacy, Mysuru. The animals were maintained under the standard condition of relative humidity (55%), temperature (22-25^{0}C) and lighting sequence of 12h light, 12 hour dark phases. All animals were fed standard rat pellet, water *ad libitum.*

Experimental design:

After acclimatization, the animals were randomly divided in to the following groups consisting of 6 rats in each groups.

Group 1: Served as control received only potable water.

Group2: Served as a negative control, received ethanol 3g/kg intraperitonelly for 7 days for inducing thrombocytopenia to all groups except Control.

Group 3 (EAM treated): Animals received *Eclipta alba* methanolic extract (200mg/kg, per oral (PO)) for 7 days after induction of thrombocytopenia.

Group 4 (EAD): Animals received *Eclipta alba*decoction (200 mg, PO) for 7 days after induction of thrombocytopenia.

Collection and preparation of plant extracts:

EA whole plant was collected from the Chandravana, Ayurvedic medicinal plants garden, Mysuru, India in the month of March 2014 and authenticated by Dr. M. N. Naganandini, Assistant Professor, Deparment of Pharmacognosy, JSS College of Pharmacy, Mysuru. The fresh sample of plant was washed and air dried for 1 week and

coarsely powered. Then 100 gm of each sample was soaked in 500 ml of 70% ethanol for 72 hrs. After extraction the extracts were separated from marc by decantation and by pressing the marc in muslin cloth to remove the extract which is left in the marc after decantation.

The extract was filtered and preserved in a well closed container. Marcleft after extraction was extracted again by the procedure said above for 3 more days with same amount of fresh solvent, and the process was repeated for one more time. i.e. the drug was extracted 3 times with a gap of 3 days each. On 10^{th} day, filtrates were pooled and concentrated to syrupy liquid under reduced pressure using rotary vaccum evaporator, dried on water bath and stored at 4^{o}C till use [16].

Preparation of plant decoction:
The decoction was made by boiling 100 grams of fresh whole plant, previously washed to remove adhered soil and debris, in 500 ml of water for 15 minutes. The decoction was vacuum filtered, stored in glass vials, and frozen for subsequent lyophilisation [17]. The percentage of yield was calculated with the lyophilized extract. The lyophilized extract was then refrigerated until use, for the phytochemical screening and *In vivo* thrombocytopenic model.

Phytochemical analysis:
Phytochemical screening of the aqueous and methanolic extracts was carried out according to the standard procedures [10]. To identify the various phyto-constituents present in them i.e. Alkaloids, Terpinoids, Glycosides, Steroids, Triterpenoids, Flavonoids, Carbohydrates, Saponins and Tannins.

Test for total phenolic content:
Total phenolic content of various extracts was carried out as per the standard procedure of (McDonalds et al. 2001). The amount of phenolic compound equivalents to Gallic acid in plants extract was determine by comparing the absorbance of the sample to the Gallic acid standard curve.

***In vitro* DPPH antioxidant activity:**
This was carried out according to the standard procedure [16], 1 ml of 0.1mM DPPH methanol solution was added to 2.5 ml of various concentration of extracts or standard (100 µg/ml, 150µg/ml, 200µg/ml 250 and 300 µg/ml) and allowed to react at room temperature for 30 min. The absorbance of resulting mixture was measured at 517 nm and percentage scavenging activity (AA %), was measured using the formula: AA% = [(Abs control – Abs sample) × 100]/Abs control. Abs = Absorbance. Methanol (2.5 ml) was used as blank. 1ml of 0.1 mM DPPH plus methanol (2.5 ml) was used as a control. Solution of Gallic acid served as positive control. This assay was carried out in triplicates for each concentration.

***In vivo* pharmacological activity:**
Group 1-4 were assessed for, platelet counts, bleeding time and clotting time on day, 0, 7^{th}, 10^{th}, 12 and 14^{th}**.**

Platelet count determination:
0**.**5 ml of blood was collected by tail vein method, from all the groups on day 0, 7^{th}, 10^{th}, 12^{th} and 14^{th}, into the EDTA k2-anticoagulent tube and was shaken properly. The platelet count was determined by manual method (MLAB 1315 hematology) and for the conformation platelet Count also was determined by cell counter (Patrick et al.2009).

Bleeding time determination:
Bleeding time was determined by Duke's method with some modifications. The exposed tail tip was cleaned with distilled water and wipe with alcohol. Then using sterile sharp surgical blade, tail tip was cut around 2mm and immediately stop watch was started. The bleeding tip was blotted on a filter paper for every 30 seconds, until no more blood was seen on the filter paper .The time from first application to the disappearance of blood was recorded. This procedure was repeated during every blood collection.

Clotting time determination:
Blood was drawn into a capillary tube. The time of appearance of the drop of the blood on the cut tail was noted. The capillary glass tube is then kept between the palms of both hands for 30 second to keep it at body temperature. After 30 seconds, the tube was taken out and small portion of the capillary tube was broken at regular intervals of 30 seconds until a thread of clotted blood appears

between the two pieces of capillary glass tube. The time interval between the appearance of the drop of the blood and the thread of the blood clot was the considered as clotting time of rats expressed in minutes [17] expressed in minutes.

Biochemical analysis:
After collecting the blood for hematological parameters, animals in all the groups were bled and serum was separated for estimation of serum biochemical parameters (SGPT &SGOT) using commercially available kit by semi auto analyzer (Microlab 300. 2009 Model, Merk)

Megakaryocytes counts:
After the animals were sacrificed by cervical dislocation, femur was isolated and bone marrow cells were collected, thin smear of same was prepared on glass slide and air dried. The bone marrow smears were stained with Leishman stain and examined for changes in the number and morphology of megakaryocytes in different groups[18]. The number of the megakaryocytes considered as normal if one megakaryocyte was observed per one to three low-power fields, increased if more than two megakaryocytes per low-power field, or decreased if one megakaryocyte per five to ten low-power fields observed [19, 20].

***In vivo* antioxidant study:**
Isolated liver of all the group were perfused with 0.9% cold physiological saline to completely remove all the red blood cells. Then it was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) cut into small pieces, and the required quantity was weighed and homogenized using a homogenizer. The homogenate was centrifuged at 10000 rpm for 20 minutes at 4^0C. The supernatant was used for the estimation of lipid peroxidation (MDA content), endogenous anti peroxidative enzymes of (Superoxide dismutase (SOD), Catalase and GSH. SOD was determined by the method of Mishra and Fridovich (1972). Catalase & GSH was determined by the method of Aebi et al [17], GSH was determined by the method of Aebi et al. 1984), Lipid peroxidation was estimated by the method of Ohkawa and Ohishi (1976) respectively. Protein content in the tissue was determined [18] (Bradford et al) using bovine serum albumin as reference standard.

Histopathological studies:
After scarification the liver and spleen were rapidly perfused and dissected out and washed immediately with saline and fixed in 10% formalin for at least 24 h. Sections of test animals were prepared and then stained with hematoxylin and eosin (H–E) dye for photo microscopic observation of sinusoidal dilation, and swelling. The sections were examined under the light microscope for histopathalogical changes and photographs were taken. All the observations and remarks of the condtion were analysed by a histopathologist. The liver was inspected for sinusoid dilation and dilation of the central vein. The findings for sinusoid dilation was categorized as +1 (10-20%), +2 (30-50%), +3 (>50%).

Statistical analysis:
All the values are expressed as Mean±SEM The data was analysed by one-way ANOVA followed by Tukey's multiple comparison test by the software Graph pad Prism version 5.03). P value <0.05 was followed as a significance.

RESULTS:
Preliminary phytochemical analysis
The preliminary phytochemical screening revealed the presence of, saponins, flavonoids, and phenolics in the water extract(decoction) *Eclipta alba*, Except alkaloids, saponins, and remaining all constituents were present in methanolic extract. The main constituents like flavonoids, alkaloids, glycosides and phenolics were present in methanolic extract.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS OF WATER AND METHANOL OF *ECLIPTA ALBA*

Sl no	Phytochemicals	*Eclipta alba*	methanol
		water	
1	Alkaloids	+	-
2	Terpenoids	+	+
3	Flavonoids	+	+
4	phenolics	+	+
5	Tannins	+	-
6	Glycosides	-	+
7	Saponins	+	-
8	steroids	+	-

+ = present, - = absent

Total phenolic content:

The total phenolic content was expressed as mg/g Gallic acid equivalent using the standard curve equation: y =0.018× - 0.156, R^2 = 0.993. The total phenolic content was found to be 26.9±0.5 and 5.9±0.23 mg of GAE/g of methanolic and water extract of *Eclipta alba* respectively.

DPPH radical scavenging activity:

DPPH radical scavenging activities of the extract was estimated by comparing the percentage of formation of Diphenyl picryl hydrazine radical by the methanolic and water extracts of *Eclipta alba* The IC_{50} values of methanolic extract (EAM) and Decoction (EAD) of *Eclipta alba* were 214.2±0.3 , 606.1±0.2 µg/ml.

Bleeding time determination:

Upon induction with the ethanol, bleeding times significantly increased to 6±0.17 min for 7 days, After the treatment with EAM and EAD, the bleeding time was decreased to 3.17± 0.11 (p=0.01] and 4.08± 0.20 minutes.

TABLE 2: EFFECT OF *ECLIPTA ALBA* EXTRACTS ON BLEEDING TIME (MIN) OF DIFFERENT DAYS OF INTERVALS

		Bleeding Time in minutes		
Groups	**Treatment**	**3rd day**	**5th day**	**7th day**
I	Normal	2.83± 0.17	2.88± 0.16	2.88± 0.16
II	thrombocytopenic	6± 0.17a	6.3± 0.15a	6± 0.17a
III	EAM	3.08± 0.30 a^{ns}, b	3.17± 0.11 a^{ns} b	3.17± 0.11 a^{ns} b
IV	EAD	4.75± 0.21a,b	4.5± 0.22a,b	4.08± 0.20a,b

Values are expressed as Mean±SEM of Six animals.

Data were analyzed by one-way ANNOVA followed by post hoc Tukey's multiple comparison test $^{a}p<0.05$ compared to Normal, $^{b}p<0.05$ compared to thrombocytopenic group.' ns' indicate not significant.

Clotting Time determination:

The effect of both EAM & EAD on extract on clotting time is shown in the **Table 3**, alcohol treatment (thrombocytopenic group) was significantly increased the clotting time(2.4±0.18) in thrombocytopenic rats when compare to normal (1.36±0.16). Conversely, a significant decrease of the clotting time in EAM treated groups at 3rd, 5th and 7th day is (1.42±0.08, 1.17± 0.10, 1.17± 0.10), respectively.

TABLE 3: CLOTTING TIME IN DIFFERENT DAYS OF INTERVAL WITH *ECLIPTA ALBA* EXTRACTS.

		Clotting Time in minutes		
Groups	**Treatment**	**3rd day**	**5th day**	**7th day**
I	Normal	1.25± 0.11	1.25± 0.11	1.36± 0.16
II	Thrombocytopenic	2.4± 0.18a	2.4± 0.18a	2.4± 0.15a
III	EAM	1.42± 0.08 a^{ns}, b	1.17± 0.10 a^{ns} b	1.17± 0.10 a^{ns} b
IV	EAD	2± 0.13a,b^{ns}	1.58± 0.08a^{ns},b	1.58± 0.10a^{ns},b

All the values are expressed in Mean±SEM, n=6.

Data were analyzed by one-way ANNOVA followed by post hoc Tukey's multiple comparison test $^{a}p<0.05$ compared to Normal, $^{b}p<0.05$ compared to thrombocytopenic group.' ns' indicate not significant.

Platelet count determination:

In the normal group platelet count was found to be 878±11.15×10^3 cells/µL., but after the induction of thrombocytopenia platelet counts comes down to 215±8.47 ×10^3 cells/µL, EAM extract treatment shows significant result in increasing the platelet count (778.5±11.98, 816±11.23, 514.67±3.77 ×10^3 cell/µL) in 3rd, 5th and 7th day of treatment respectively, with the p value <0.01. EAD shows (340.83±16.79, 497.17±3.64, 514.67±3.77 ×10^3 cell/µL).

TABLE 4: PLATELETS COUNT IN DIFFERENT DAYS IN DIFFERENT GROUPS OF ANIMALS.

Sl no	**Groups**	**Platelet counts×10^3 cell/µL**		
		3 days of the treatment	**5 days of the treatment**	**7 days of the treatment**
1	Normal	878±11.15	870±9.6	874±1.11
2	Thrombocytopenic	215±8.47^{a}	221±11.1^{a}	220±8.7^{a}
3	EAM	778.5±11.98^{a,b}	816±11.23^{a,b}	816.17±6.81^{a,b}
4	EAD	340.83±16.79^{a,b}	497.17±3.64^{a,b}	514.67±3.77^{a,b}

All the values are expressed in Mean±SEM, n=6.

Data were analyzed by one-way ANNOVA followed by post hoc Tukey's multiple comparison test $^{a}p<0.05$ compared to Normal, $^{b}p<0.05$ compared to thrombocytopenic.

Effects of extracts on MDA, SOD, CAT, GSH LEVELS:

TABLE 5: EFFETC OF EA ON LIPID PEROXIDATION (µg/mg OF PROTEIN), ENDOGENOUS ANTIOXIDANTS AND SERUM MARKERS

Groups	Lipid Peroxidation	Endogenous Antioxidants			Serum parameter	
		SOD	CAT	GSH	SGOT	SGPT
Normal	11.16±0.10	10.16±0.10	293.09±0.52	49.74±0.42	62.5±0.76	72.5±0.56
Thrombocytopenic	15.63±0.27[a]	1.74±0.29[a]	131.19±1.73[a]	20.26±0.19[a]	171.33±1.61[a]	160.17±1.22[a]
EAM	10.22±0.12[a,b]	10.310.61[b]	285.07±4.98[b]	42.72±1.22[a,b]	59.83±2.10[b]	83±2.65[a,b]
EAD	6.11±0.05[a,b]	10.01±0.05[b]	288.32±1.11[b]	38.00±0.33[a,b]	46.33±2.06[a,b]	62.17±3.52[a,b]

All the values are expressed in Mean±SEM, n=6.

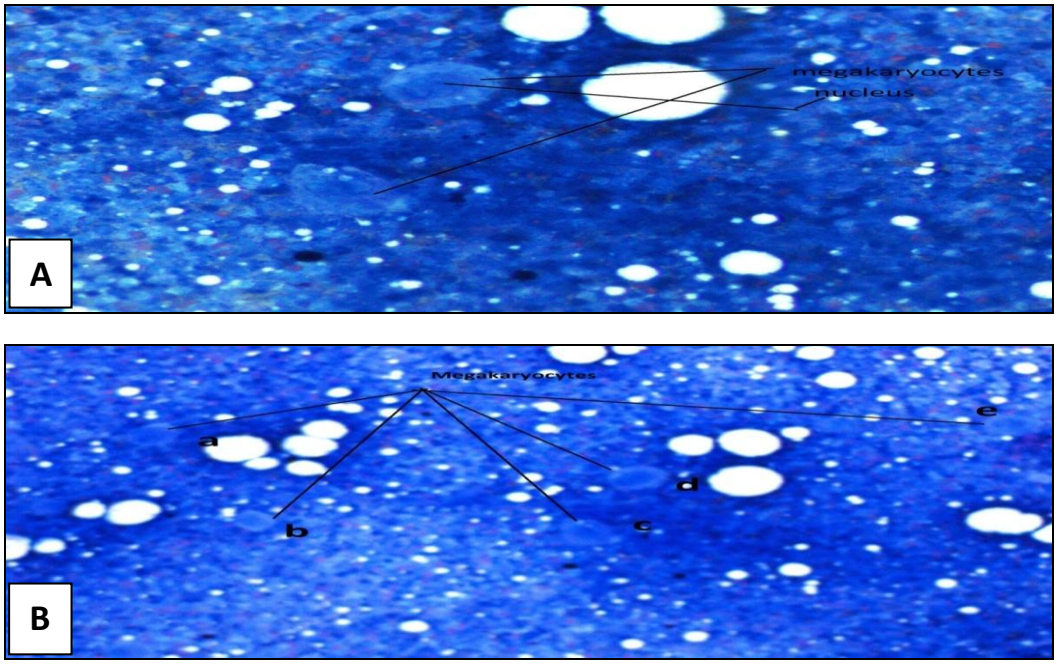
Data were analyzed by one-way ANNOVA followed by post hoc Tukey's multiple comparison test [a]p<0.05 compared to Normal, [b]p<0.05 compared to thrombocytopenic.

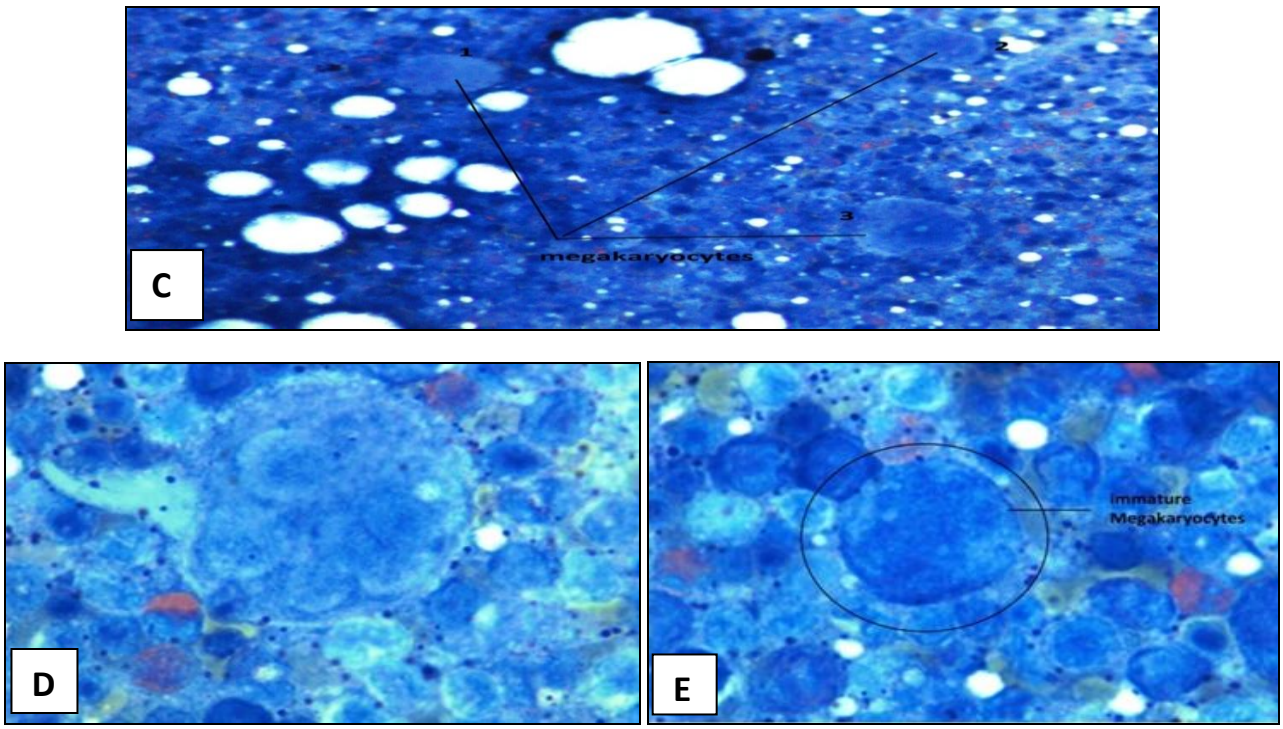
Result of the study clearly revealed increase in the levels of MDA in thrombocytopenic rats (15.63±0.27) and SOD (µg/mg of protein) CAT (µg/mg of protein) GSH (µg/mg of protein) levels decreased (1.74±0.29, 131.19±1.73, 20.26±0.19) when compared to normal group. Treatment with EAM significantly prevented this raise in LPO levels (10.22±0.12). GSH, SOD and CAT contents have significantly increased in extract treated groups (42.72±1.22, 10.310.61, 285.07±4.98) respectively. It was observed that ethanol administration resulted in the significant elevation in the levels of SGOT(171.33±1.61 U/L), SGPT (160.17±1.22 U/L) when compared to that of normal group which is having SGOT(62.5±0.7U/L) and SGPT (72.5±0.5 U/L). After the treatment with 200mg of EA extract for 7 days prior to ethanol administration dose dependently restored the activities of SGOT (U/L) SGPT (U/L). The maximum significant reduction in the elevated biomarker enzymes found in the methanolic extract of *Eclipta alba.* In cases of EAM included group SGOT&SGPT levels were found to be (59.83±2.10U/L) and (83.2±2.65 U/L)

Megakryocytes counts in plants extract treated and thrombocytopenic rats:

TABLE 6: MEGAKARYOCYTES COUNTS IN THE DIFFERENT GROUPS OF ANIMALS

Sl no.	Groups	No. of megakaryocytes
I	Normal	1-2
II	Thrombocytopenic	4-5
III	EAM	2-3
IV	EAD	0-1





A) Megakaryocytes in Control (40x) (B).Megakaryocytes in thrombocytopenic (C). Megakaryocytes in EAM treated groups (D). Megakaryotes in (100x). (E)Immature megakaryocytes in thrombocytopenic.

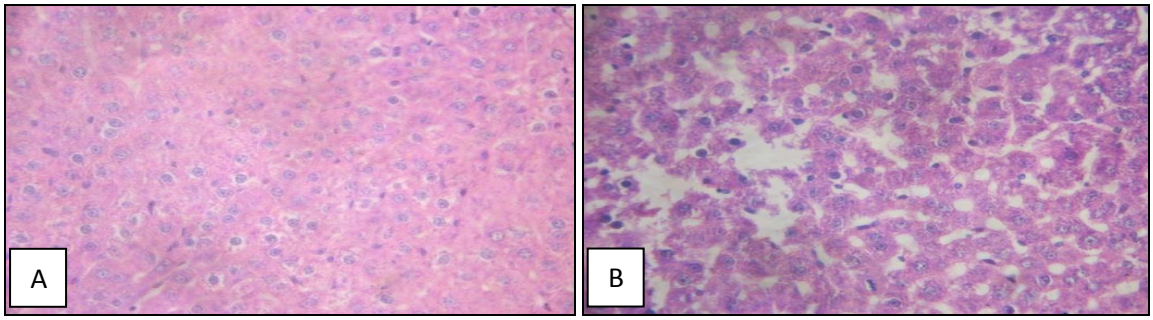
FIG.1: MEGAKARYOCYTES IN BONE MARROW

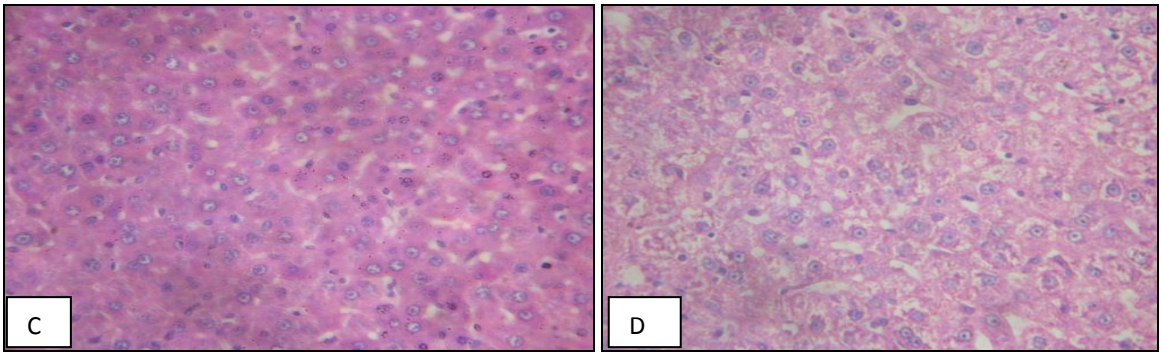
Histipathalogical study of liver cell:

Histology of the liver sections of control animals (Group A) showed normal hepatic cells without any sinusoidal dilation & hepatic vein hypertension. The liver Sections of thrombocytopenic rats showed more than 50% of sinusoidal dilation. The dilated sinusoids of the liver of the EAM group, thrombocytopenic group, and the control group were significantly different. The EAM group differed and significant from the thrombocytopenic group, but was similar to the control group indicating an almost complete reversal of damage by the alcohol. Only ethanol group was marked with dilations that are 2+ and 3+ while EAM group and the control group only had the presence of 1+ and 2+ dilations. **Fig. 2** and **Table 7** showed the sinusoidal dilation of thrombocytopenic, EAM group and the control group. Dilation of the central vein of the liver and cloudy swelling of the liver was found to be different for all the groups. EAM treated groups showed noteworthy result towards the sinusoidal dilation compared to EAD.

TABLE 7: EFFECT OF TRAETMENT ON LIVER SINUSOIDAL DILATION

Groups	1+(10-20%)	2+(30-50%)	3+(>50%)
Control	0%	6.79%	0%
Thrombocytopenic	0%	11.12%	44.65%
EAM	7.4%	5.54%	0%
EAD	9.32%	16.5%	0%





Histopathology of liver tissues. (A) Section shows normal liver architecture. (B) Section shows Sinusoid dilation in thrombocytopenic group. (C) Section show dilation recovers in EAM treated group almost near normal. (D) EAD treated group not showed significant result

FIG. 2: SHOWED LIVER SINUSOIDAL DILATION IN THROMBOCYTOPENIC AND PLANTS EXTRACTS TREATED GROUPS

DISCUSSION: Platelets are an important factor for the blood clotting system. These are associated with some proteins like fibrinogen and some factors V, VII and VIII more, which are responsible for the blood clotting. In this study thrombocytopenia was induced by the ethanol administration. Thus, apart from the AIDS, alcoholism probably is the leading cause of thrombocytopenia [21]. Administration of alcohol to the rats, causes the liver damage, where sinusoidal dilation occur and when sinusoid get damaged the production of thrombopoeitin level decreases. Thrombopoeitin is the main signal to the megakaryocytes to produce the platelets [22].

Whole plant of *Eclipta alba* was extracted by cold maceration and decoction method. That was used for the investigation of phytochemical analysis. The investigation revealed that that the plant contained Alkaloids, Terpenoids, Flavonoids, Phenolics, and Tannins. These compounds may be responsible for beneficial effect of various biological properties of the plant in the body.

The total phenolic content of the plant extracts was determined by Folin-Ciocalteu assay. This method is based on the reduction of tungsten and molybdenum oxides by the phytochemicals which have reducing property, yielding a color change from yellow to blue. The result from this assay indicated the reducing property of substances (i.e antioxidant potential). Phenolics were reported to be to very good reducing agents and reported to have excellent antioxidant properties [23]. So this assay indicates the quantum of antioxidant phytochmeicals present in the extract calculated as phenolics. Scavenging of DPPH radical is related to inhibition of lipid peroxidation [22]. DPPH radical involves a hydrogen atom transfer process [24]. This study suggested that, the good antioxidant activity of DPPH radical of EAM may be attributed to a direct role in trapping free radicals by donating hydrogen atom.

Chronic alcohol consumption increases the bleeding and clotting time (**Table 2 and 3**), because alcohol can interfere with the process called fibrinolysis [25] i.e. when there is abnormal low platelet count, availability of platelets to blood stream for fibrinolysis or for the process of platelet plugging is decreased. And also, alcohol causes impaired platelet aggregation, decreased secretion or activity of platelet derived proteins involved in the blood clotting and causes prolongation of bleeding [26]. These effects can have serious medical consequences such as, an increased risk of strokes. Also concomitant use of alcohol and aspirin greatly increases the patients risk for gastrointestinal bleeding and alcohol enhances the aspirin induced fecal blood loss. Bleeding time was significantly increased in the alcohol treated groups. Increasing the bleeding time was significantly decreased in the EAM treated group (**Table 2**).

Because of low platelet count in thrombocytopenic condition blood clotting time increased. In the present study, after ethanol treatment the clotting time was increased in all the groups. This increased clotting time was reversed with treatment of 200mg/kg of plant extract EAM and EAD. EAM

extract showed significant decrease in clotting time ($p<0.01$) at 3rd day treatment when compare to thrombocytopenic group. There was a negligible change between 5th and 7th day of clotting time in EAM group. EAD treated groups also demonstrated significant results after 5th and 7th day of treatment, but not as potent as EAM (**Table 3**).

Chronic alcohol administration in the rats causes damage in the liver sinusoidal cells (**Fig.2)** and so production of thrombopoeitin level in the liver decreases, so there is no signal for the bone marrow megakaryocytes for the production of platelets and causes thrombocytopenia. When thrombocytopenic animals were treated with plant extracts, platelet count was increased. EAM showed significant result in increasing the platelet counter in thrombocytopenic condition when compared to EAD within the 3 days of treatment. This reversal of alcohol induced thrombocytopenia in the extracts could be due to the reasons explained here under. *Eclipta alba* know to be one of the potent hepatoprotechtive agent from ages [27].

This hepatoprotechtive activity may due to presence of phenolics. Olas et al noted that, induced the platelet oxidation by peroxynitrite, a highly reactive oxidizing species, and caused the Inhibition of platelet activation and activation, Inhibiting the adhesion of platelet to collagen and fibrinogen, and reducing the platelet aggregation and secretion. It was found that peroxynitrite reacts with the thiol groups of the blood proteins. Furthermore, the prevention of further oxidation was countered by the radical scavenging abilities of antioxidants, namely tea polyphenolics.

In our study, total phenolic content obtained by Folin-Ciocalteu was 26.9 mg GAE/g of extract indicates the presence of high phenolic content in EAM. So the effect of *Eclipta alba* can be consider as analogous to the application of antioxidants to counter platelet oxidation and Platelet dysfunction by reducing platelet Oxidation by ROS. platelet function and lifespan are maintained. Thus improving platelet count, bleeding and clotting time. And also phenolics have the property to interact with the receptor like GPIIb and GPIIIa present on the surface of the platelets responsible for the platelet activating pathway [28].

In the condition of thrombocytopenia the megakaryocytes number and size is altered in the bone marrow (**Fig.1**). Because of low availability of the platelets in the peripheral system the megakaryocytes get stimulated for the production of the platelet [29]. So number of megakaryocytes is increased in the bone marrow. After the treatment with EAM the megakaryocytes decreased because of increase in the platelet count in blood peripheral system.

SGOT and SGPT have been reported to be sensitive indicators of liver injury. The disturbance in the transport function of the hepatocytes as a result of hepatic injury and causes enzymes leakage From cells due to altered permeability of membrane. This results in increased levels of SGOT, SGPT in the serum. The present study revealed a significant increase in the levels of SGOT and SGPT, and decrease in level of serum total protein after exposure to the Ethanol treatment, indicating considerable hepato-cellular injury. Treatment of the animals with EAM and EAD decreased the levels of the serum marker enzymes SGOT and SGPT by recovering the sinusoidal damage in the liver and increased the level of Serum thrombopoeitin (**Table 5**). This indicates that extracts showed the potent hepatoprotechtive activity by recovering the sinusoids dilation and regulate the serum SGPT and SGOT levels. EAM was found to be more potent than EAD in reversing the thrombocytopenic condition in alcohol treated animals.

Ethanol induced thrombocytopenia is also evidenced by the elevation of TBARS and decrease in the activity of free radical scavenging enzymes, SOD, CAT and GSH. These enzymes constitute a mutually supportive team of defense mechanism against the harmful effects of the reactive oxygen species (ROS) and free radicals in biological systems [30]. Lipid peroxidation also yields a wide range of cytotoxic products most of which are aldehydes, as exemplified by MDA, which can be measured following the method of Yagi et al. Moreover, the lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Free radicals are increased in the alcoholic condition and

causes the liver damage. Scavenging of free radicals is one of the major antioxidants mechanisms to inhibit the chain reaction of lipid peroxidation. EAM significantly found to reverse the hepatotoxicity caused by ethanol [31]. This was demonstrated by a significant decrease in the levels of lipid peroxidation and restoring the elevated antioxidants enzymes and by preventing liver damages as shown in the histopathalogical studies(**Table 5**).

Histopathalogical examination of the liver provided further evidence for the thrombocytopenia caused by ethanol treatment. It also leads to increase in the blood pressure of the portal veins, which impedes the flow of blood out of the spleen and liver [32]. Also as per the study of Karker et al the dilation of the sinusoids of organs is often the result of poor venous outflow. When ethanol is administered continuously, liver necrosis occurs which is the major approach to cause the thrombocytopenia (**Fig.2).**Treatment of plants extracts with 200mg/kg body weight for 7 days showed recovery in the liver sinusoidal damage. In thrombocytopenic condition liver damage was found to be 44.65% in category +2. EAM extracts showed significant result in recovering the liver damage to 0% in +3 and 5.54% in +2 category (>50%damage) when compared to the thrombocytopenic group.

With reference to the above mentioned findings *Eclipta alba* methanolic extract showed the significant results in enhancing the platelets counts. This may be due to rich antioxidants and polyphenolics presents in the plants. Anti oxidants helps in scavenging the free radicals and reduce the sinusoidal damage in liver, and polyphenolics have the property to interact with receptor presents on the platelets surface and activate the sequester platelets in the spleen. This could be reasons for the increases in the platelets counts in the thrombocytopenic model.

CONCLUSION: Present study demonstrated that chronic alcohol treatment caused the thrombocytopenia in rats as evidenced by increasing bleeding and clotting time, low platelet count, increased level in serum marker and reduction in the antioxidant enzymes and histopathlogical changes. The present study revealed that *Eclipta alba* methanolic extract maintained the antioxidant enzymes level and decrease the bleeding and clotting time within 3 days of treatment with 200mg/kg body weight and increased the platelet count more than 60%. *Eclipta alba* showed promising result in increasing the platelet counts in thrombocytopenic condition. However, further work is needed to isolate and identify the active principles present in *Eclipta alba* which may be responsible for enhancement of platelet count.

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