Methanol extract of *Emblica officinalis* attenuates Cisplatin induced myelosuppression in wistar rats by exhibiting free radical scavenging potential

Ramesh C[1*], Anusha B N[1], Girish B[2], Sushma Bai B[3], Rakesh S A[4] , Amith Kumar[5] and Pratiksha C C[6]

[1]Department of Pharmacology, GM Institute of Pharmaceutical Sciences, Davangere-577006[1]
[2]Center for Excellence and Innovative Ideas. GM University, Davangere-577006
[3]Department of Pharmacology, East West College of Pharmacy, Bangalore
[4]Department of Pharmacognosy, GM Institute of Pharmaceutical Sciences, Davangere-577006
[5]Assistant Dean-Research, GM University, Davangere-577006
[6]Department of Pharmaceutics, GM Institute of Pharmaceutical Sciences, Davangere-577006

Mobile: +91-9901514401

Abstarct. The *Emblica officinalis* commonly known as Indian gooseberry or Amla has superior value in entire indigenous traditional system of medicine including Ayurveda and folklore. The current research was undertaken to check beneficial properties of methanol extract of *Emblica officinalis* against cisplatin induced myelotoxicity in rats. The cisplatin induced myelotoxicity in rats model was used for the current study in which all animals were administered with cisplatin on 1st, 3rd, 5th and 07th day to induce bone marrow toxicity and rats were treated with methanol extract of *Emblica officinalis* for 21 days. Blood samples were collected from all the animals on day 1st, 7th, 14th and 21st after one hour before the administration of the drugs and hematological parameters like erythrocytes, leukocytes, differential leukocytes, clotting and bleeding time were estimated. At 21st, bone marrow samples were collected after the scarification of all rats under investigation. The part of bone marrow sample was used for the determination of antioxidant enzymes and remaining was subjected to histopathological examination. The therapeutic rats administered with extract of *Emblica officinalis* have shown normal hematological parameters, bleeding & clotting time on 14th and 21st day. Administration of extract significantly enhanced tissue antioxidant enzymes and could regenerate bone marrow cells. The methanol extract of *Emblica officinalis* of has shown significant protective effects against cisplatin induced myelosuppression in wistar rats.
Key words: Emblica officinalis, Cisplatin, Myelosuppression, Hematological parameter, Lipid peroxidation, Glutathione peroxidase, Antioxidant enzymes.

1.0 INTRODUCTION

Antineoplastic drugs are toxic to normal cells also when we administer them to treat cancer which may results in various noxiousness such as bone marrow toxicity, lympho-reticular damage, liver toxicity, kidney damage, gonadal toxicity, cardiotoxicity and skin toxicity. Hence patients agonize from various post chemotherapy complications. Among numerous toxicities of cancer chemotherapy, bone marrow noxiousness are very common which results in various complications. Bone marrow toxicity also known as myelosuppression which commonly causes

aplastic anaemia, leukopenia, agranulocytosis and thrombocytopenia [1,2]. The above mentioned complications reduce quality of life of cancer survivors and can cause failure of chemotherapy. Hence, considerable supportive treatment is always necessary to enable cancer patients to tolerate effects of chemotherapy and achieve maximum benefit. Several approaches are practicing for the effective management of chemotherapy-induced such complications like administration of Colony stimulating factor for myelosuppression, gastroprotective for GIT intolerance and etc which are costly and not much effective. Hence managing of post cancer chemotherapeutic complications still remains problematic as there is no satisfactory drug available [3,4].

Most of the untoward effects of cancer chemotherapy are due to their free radical nature and administration of antioxidant drugs may helpful to counter them. In this regard several plants and poly herbal formulations such as *Triphala* were widely used in the Ayurveda for the management of complications of chemotherapy [5].

In this regard herbal medicine plays vital role due to their effectiveness in reducing cellular toxicity due to their antioxidant properties with least possible side effects. The *kshara-karma* is the approach which involves use of chemicals for the management of cancer (Chemotherapy) and *kshara-vyapada* is term used to describe chronic toxicities of chemotherapy in Indian traditional system Ayurveda. In this regard several plants and poly herbal formulations such as *Triphala* were widely used in the Ayurveda for the management of complications of chemotherapy [6].

Emblica officinalis commonly called as *Amla* (Indian gooseberry) is one of the most widely studied plant drug for various purposes. Plant parts of *Emblica officinalis* shown antibacterial, antioxidant, antidiabetic, hypolipidemic, anticancer, hepatoprotective, gastro protective, anti-ulcerogenic, nephroprotective and chemo-preventive properties. The fruits *Emblica officinalis* is important ingredient of *Triphala* an ayurvedic formulation and have the ability to protect the cells against free radicals and can counter the complication of anticancer drugs. The *Triphala* in which the *Emblica officinalis* was the essential ingredient was extensively used in Ayurveda for treating chemotherapy induced complications but lack scientific evidence for the same [6,7,8]. Hence, present investigation was designed to evaluate and establish scientific data for the protective effects of *Emblica officinalis* against cisplatin induced myelosuppression.

2.0 MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The fruits of the *Emblica officinalis* was purchased from local market and authenticated by Dr. Venkateshappa, HOD, Department of Botany, East West College of Science, Bangalore. The specimen herbarium was preserved at institute herbarium library (Herbarium number-119).

2.2 Preparation of ethanol extract

The fruits of *Emblica officinalis* were dried under shade immediately, milled and the coarse powder was collected. About 250gm of dried coarse powder of *Emblica officinalis* was defatted first using petroleum ether (40°-70°C) by hot continuous percolation using Soxhlet apparatus the left over marc was kept in the hot air over (90°C) and the defatted product was collected. The defatted coarse powder was again subjected for extraction with methanol for 72 hours [9].

2.3 Preliminary phytochemical investigation

The methanol extract of *Emblica officinalis* (MEEO) was evaluated for the preliminary phytochemical compounds according to per procedure prescribed in previous research [10].

2.4 Estimation of total phenol content in extract

The Folin Ciocalteu reagent was used to estimate total phenolic content of methanol extract of *Emblica officinalis*. In brief, the determination was is carried out as follows: 1 mL of solution containing 1mg of methanol extract was delivered into 100-mL Erlenmeyer flask and then adjusted to the final of 46 mL with the addition of distilled water. The above reaction mixture was made to react with Foline Ciocalteu (1mL) reagent and 3 mL of 2% of sodium carbonate after 3 minutes. Then at the room temperature, above mixture was shaken using a shaker for 2 hours its absorbance was measured at 760 nm. The amount of the total phenolic content in MEEO was expressed as micrograms of pyrocatechol using following equation that was obtained from standard pyrocatechol graph [11,12].

$$\text{Absorbance} = 0.001 \times \text{Pyrocatechol } (\mu g) + 0.0033.$$

2.5 Animals

The wistar male rats were acclimatized for 7 days under husbandry with standard conditions (25 ± 1^0 C and relative humidity 45 to 55%) in 12:12h light/ dark cycle. The standard rat pellet was given to experimental rats which were free to access to water supplied *ad libitum* under strict hygienic conditions. The present experimental protocol was designed according to ethical principles of CPCSEA, New Delhi and the study was approved by the Institutional Animal Ethics Committee, East West College of Pharmacy, Bangalore (Ref No.- EWCP/CPCSEA/IAEC/V/02).

2.6 Evaluation of methanol extract of *Emblica officinalis* against cisplatin induced toxicity

The study design for the evaluation of myeloprotective effects of MEEO consists of five group of albino rats containing six in each group. The doses of methanol extract of *Emblica officinalis* are selected based on previous studies. The protocol of treatment given in study is given in the following table.

Group	Group	Treatment
1.	**Normal**	Animals are given with Normal Saline (2ml/kg,p.o)
2.	**Toxic control**	Animals are given with Cisplatin (5mg/kg, i.p) for initial 4 consecutive days and given 2% tween 20 for 1^{st} to 21 days.
3.	**Standard**	Treated with Cisplatin (5mg/kg, i.p) for first 4 consecutive days and Standard drug
4.	**MEEO-100mg/kg**	Animals are given with Cisplatin (5mg/kg, i.p) for initial 4 consecutive days and low dose of *Emblica officinalis* methanol extract by oral for 21 days.
5.	**MEEO-200mg/kg**	Animals are given with Cisplatin (5mg/kg, i.p) for initial 4 consecutive days and medium dose of *Emblica officinalis* methanol extract by oral for 21 days.
6.	**MEEO - 400mg/kg**	Animals are given with Cisplatin (5mg/kg, i.p) for initial 4 consecutive days and high dose of *Emblica officinalis* methanol extract by oral for 21 days.

2.6.1 Induction of Cisplatin induce myelosupression

The methanol extract of *Emblica officinalis* was evaluated for its protective effects against cisplatin induced toxicity. The Myelosuppression and neurotoxicity was induced in experimental wistar rats by administering cisplatin (5mg/kg,i.p) on 1st, 3rd, 5th and 7th days of study [13].

2.6.2 Protective effects of methanol extract against cisplatin induced myelosuppression

The blood samples from all the experimental rats were collected before the administration of the extract and cisplatin under ether anesthesia at Day 1. And the blood samples were collected from each rat one hour after the administration of the drugs under ether anesthesia at 7th, 14th and 21st day. The blood samples were subjected to hematological analysis for following parameters [14,15,16].

- Complete blood count: Includes the estimation of Red Blood Cells, White Blood Cells, Thrombocytes, Differential leukocyte count, Hemoglobin and haematocrit.
- Bleeding time
- Clotting time
- Histopathology of bone marrow

All animals were sacrificed on 21st day and bone marrow samples were collected and examined for the histopathological changes.

2.6.3 Histopathological examination

The bone marrow and brain samples were taken from rats from all groups and fixed in 10% formal saline (PH 7.2) for 24 h. Serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Paraffinized blocks were made for sectioning at 4 µm thickness by slide microtome. Tissue sections were mounted on glass slides and stained by hematoxylin & eosin stain for histopathological assay using the light electric microscope [17].

2.6.4 Determination of Lipid peroxidation in bone marrow samples

Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) in bone marrow of rats to determine the thiobarbituric acid reactive species (TBARS) **[**18**].**

2.6.5 Determination of antioxidant enzymes in bone marrow samples

Catalase peroxidase assay (CAP)

CAT activities were determined in the bone marrow samples with reaction solution contained: 2.5 ml of 50 mmol phosphate buffers (pH 5.0), 0.4 ml of 5.9 mmol H_2O_2 and 0.1 ml tissue homogenate. Changes in absorbance of the reaction solution at 240 nm were determined after one minute. One unit of catalase activity was defined as an absorbance change of 0.01 as units/min [19].

Superoxide dismutase assay (SOD)

The activity superoxide dismutase was estimated in bone marrow samples. Reaction mixture of this method contained: 0.1 ml of phenazine methosulphate (186 µmol), 1.2 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0), 0.3 ml of the supernatant after centrifugation (1500 × g for 10 min followed by 10,000 × g for 15 min) of homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 µmol) and stopped after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein [20] .

Glutathione-S-transferase assay (GST)

For estimation of Glutathione S Transferase in bone marrow samples of experimental rats of all the groups [21]. The Glutathione-S-transferase activity mixture was consisting of 1.475 ml phosphate buffer (0.1mol, pH 6.5), 0.2 ml reduced glutathione (1mmol), 0.025 ml 1-Chloro-2,4-dinitrobenzene (CDNB) (1mmol) and 0.3 ml of homogenate in a total volume of 2.0 ml. The

changes in the absorbance were recorded at 340 nm and enzymes activity was calculated as nmol CDNB formed/min/mg protein using a molar extinction coefficient of 9.6×10^3 $M^{-1}cm^{-1}$.

Glutathione reductase assay (GRD)

Glutathione reductase activity was determined in bone marrow samples [22] . The reaction mixture consisted of 1.65 ml phosphate buffer: (0.1 mol; pH 7.6), 0.1 ml ethylene diamine tetra acetic acid (EDTA) (0.5 mmol), 0.05 ml oxidized glutathione (1 mmol), 0.1 ml nicotinamide adenine dinucleotide phosphate (NADPH) (0.1 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. Enzyme activity were quantitated at 25°C by measuring disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione peroxidase assay (GPX)

Glutathione peroxidase activity was in bone marrow samples assayed by the method of Mohandas et al [23,24]. The reaction mixture was consisting of 1.49 ml phosphate buffer (0.1 mol; pH 7.4), 0.1 ml EDTA (1 mmol), 0.1 ml sodium azide (1 mmol), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mmol), 0.1 ml NADPH (0.2 mmol), 0.01 ml H_2O_2 (0.25 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25°C. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Statistical analysis

The data obtained from the present investigation was analyzed by ANOVA followed by post hoc Dunnett's t-test with the help of Graph pad prism5 software. All the values were shown as mean ± standard error of mean (S.E.M.).

3.0 RESULTS

3.1 Preparation of extract

The percentage yield of methanol extract of *Emblica officinalis* was 9.45% and it was light brown in colour.

3.2 Preliminary phytochemical study

The investigation for primary phytochemicals performed for the methanol extract of *Emblica officinalis* (MECD) has revealed the presence of various phyoconstituent flavonoids (FLV), glycosides (GLS), alkaloids (ALK), poly phenols (PPL), tannins (TNS), steroids (ST), and carbohydrates (CH) [Table No.1].

Table No 1: Results of preliminary phytochemical investigation on MEEO

Sl. No	Phyto-constituent	Presence
1.	PPL	+++
2.	FLV	+++
3.	GLS	+++
4.	ALK	-
5.	CH	++
6.	Proteins	++

3.3 Estimation of phenolic content in MEEO

The amount total phenolic components of the methanol extract of *Emblica officinalis* was found to be expressed as 129.51mg pyrocatechol/g of MEEO.

3.4 Evaluation of protective effects of MECD against bone marrow suppression

The current research study was performed to test the protective potentials of *Emblica officinalis* against chemotherapy induced toxicity in albino wistar rats.

3.4.1 Effect of MECD on hematological parameters Day 1

All the hematological parameters namely RBC, WBC, platelets, hemoglobin, hematocrit, lymphocytes, neutrophils, basophils and eosinophils were determined before the administration of cisplatin on day 1 and hence all the hematological parameters were normal in all group animals. The bleeding and clotting time were also normal in all animals [Figure No.:1 & Figure No.:9].

3.4.2 Effect of MECD on hematological parameters on Day 7

Administration of cisplatin in all group of animals except normal could induce bone marrow damage and reduced the synthesis of all blood cells which was evidenced by the disturbed haematological parameters on 7^{th} day of the study. Hence there was significant reduction in all haematological parameters was observed in toxic control and all group of rats treated with low (100 mg/kg), medium (200 mg/kg) and high (400 mg/kg) dose of extracts of *Emblica officinalis* compare to normal animals. There was no significant change was found in haematological parameters of therapeutic groups treated with extracts in comparison with toxic control animals. And there was significant prolongation of bleeding and clotting time in all animals except normal group. [Figure No.:1 & Figure No.:9].

3.4.3 Effect of MECD on hematological parameters on Day 14

Administration of methanol extract of *Cedrus deodara* could reverse bone marrow damage caused by cisplatin only in therapeutic groups of animals by 14^{th} day probably due to regeneration bone marrow cells. Hence. the experimental rats treated with cisplatin alone have exhibited the significant ($p<0.01$) decline in the haematological parameters in toxic control animals when compare to normal group of animals. But therapeutic group of rats treated with medium (200 mg/kg) and high (400mg/kg) dose of methanol extract of *Emblica officinalis* have shown significant ($p<0.01$) rise in haematological parameters compare to toxic animals. There was significant reduction in bleeding and clotting time was found in therapeutic groups compare to toxic group due to rise in platelet count. While the significant change was not found in animals treated with low dose of MECD (100mg/kg). [Figure No.:1 & Figure No.:9].

3.4.4 Effect of MEEO on hematological parameters on Day 21

Administration of methanol extract for 21 days could bring back damaged bone marrow to normal which was evidenced by the normalization of all haematological parameters in therapeutic groups. The animals of toxic control have shown significant reduction in all haematological parameters ($p<0.01$) when compare to normal group. But the administration of medium (200mg/kg) and high (400mg/kg) dose of MECD could significantly ($p<0.01$) raised haematological parameters in therapeutic group of animals compare to toxic group. As a result of this, bleeding and clotting time was also decreased in extract treated animals compare to toxic animals. But there was no significant change found in animals treated with low dose of extract (100mg/kg) [Figure No.1 to.9].

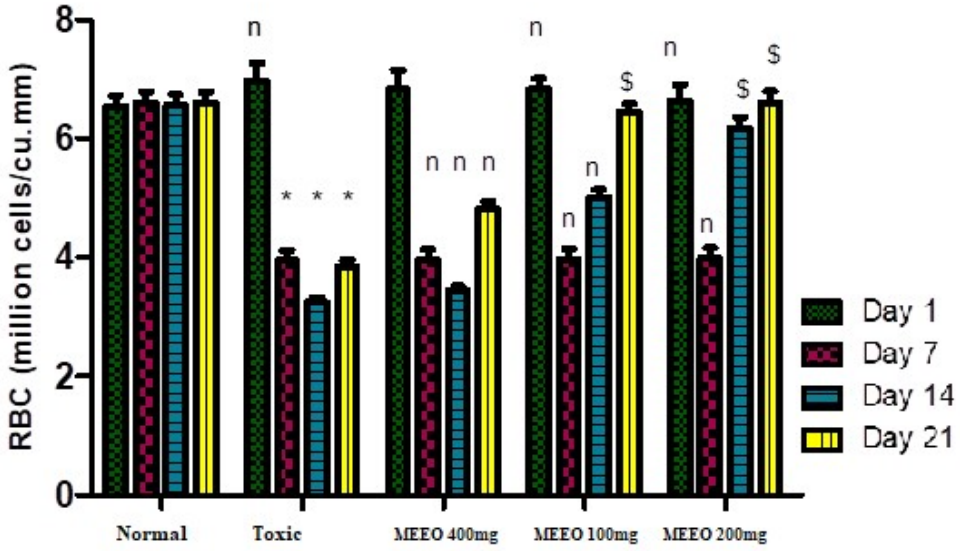
3.4.5 Effect on Lipid peroxidation of bone marrow sample

The lipid peroxidation is process in which free radicals cause damage to cell membrane and lead to cytotoxicity. There are several antioxidant mechanisms such as glutathione reductase, superoxide dismutase, catalase peroxidase and glutathione peroxidase which can counter the free

radicals. The administration of methanol extract could reduce lipid peroxidation indicating the its ability to supress cisplatin toxicity on bone marrow cells.

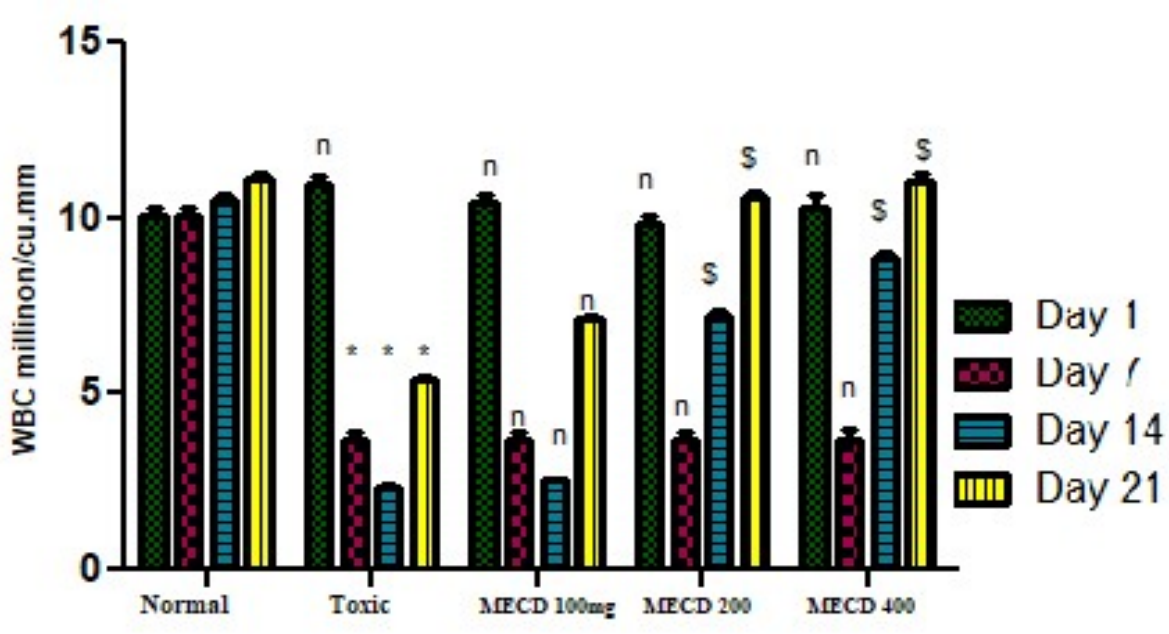
Administration cisplatin can cause bone marrow damage and hence increases level of lipid peroxidation in bone marrow sample due to its free radical mediated damage. Hence, there was found to be significant (P<0.001) elevation of concentration of lipid peroxidation in bone marrow seen in toxic control group treated with cisplatin alone compare to that of normal rats. But animals of therapeutic groups treated with MECD (200mg/kg and 400 mg/kg), have exhibited significant (P<0.001) decline in concentration of lipid peroxidation compare to toxic control [Table No.2].

Figure No.1: Effect of methanol extract of *Emblica officinalis* RBC against cisplatin induced myelosuppression



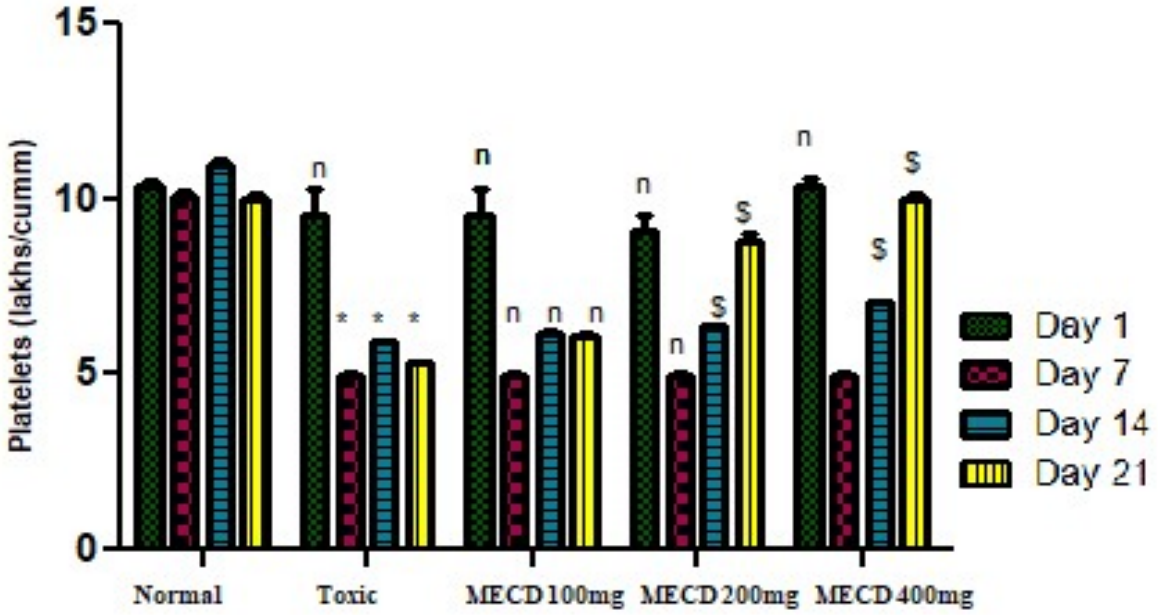
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control,

Figure No.2: Effect of methanol extract of *Emblica officinalis* Leukocytes against cisplatin induced myelosuppression



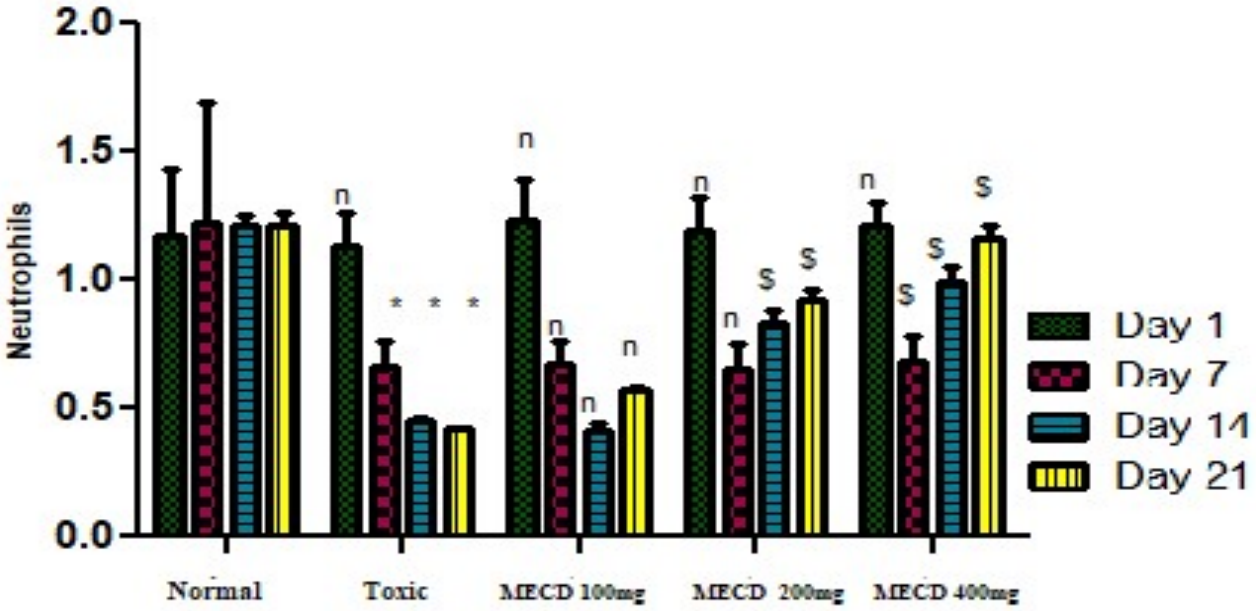
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control

Figure No.3: Effect of methanol extract of *Emblica officinalis* platelets against cisplatin induced myelosuppression



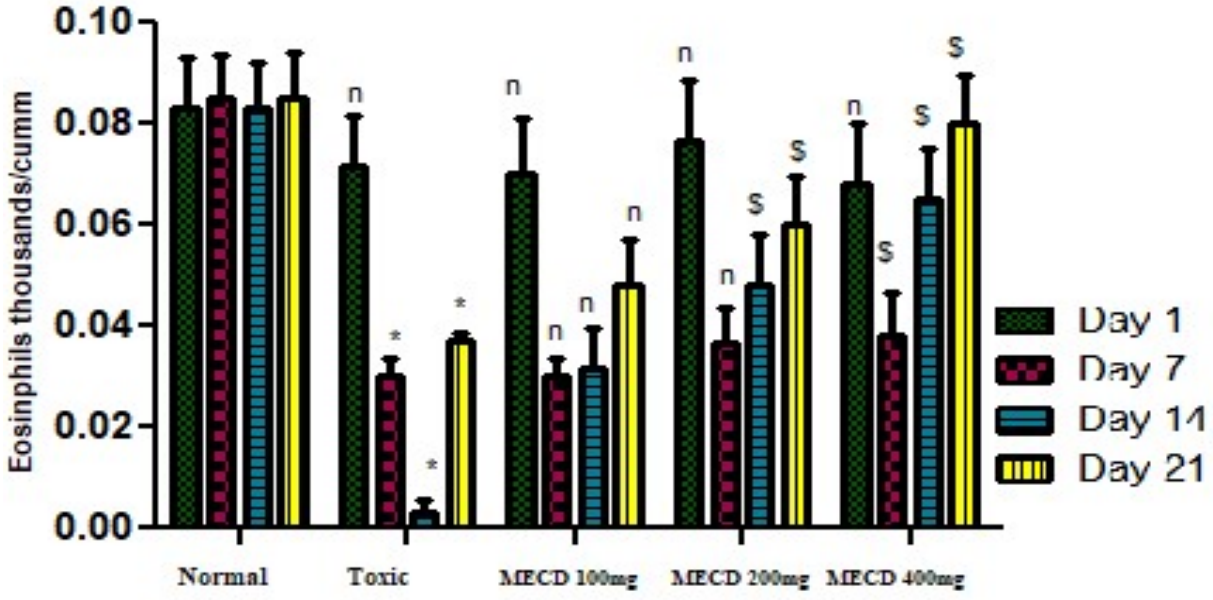
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control

Figure No.4: Effect of methanol extract of *Emblica officinalis* neutrophils against cisplatin induced myelosuppression



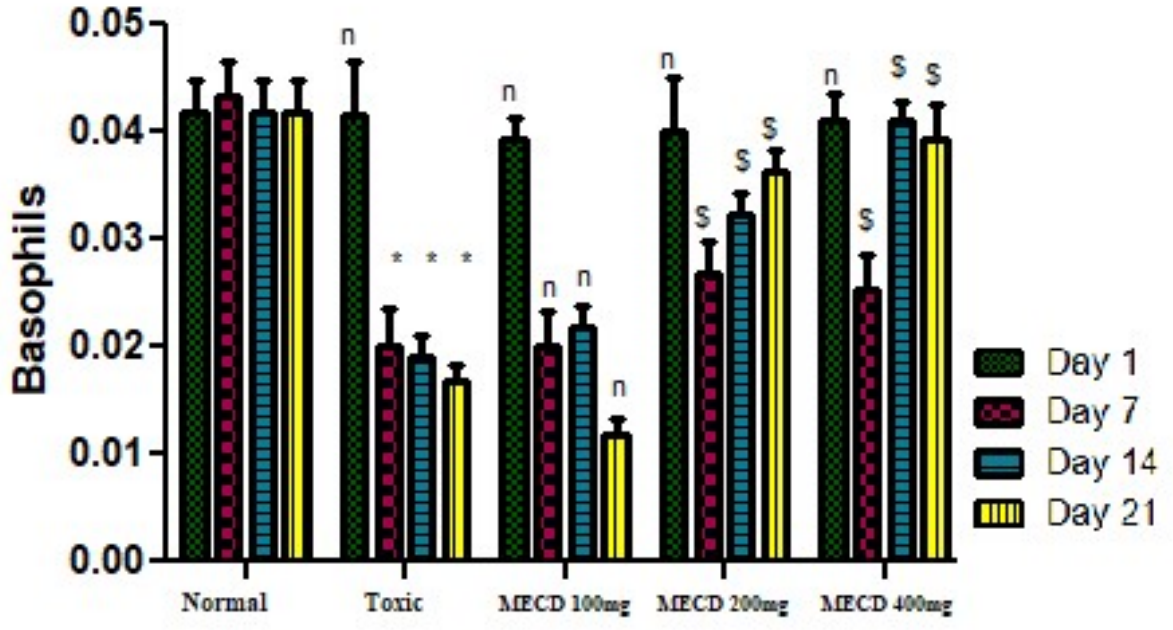
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control

Figure No.5: Effect of methanol extract of *Emblica officinalis* acidophils against cisplatin induced myelosuppression



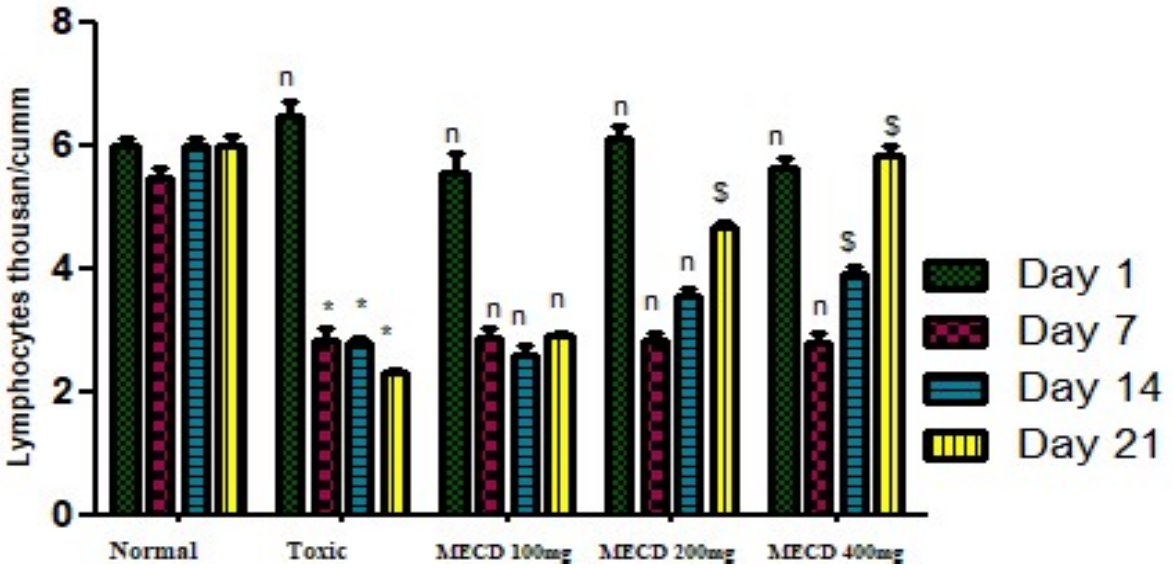
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control

Figure No.6: Effect of methanol extract of *Emblica officinalis* basophils against cisplatin induced myelosuppression



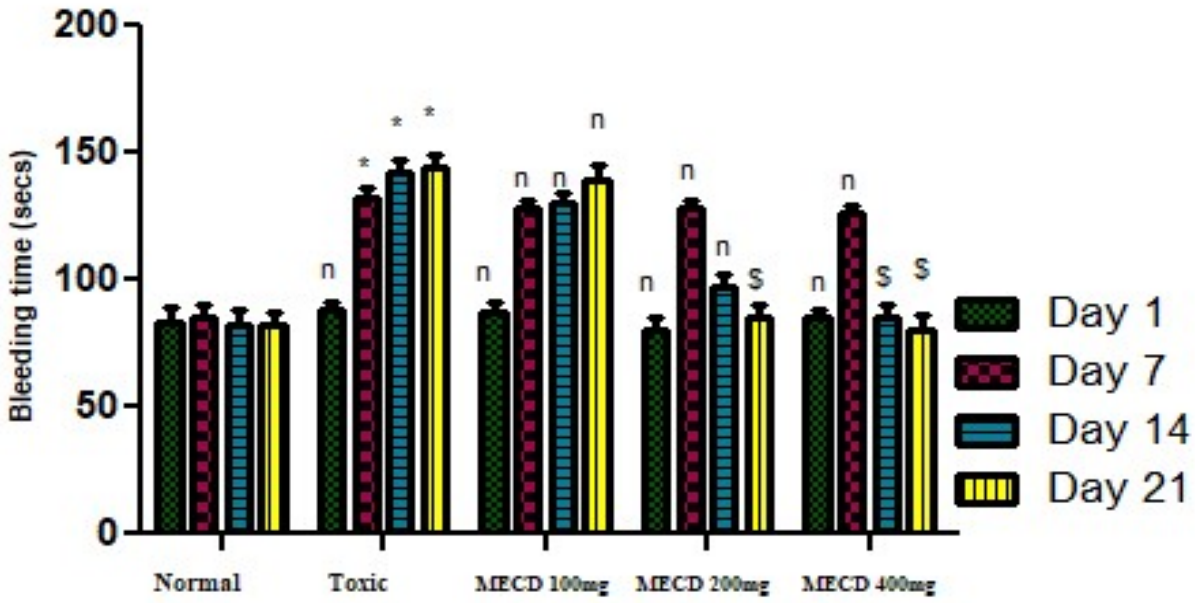
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control,

Figure No.7: Effect of methanol extract of *Emblica officinalis* lymphocytes against cisplatin induced myelosuppression



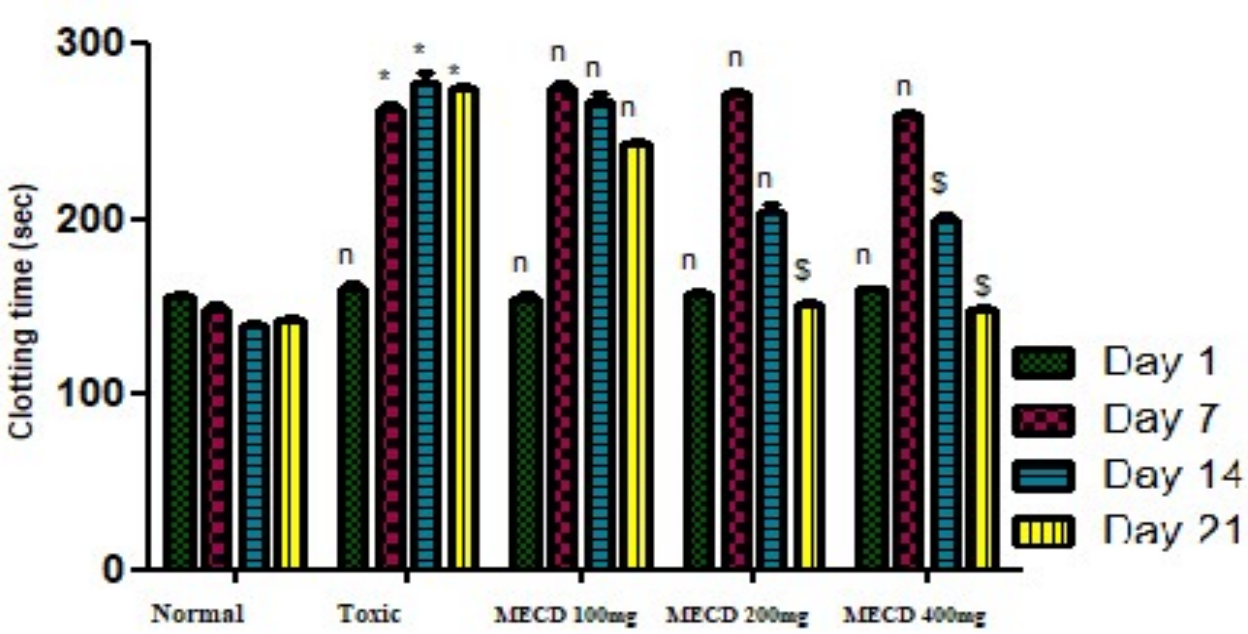
Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control,

Figure No.8: Effect of methanol extract of *Emblica officinalis* bleeding time against cisplatin induced myelosuppression



Values are mean ± S.E.M, n=6 symbols represent statistical significance.
* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control,

Figure No.9: Effect of methanol extract of *Emblica officinalis* clotting time against cisplatin induced myelosuppression



Values are mean ± S.E.M, n=6 symbols represent statistical significance.

* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control

3.4.6 Effect on antioxidant enzymes of bone marrow sample

There are several endogenous antioxidant systems such as Catalase peroxidase (CAP), Superoxide dismutase (SOD), Glutathione S transferase (GST), Glutathione reductase (GRD) and Glutathione peroxidase (GPX) present in the living system which can able to counter the free radical mediated damage. These antioxidant systems can be activated with the help of suitable treatment. In the present study, significant (P<0.001) reduction of concentration of antioxidant enzymes was found in bone marrow samples of toxic control animals treated with cisplatin alone when compare to normal. While animals of therapeutic groups treated with MECD (200mg/kg and 400 mg/kg), have exhibited significant (P<0.001) rise in liver antioxidant enzyme compare to toxic animals [Figure No.10].

3.4.5 Histopathological examination of bone marrow samples

The bone marrow samples collected from experimental animals were examined for the histological changes. The results of histopathological examination are as follows.

Normal control: The bone marrow sample of this group shows bone trabeculae with marrow space consisting of hematopoietic cells [normal cellularity] in varying proportions. Fat to Cell ratio is 1:4 [20:80]. Myeloid: Erythroid ratio [M: E - 2.5:1] appears normal Megakaryocytes appear intact. The trabecular bone consists of several osteocytes with few showing lacunae and are rimmed by osteoblasts [Figure No.10].

Toxic control: The Section belongs to toxic control group have shown bone trabeculae with marrow space consisting of hematopoietic cells [Hypo cellularity] in varying proportions. Fat to Cell ratio is increased with 3:1 [75:25]. Myeloid: Erythroid ratio [M: E 2:1] appears mildly Megakaryocytes are < absent. The trabecular bone consists of several osteocytes with few showing lacunae and are rimmed by osteoblasts reduced [Figure No.10].

MECD 100mg group: The bone marrow section belongs to this group shows bone trabeculae with marrow space consisting of hematopoietic cells [normal cellularity] in varying proportions. Fat to Cell ratio is 1:4 [80:20]. Myeloid: Erythroid ratio [M: E - 2.5:1] appears normal Megakaryocytes appear intact. The trabecular bone consists of several osteocytes with few showing lacunae and are rimmed by osteoblasts [Figure No.10].

Table No: 2. Potentials of *Emblica officinalis* 's methanol extract on liver antioxidant enzymes and lipid peroxidase against cisplatin induced myelosuppression-Day 21

Treatment	Antioxidant enzymes					
	GPX (mg/G)	CAP (mg/G)	SOD (mg/G)	GST (mg/G)	GRD (mg/G)	LOP (mg/G)
Normal Control	9.01±0.321	59.35±2.31	9.572±1.27	7.274±0.462	4.254±0.402	7.83±0.1897
Toxic Control	4.942[*]±0.29	31.21[*]±1.52	5.618[*]±1.58	3.552[*]±0.243	2.362[*]±0.262	18.22[*]±0.46
MEEO 100 mg/kg	4.912[n]±0.35	32.68 [n] ±1.12	5.784 [n]±1.55	3.672 [n] ±0.5	2.923 [n] ±0.24	18.02 [n] ±0.29
MEEO 200 mg/kg	6.12[$]±0.234	45.29[$]±4.09	7.228[$]±1.81	5.372[$]±0.46	3.782[$]±0.42	12.86[$]±0.26
MEEO 400 mg/kg	8.315[$]±0.25	51.58[$]±2.29	8.931[$]±2.01	6.91[$] ±0.28	4.371[$]±0.44	10.24[$]±0.47

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

* p<0.05-Toxic control vs Normal, [n]p>0.05-Treatment group vs Toxic control, [$] p>0.05-Treatment group vs Toxic control

MECD 200mg group: The section obtained from MECD (200 mg/kg) shows bone with marrow space consisting of hematopoietic cells [Hypercellularity] in varying proportions. Fat to Cell ratio is decreased1:9 [30:70]. Myeloid: Erythroid ratio [M: E 3:1] appears normal. Megakaryocytes appear intact. The trabecular bone consists of several osteocytes with few showing lacunae and are rimmed by osteoblasts [Figure No.10].

MECD 400mg group: The bone marrow sample of rat treated with high dose of extract have shown bone trabeculae with marrow space consisting of hematopoietic cells [normal] in varying proportions. Fat to Cell ratio is 1:2.3 [20:80]. Myeloid: Erythroid ratio appears normal. Megakaryocytes appear intact. The trabecular bone consists of several osteocytes with few showing lacunae and are rimmed by osteoblasts [Figure No.10].

4.0 DISCUSSION

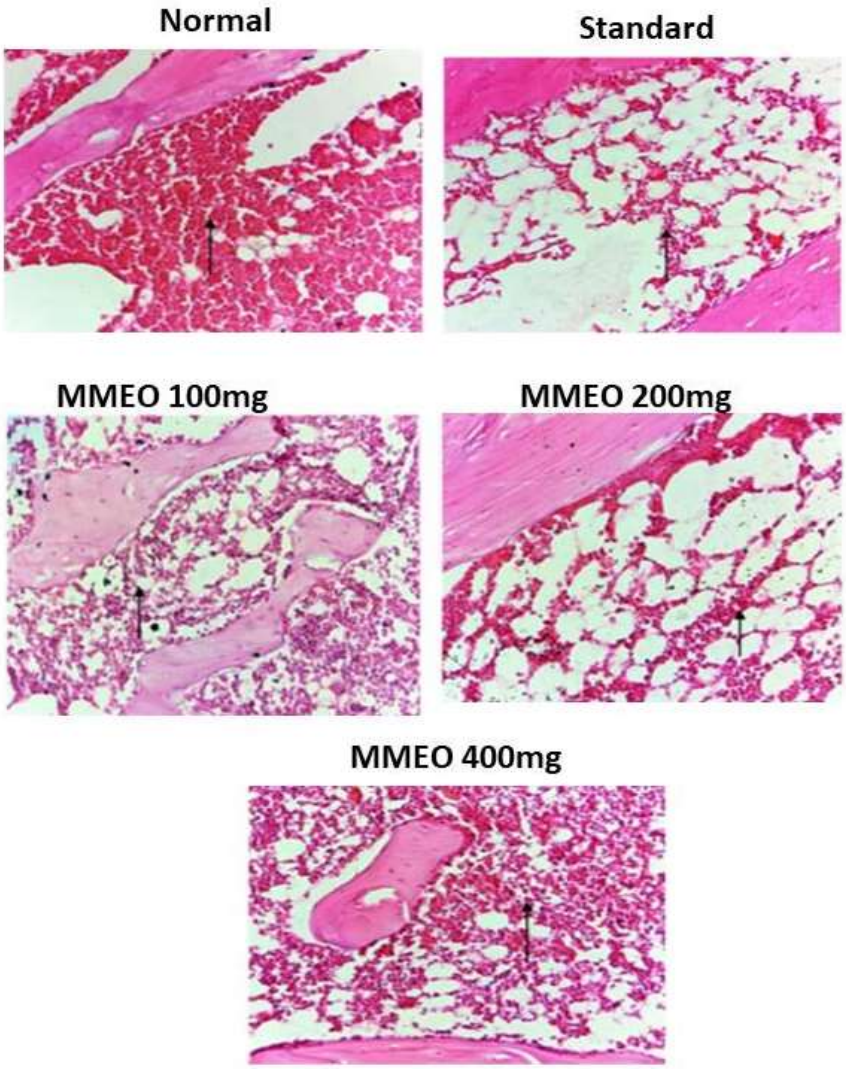
Most of the drugs used for the management of cancer or their metabolites are highly reactive free radicals and hence can cause oxidative damage to normal cells also which ultimately lead to toxicities. The common toxicities of cytotoxic drugs are bone marrow damage, Lympho-reticular toxicity, gonad toxicity, neurotoxicity and etc. Among several toxicities, bone marrow toxicity is very common complication due to the administration of most of the anticancer drugs. The cytotoxic agents destroy bone marrow cells and leads to the reduced synthesis of all the three type of blood cell. Hence bone marrow damage is characterized by the alteration in a hematological parameter which ultimately causes aplastic anemia, thrombocytopenia, leucopenia and agranulocytosis. The prolongation of bleeding and clotting time is the secondary complication of the thrombocytopenia [26,27]. In the present study, cisplatin a widely used cytotoxic was given to albino wistar rats to induce myelosuppression and hematological parameters like RBC, WBC, thrombocytes, hemoglobin, hematocrit, lymphocytes, neutrophils, basophils and eosinophils were determined and also bleeding and clotting time were recorded. The bone marrow sample was studied for histopathological changes, lipid peroxidation and antioxidant enzymes.

The animals of toxic control group have shown significant reduction of hematological parameters. The bleeding time and clotting time were prolonged as consequences of decreased platelet count. But the administration of methanol extract of *Emblica officinalis* at medium and high dose could significantly increases above hematological parameters in therapeutic groups. The reduction of bleeding and clotting time were observed as platelets number was increased. The effect maybe associated with protective effect of *Emblica officinalis* extract against cisplatin damage on bone marrow toxicity.

The present study also revealed the potentials of methanol extract to increase concentrations of antioxidant enzymes in therapeutic indicates its ability to counter the free radical injury to bone marrow [Ellman et al 1961]. The histopathological examination of bone marrow samples exhibited significant regeneration of bone marrow cells in rats treated with methanol extract. The cisplatin and its metabolites are reactive free radicals and can cause free radical mediated destruction of normal cell and causes bone marrow toxicity [Curtis et al 1991]. The agents such as antioxidants which inactivate free radicals can be suitable approach to manage the chemotherapy induced complications [Tripathi et al 2008]. In the present investigation, administration of cisplatin in toxic control animals could damage the bone marrow cell may be due to oxidative damage and caused significant alteration in the hematological parameters and

structural damage to bone marrow samples. Administration of methanol extract of *Emblica officinalis*. could normalize the hematological parameters and could regenerated bone marrow cells which was evidenced by the reduction in lipid peroxidation and increase in antioxidant enzymes. The previous studies performed on the extract of *Emblica officinalis* have been reported for its *in vitro* and hence antioxidant potential of plant which may be the reason for its protective activity in the present study. Hence further investigation can be design to understand mechanism of action of methanol extract of *Emblica officinalis* and also to isolate phytoconstituents and to evaluate its beneficial effects.

Figure No. 10: Histopathology of Bone marrow samples in evaluation of myleoprotective property of MEEO



4.0 CONCLUSION

In the present study, administration of methanol extract of *Emblica officinalis* significantly increased the amount of antioxidant enzymes and regenerated cells of bone marrow which was confirmed by histopathological examination. Hence there was improvement in hematological parameters and increased bleeding and clotting time. Hence, the results of the current research study propose that the methanol extract of *Emblica officinalis* of have substantial protective

assets against bone marrow toxicity induced by cisplatin in albino wistar rats. Additional study should be undertaken to determine the mechanism of action of extract for its protective property.

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Declaration of competing interest. We are by declare that there is no conflicts interest.

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