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PROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *AEGLE MARMELOS* FRUIT IN CYCLOPHOSPHAMIDE INDUCED TOXICITY IN RATS

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Keywords:

Aegle marmelos, Cyclophosphamide, Protective effect, Toxicity, Neutrophil adhesion assay

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ABSTRACT: *Aegle marmelos* commonly known as Bael has been widely used traditionally due to its various medicinal properties. Cyclophosphamide is an alkylating agent used in the treatment of various types of cancer by slowing or stopping the growth of cancer cells but also affecting the normal cells leading to immunosuppressant effect. The present study has been aimed to see the protective effect of hydroalcoholic extract of *Aegle marmelos* fruit (AME) in normal and immunosuppressed rats. Different doses of AME *i.e.* 100, 250 and 500 mg/kg were administered orally for 14 days. On 15th day cyclophosphamide was given at a dose of 180 mg/kg subcutaneously. On the 18th day, hematological parameters like red blood cells (RBC), white blood cells (WBC), platelets, and neutrophil adhesion assay were carried out. AME showed a significant increase in white blood cell count, neutrophil count, eosinophil count, lymphocytes count and Neutrophil adhesion assay in cyclophosphamide treated rats. The results indicate the potential protective effect of AME in cyclophosphamide induced bone marrow suppression in rats.

INTRODUCTION: The Immune system works as a host defense system that protects the body against various infections and diseases. It detects a wide variety of agents, *i.e.* Pathogens, from viruses to parasitic worms, recognizes them, and produces antibodies as a response. The immune system is responsible for the immune response, which recognizes infected or damaged cells and destroys them and secrets pro-inflammatory mediators such as interleukins (ILs) and interferon (INFs) [1]. Cyclophosphamide (cytophosphane) belonging to nitrogen mustard family is widely used in chemotherapy for treating various types of cancer such as lymphoma, multiple myeloma, neuroblastoma, leukemia.

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It also works as an immunosuppressive agent. However, it had been widely used in chemotherapy but also has various side effects such as vomiting, hair loss, allergic reactions and suppressed immunity. This cytotoxic agent suppresses the immunity by activating T-cells or inhibiting activation of helper cells [2]. *Aegle marmelos*, a traditional plant from the Rutaceae family is known to treat a variety of disorders. It is a perennial tree, wild in the sub-Himalayans tract, central and South India. The dried pulp of the fruit is honey-colored, slightly acrid but has an agreeable taste, faintly aromatic, very sticky or glutinous in nature [3]. The fruit of *Aegle marmelos* is reported to contain many compounds such as Coumarins *viz*., marmesin, imperatorin, alloimperatorin, *etc*.; Alkaloids, polysaccharides yielding galactose, arabinose, uronic acid and L-rhamnose on hydrolysis. It contains many vitamins like vitamin C, vitamin A, thiamine, riboflavin, niacin and minerals like calcium and phosphorus [4]. Therefore, the chemical profile indicates *Aegle marmelos* as a good source of the protective agent.

It has reported activities such as antifungal, antidiarrheal, antiulcer, hypoglycemic, antipyretic. Thus, this study was carried out to evaluate the protective activity of hydroalcoholic extract of *Aegle marmelos* fruit in cyclophosphamide induced toxicity in rats.

MATERIALS AND METHODS:

Experimental Animals: Laboratory bred Wistar albino male rats (300-350 g) were housed at 25 °C ± 5 °C in the well-ventilated animal house under 12/12 h light/dark cycle. The animals were given food pellets; bedding material was removed and replaced with fresh paddy husk as often as necessary to keep the animals clean and dry. The animals were kept under standard conditions in animal house.

All studies were performed according to the guidelines on the regulation of scientific experiments as approved by CPCSEA (Reg no: 921/PO/ ReBi/S/05/ CPCSEA) as per Protocol no: PIPH 05/18 and have been verified from the records by IAEC. The animals were kept for quarantine (15 days) prior to the experiment.

Procurement of Plant Material and Extraction: Unripe fruit of *Aegle marmelos* was collected from local areas of Vadodara. The fruit was authenticated by Mrs. Usharani S. Suvarna, Associate professor and HOD of Botany, MGM College, Udupi. The specimen (no: PP 622) was submitted to the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka.

The fruit was cut into pieces and was shade dried for 5 days; it was then made into coarse powder by using mechanical grinder and was sieved to obtain fine powder. It was stored in well-sealed polythene bags.

Extraction: 200 g powder of dried unripe fruit of *Aegle marmelos* was soaked in 1000 ml of water and allowed to macerate for 3 days; it was then filtered using Whatman's filter paper no. 1. The extract obtained was stored in well-closed glass container, the residue was dried and soaked into 400 ml of Ethanol and allowed to macerate for 3 days, it was then filtered using Whatman's filter paper no. 1. The extract was stored into a well-closed glass container.

Both, aqueous and alcoholic extract were then subjected to evaporation using a water bath (60-70 °C) for 6-8 h to obtain a semi-solid mass which was then dried and crushed using mortar pestle to obtain a fine powder, it was sieved using sieve 80# size and was stored in airtight glass vials separately. The dried powder was mixed in 70:30 (aqueous: alcoholic) proportion.

Chemicals: Nylon fibers were obtained from GUJCON-PRF (GSFC, Kosamba, Surat). Cyclo-phosphamide (Endoxan-N 500 mg) was obtained from Zydus oncosciences, Cadila healthcare limited, Ahmedabad. Levamisole (Vitilex-150) was obtained from Glowderma lab Pvt., Ltd., Mumbai.

Phytochemical Tests: [5] The freshly prepared hydroalcoholic extract of *Aegle marmelos* fruitwas subjected to phytochemical screening tests for various constituents. The procedures are as follows

Test for Saponins:

Foam Test: 2 ml of extract was taken and a small amount of water was added, shake well, stable froth (foam) is formed.

Test for Tannins:

Ferric Chloride Test: A small amount of test solution treat with a ferric chloride solution; blue color appears if hydrolyzable tannins are present and green color appears if condensed tannins are present.

Test for Amino Acids:

Millon's Test: To the test, the solution adds 2 ml of millions reagent, white precipitate indicates the presence of amino acid.

Ninhydrin Test: To the test solution add ninhydrin solution, boil; violet color indicates the presence of amino acid.

Test for Proteins:

Biuret Test: To the test solution (2 ml) add Biuret reagent (2 ml); violet color indicates the presence of proteins.

Xanthoproteic Test: To the 5 ml of a test solution, add 1 ml of concentrated nitric acid and boil, a yellow precipitate is formed. After cooling it, add 40% sodium hydroxide solution, the orange color is formed.

Glycosides:

Keller Killiani Test: The test consists of boiling about 1 gm finely powered sample with 10 ml 70% alcohol for 2 to 3 min. The extract is filtered. To the filtrate is added, 5 ml water and 0.5 ml strong solution of lead acetate. Shake well and separate the filtrate. The clear filtrate is treated with an equal volume of chloroform and evaporated to yield the extractive. The extractive is dissolved in glacial acetic acid and after cooling, 2 drops ferric chloride solution is added to it. These contents are transferred to a test tube containing 2 ml concentrated sulphuric acid. A reddish-brown layer turns bluish-green color after standing.

Legal Test (Cardiac Glycosides): The extract is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline-pink or red color is produced.

Modified Borntrager's Test: To the extract adds Ferric Chloride solution and immerse the mixture in boiling water for about 5 min; after cooling the mixture was extracted with equal volume of benzene. The separated benzene layer was treated with ammonia solution. Ammonical layer turns rose-pink color, which indicates presence of anthranol glycosides.

Test for Alkaloids:

Dragendroff's Test: A few drops of dragendroff's reagent were added to 2-3 ml of filtrate. An orange-brown precipitate is formed.

Mayer's Test: 2-3 ml filtrate with few drops of Mayer's reagent gives a precipitate.

Hager's Test: The 2-3 ml filtrate, add few drops of Hager's reagent, gives a yellow precipitate.

Test for Carbohydrates:

Molisch's Test: It consists of treating the compounds with alpha-naphthol and concentrated sulphuric acid which gives a purple color ring at the junctions of two-layer.

Fehling's Test: To the solution of the sample, an equal quantity of Fehling's solutions A and B is added. After heating, a brick red precipitate is obtained.

Test for Flavonoids:

Shinoda Test: To dry powder or extract, add 5 ml 95% ethanol, few drops concentrated HCl and 0.5 gm magnesium turnings. The pink color observed.

Test for Steroids: 1 ml extract was dissolved in 10 ml of chloroform & equal volume of conc. sulphuric acid was added from the side of test tube to prevent mixing. The upper layer of the solution turns red while Sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of Steroid.

Experimental Design: The albino rats were randomized into six groups (n = 4). First and second group were treated with saline, second group was induced toxicity by cyclophosphamide administration, other three groups (AME group) were treated with the hydroalcoholic extract of *Aegle marmelos* at a dose of 100 mg/kg, 250 mg/kg, 500 mg/kg respectively and sixth group was treated with Levamisole 50 mg/kg. The animals were treated for 14 days *via* oral intra-gastric tube, and on the 15th day, all groups except the normal control were treated with Cyclophosphamide 180 mg/kg s.c to induce toxicity.

TABLE 1: EXPERIMENTAL STUDY PLAN

Study plan						
Group	**I(N=4)**	**II(N=4)**	**III(N=4)**	**IV(N=4)**	**V(N=4)**	**VI(N=4)**
Drug and Dose for day 1 to 14	Saline (1 ml, p.o)	Saline (1 ml, p.o)	AME (100 mg/kg, p.o)	AME (250 mg/kg, p.o)	AME (500 mg/kg, p.o)	Levamisole (50 mg/kg, p.o)
CYP treatment	-	180 mg/kg s.c. on 15th day				
Statistical analysis	ANOVA followed by the Dunnett's post-test					

Hematological Parameters: The blood samples from the control and treated rats were obtained from retro-orbital plexus on the 18th day of the experiment. Rats were lightly anesthetized using ether for this purpose. The blood was collected in EDTA vacutainer tubes.

The blood samples were analyzed for various hematological parameters (Hb, RBC, WBC, Platelets) in Medonic hematological autoanalyzer; all tests were done on Medonic three-part cell counter in Parul sevashram hospital central laboratory.

Neutrophil Adhesion Test: [6] The collected blood samples were analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg nylon fibers/ml for 15 min at 37 ºC. The incubated blood samples were again analyzed for TLC and DLC, respectively to give a neutrophil index of blood samples. The percent of neutrophil adhesion was calculated as follows:

$$\text{Neutrophil adhesion (\%)} = NI_U - NI_T \times 100 / NI_U$$

Where NI_U is the neutrophil index of untreated blood samples and NI_T is the neutrophil index of treated blood samples.

Physical Observation: The animals were physically observed every day for their general health status and presence of specific toxicity like hemorrhagic cystitis or bleeding from other parts of the body. The animals were also observed for their mortality.

Statistical Analysis: The statistical significance was assessed using a one-way analysis of variance (ANOVA) followed by Dunnett's post-test in GraphPad Prism (version 8.0). The values were expressed as Mean ± SEM and $P < 0.05$ was considered significant.

RESULTS:

Phytochemical Analysis of Fruit Extract: The hydroalcoholic extract of *Aegle marmelos* fruit showed the presence of alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, glycosides, proteins, phenols, saponin, tannins. Due to the presence of many bioactive molecules in the extract, it has a medicinal property to cure many ailments.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF HYDRO-ALCOHOLIC FRUIT EXTRACT OF *AEGLE MARMELOS*

S. no.	Phytochemicals	Presence (±) or Absence (-)
1	Alkaloids	±
2	Amino acids	±
3	Carbohydrates	±
4	Cardiac glycosides	±
5	Flavonoids	±
6	Glycosides	±
7	Proteins	±
8	Saponin	±
9	Steroids	-
10	Tannins	±

Effect on Body Weight of Animals:

TABLE 3: % BODY WEIGHT DEVIATION

S. no.	NC	DC	AME-1	AME-2	AME-3	LM
1	-2.565 ± 3.766	-3.545 ± 0.5911	6.502 ± 1.208*	10.21 ± 2.632**	-0.3914 ± 3.716	3.179 ± 0.4508

Values are expressed as Mean ± SEM, N = 4, Analyzed by One-way ANOVA followed by Dunnett's Test *Values differ significantly from disease Control (DC) group *($p< 0.1$), ** ($p<0.01$). NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50mg/kg bw.

There was a significant difference ($p < 0.1$) in ± % body weight deviation of cyclophosphamide-treated AME-1 and AME-2 group when compared to the control group. However, there was increase in body weight of AME-3 and Levamisole treated group but was not significant.

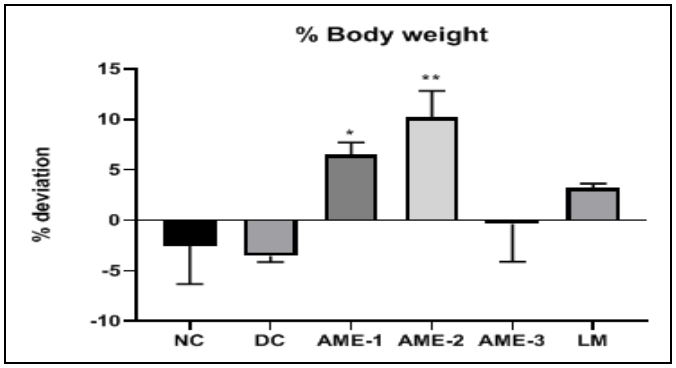

FIG. 1: % BODY WEIGHT DEVIATION

Values are expressed as Mean ± SEM, n = 4, Analyzed by One-way ANOVA followed by Dunnett's Test *Values differ significantly from disease Control (DC) group *($p< 0.1$), ** ($p<0.01$). NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

Effect on Hematological Parameters:

TABLE 4: EFFECT OF EXTRACT ON HEMATOLOGICAL PARAMETERS

Parameters	NC	DC	AME-1 (100 mg/kg)	AME-2 (250 mg/kg)	AME-3 (500 mg/kg)	LM (50 mg/kg)
RBC	9.863 ± 0.492	9.185 ± 0.247	9.09 ± 0.274	8.995 ± 0.335	9.958 ± 0.705	8.603 ± 0.493
WBC	6850 ± 427.2	2383 ± 641.5$^{\$\$\$}$	3650 ± 695.8	3825 ± 476.8	2575 ± 515.4	4275 ± 587.9
Neutrophils	3030 ± 707.3	1280 ± 444.9	1921 ± 403.6	2468 ± 325.6	1561 ± 373.6	2579 ± 347.5
Eosinophils	137 ± 8.544	52.25 ± 12.47$^{\$\$}$	96.75 ± 14.27	172 ± 23.82****	51.50 ± 10.31	85.50 ± 11.76
Lymphocytes	3424 ± 966.8	957 ± 442$^{\$}$	1474 ± 380	1059 ± 194.7	875.3 ± 249.7	1440 ± 329.2
Platelets	588250 ± 38530	244250 ± 57433$^{\$\$\$\$}$	321000 ± 13699	392750 ± 29389*	361250 ± 22035	391000 ± 33801*

Values are expressed as Mean ± SEM, N= 4, Analyzed by One way ANOVA followed by Dunnett's Test. $^{\$}$ Values differ significantly from NC ($p< 0.05$), $^{\$\$}$($p< 0.01$), $^{\$\$\$}$($p< 0.001$), $^{\$\$\$\$}$ ($p< 0.0001$).* Values differ significantly from disease Control (DC) group ****($p< 0.0001$), * ($p< 0.05$), NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw. RBC (millions/mm^3): Red blood cells, WBC (per mm^3): White blood cells, Lymphocytes (per mm^3), Platelets (per mm^3).

There was a significant difference ($p < 0.0001$) in Platelets of cyclophosphamide-treated AME-2 group when compared to control group. There is a marked increase in WBC counts in AME treated group **Fig. 2**. Also, there is a difference in the Neutrophil & Lymphocytes count between the AME treated group and disease control group. There was no significant difference in the RBC count of cyclophosphamide treated group when compared to the disease control group.

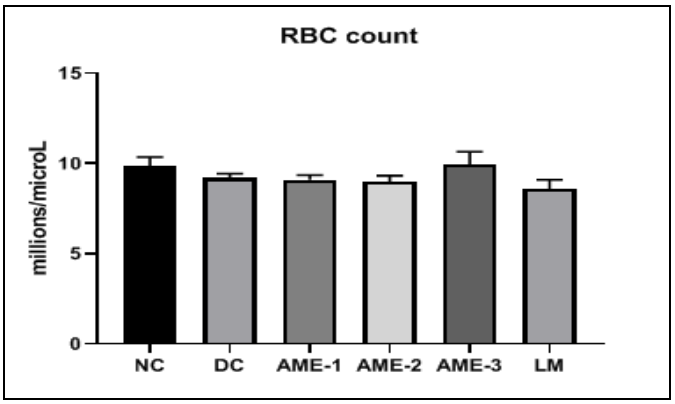

FIG. 2: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON RBC COUNT

Values are expressed as Mean ± SEM, N = 4, Analyzed by One way ANOVA followed by Dunnett's Test NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

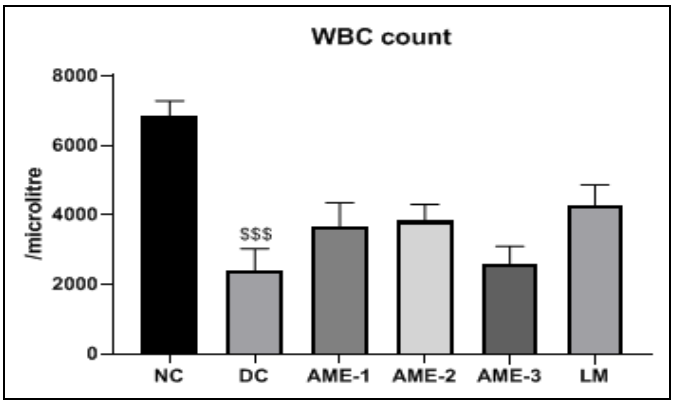

FIG. 3: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON WBC COUNT

Values are expressed as Mean ± SEM, N= 4, Analyzed by One way ANOVA followed by Dunnett's Test. $^{\$}$ Values differ significantly from NC ($p< 0.05$), $^{\$\$\$}$($p< 0.001$), NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

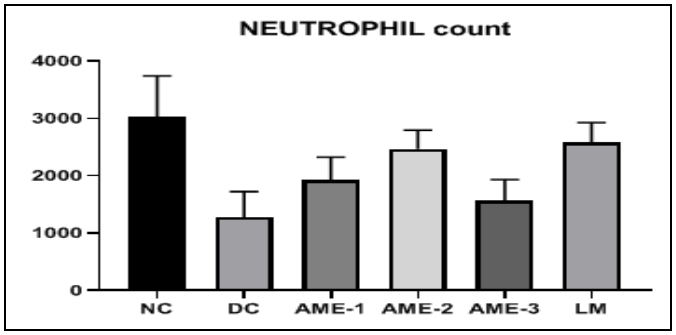

FIG. 4: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON NEUTROPHIL COUNT

Values are expressed as Mean ± SEM, N= 4, Analyzed by One-way ANOVA followed by Dunnett's Test NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

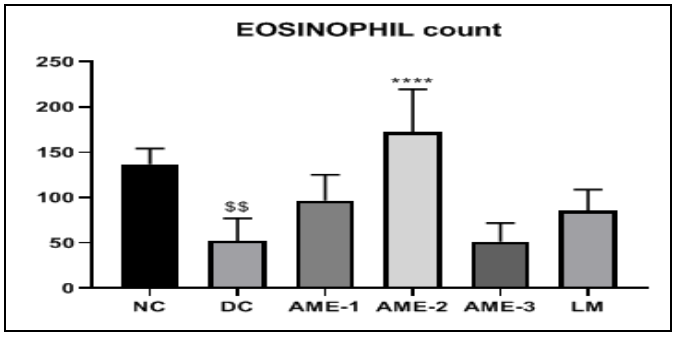

FIG. 5: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON EOSINOPHIL COUNT

Values are expressed as Mean ± SEM, N= 4, Analyzed by One-way ANOVA followed by Dunnett's Test. $ Values differ significantly from NC ($p<0.05$), $$ ($p< 0.01$). * Values differ significantly from disease Control (DC) group **** ($p< 0.0001$). NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

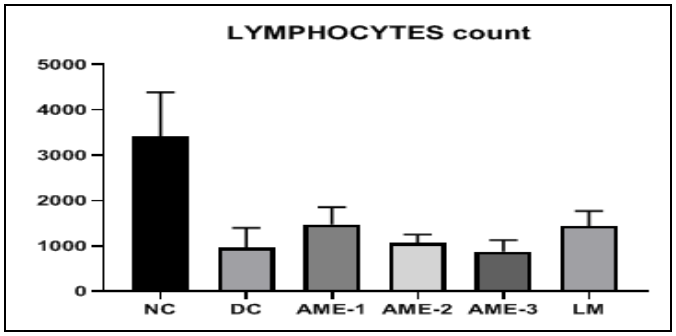

FIG. 6: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON LYMPHOCYTE COUNT

Values are expressed as Mean ± SEM, N= 4, Analyzed by One way ANOVA followed by Dunnett's Test NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

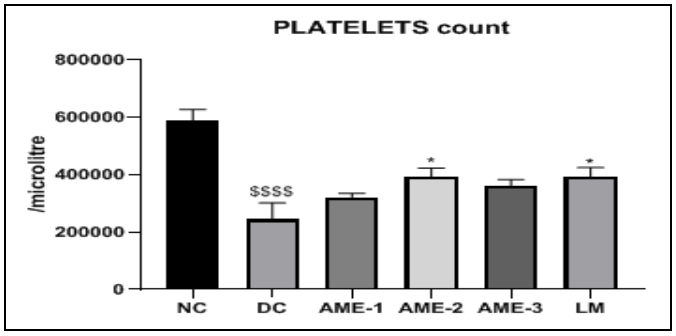

FIG. 7: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON PLATELETS COUNT

Values are expressed as Mean ± SEM, N= 4, Analyzed by One-way ANOVA followed by Dunnett's Test. $ Values differ significantly from NC ($p< 0.05$), $$$$ ($p< 0.0001$). * Values differ significantly from disease Control (DC) group * ($p< 0.05$), NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

Neutrophil Adhesion Assay: Incubation of blood with nylon fibers resulted in a decrease in neutrophil counts due to adhesion of neutrophils to the fibers. The comparison of Neutrophil adhesion (%) is given in **Table 5**.

TABLE 5: EFFECT OF EXTRACT ON NEUTROPHIL ADHESION PERCENT

Parameters	NC	DC	AME-1 (100mg/kg)	AME-2 (250mg/kg)	AME-3 (500mg/kg)	LM (50mg/kg)
Neutrophil adhesion assay (%)	11.94 ± 7.486	5.883 ± 2.796	27 ± 8.454	26.61 ± 5.859	29.25 ± 12.95	18.51 ± 9.746

Values are expressed as Mean ± SEM, one-way ANOVA followed by Dunnett's Test, Treated groups are compared with the control group.NC= Normal control, DC= Disease control, AME= *Aegle marmelos* extract, LM= Levamisole.

There was a marked difference in neutrophil adhesion % when compared to cyclophosphamide treated AME group and controlled group.

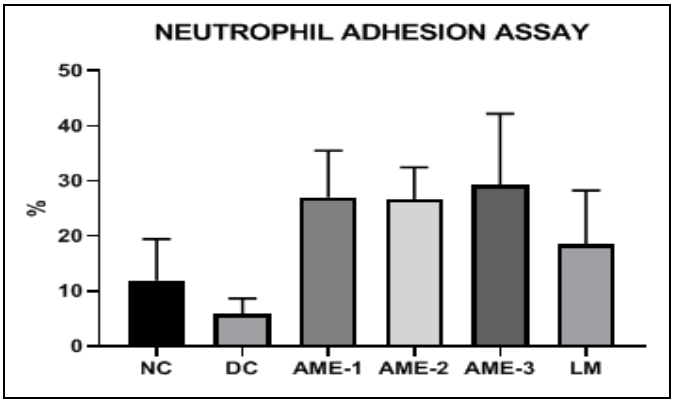

FIG. 8: EFFECT OF *AEGLE MARMELOS* FRUIT EXTRACT ON NEUTROPHIL ADHESION (%)

Values are expressed as Mean ± SEM, N= 4, Analyzed by One way ANOVA followed by Dunnett's Test NC: Normal control, DC: Disease control, AME-1: *Aegle marmelos* extract treatment at 100 mg/kg bw, AME-2: *Aegle marmelos* extract treatment at 250 mg/kg bw, AME-3: *Aegle marmelos* extract treatment at 500 mg/kg bw, LM: Levamisole treatment at 50 mg/kg bw.

As seen above there is a decrease in neutrophil adhesion in the disease-controlled group because of a decrease in neutrophil count due to the administration of cyclophosphamide, as cyclophosphamide suppress the bone marrow which is responsible for the production of various blood cells thus leading to decrease in neutrophil count. Administration of *Aegle marmelos* in cyclophosphamide treated rats produced a marked increase in adhesion of neutrophils.

Physical Observation: There was an increase in body weight of AME and Levamisole treated group during the treatment period while no significant difference in body weight of normal and disease control group. After cyclophosphamide administration, the animals from the disease control group showed bleeding from eyes and nose, bloody diarrhea, hair loss, and significant body weight loss. The animals from the disease control group died after 4 days of cyclophosphamide administration.

FIG. 9: BLEEDING FROM EYES OBSERVED IN CYCLOPHOSPHAMIDE TREATED DISEASE CONTROL GROUP

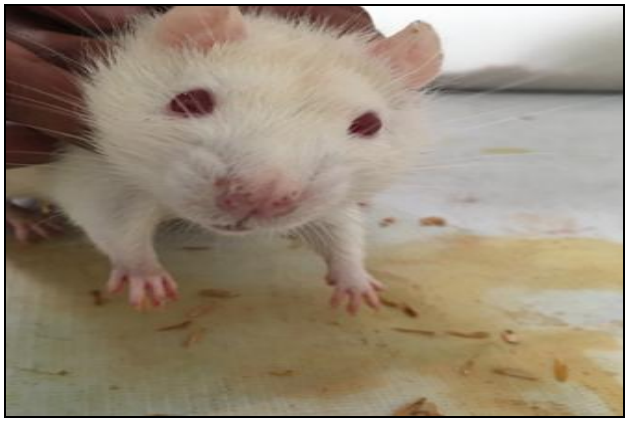

FIG. 10: BLEEDING FROM NOSE OBSERVED IN CYCLOPHOSPHAMIDE TREATED DISEASE CONTROL GROUP

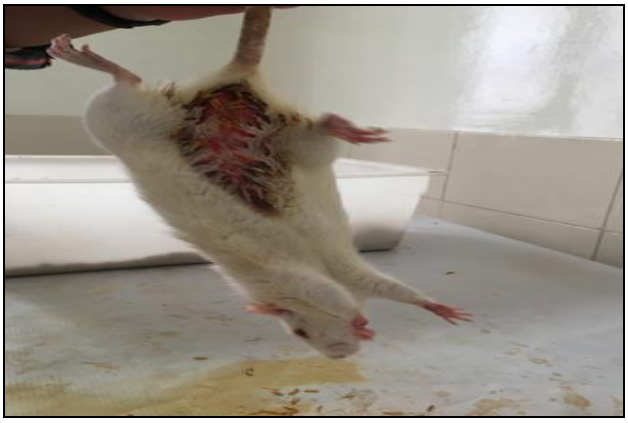

FIG. 11: BLOOD IN URINE OBSERVED IN CYCLOPHOSPHAMIDE TREATED DISEASE CONTROL GROUP

FIG. 12: HAIR LOSS OBSERVED IN CYCLOPHOSPHAMIDE TREATED DISEASE CONTROL GROUP

FIG. 13: DEATH ON THE 3rd DAY OF CYCLOPHOSPHAMIDE TREATMENT IN DISEASE CONTROL GROUP

AME and Levamisole treated groups were not significantly affected after cyclophosphamide administration however there was a slight reduction in body weight. One rat from AME-2 and AME-3 group died on the 6th day after cyclophosphamide administration. There was no mortality from AME-1 and Levamisole treated group.

DISCUSSION: Cyclophosphamide- an anti-cancer drug widely used in the treatment of multiple lymphoma, leukemia and various other cancers. However, there are many side effects associated with it like thrombocytopenia, leukopenia, bone marrow depression. Hemorrhagic cystitis is a major drawback associated with cyclo-phosphamide. Various protective drugs are being studied to overcome the side effects of cytotoxic drugs. There was a decrease in body weight of disease control group after cyclophosphamide administration. However, there was significant increase in body weight of AME-1 and AME-2 groups when compared to control group.

Cyclophosphamide administration in animals' decreased WBC count in the disease control group while in AME treated groups, the extract showed protective action against toxicity, which is confirmed by an increase in WBC count in AME groups compared to the disease control group. Other blood parameters like Platelets, Eosinophils, monocytes, neutrophils, and lymphocytes were also increased by the *Aegle Marmelos* extract administration which concludes to the protective activity of the extract.

There was an increase in neutrophil adhesion percentage in AME treated groups when blood was incubated with nylon fibers. Neutrophils are circulating in the vascular system of the body in a passive state and become more adhesive upon stimulation at sites of inflammation which is followed by phagocytosis. When the pathogens invade the body, macrophages detect the pathogen and release neutrophils in order to eliminate the pathogens from the body.

The increased neutrophils in AME treated group may be due to the upregulation of β2 integrins which helps in adhesion of neutrophils to nylon fibers. Thus, it confirms the stimulation of neutrophils towards the site of inflammation by fruit extract of *Aegle Marmelos.*

CONCLUSION: *Aegle marmelos* fruit extract contains alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, glycosides, proteins, phenols, saponin. These constituents may be responsible for its protective effect against cyclophosphamide toxicity. The exact mechanism of action is not known, however further studies may be carried out using a greater number of animals to get more data. The results of the present study done on animals confirm the Protective effect of hydroalcoholic extract of *Aegle marmelos* fruit in cyclophosphamide induced toxicity in rats.

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