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Research Article

Phytochemical Profiling (GC-MS) and Hematological modulating effects of Ethanol Leave Extract of *Eucalyptus Camaldulensis in* Wistar rats

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Abstract

Background: The extract of the leaves of *E. camaldulensis* has been used for centuries in traditional medicine, its leaves and essential oils have found various application in everyday life due to their antiseptic, anti-inflammatory and antipyretic properties.
Aim: This study evaluates phytochemical profiling (GC-MS) and the effect of ethanol leaf extract of *E. camaldulensis* on some haematology parameters (white blood cell, red blood cell, MCV, haemoglobin, packed cell volume, mean cell hemoglobin concentration, mean corpuscular hemoglobin, red blood cell distribution, platelets) of wistar rats.
Methods: Forty-two wistar rats weighing 100g-280g were randomly divided into six groups (A-F), with seven rats per group. Normal control (group A) was administered (orally) distilled water, DMSO and feed, group B to F was administered (orally) 150mg/kg body weight, 250mg/kg body weight, 350mg/kg body weight, 450mg/kg body weight and 550mg/kg body weight respectively. Extraction of the plant was done using 90% ethanol and soxhlet apparatus. All biochemical analysis were carried out using standard laboratory techniques. This experiment lasted for 21 days.
Results: The findings demonstrated that the *E. camaldulensis* extract significantly altered the packed cell volume (PCV) of the administered groups in comparison to the normal control group at a significance level of ($P < 0.05$). In contrast to the control group, the administered group's white blood cell count (WBC) did not significantly rise ($P < 0.05$). Phytochemical screening revealed the presence of alkaloids,saponins,terpenoids, catecholic tanninsn, carbohyrades, glycosides, fats and oils in *E. camaldulensis* leaf extract. The plant extract's GC-MS examination revealed that it contains compounds like 1H-Cycloprop[e]azulene, decahydro, 1,2-Benzenedicarboxylicacid, mono, 9-Octadecenoicacid, methylester, 7-Hexadecenal,(Z)-, 1,2-Benzenedicarboxylicacid, 2-eth etc.
Conclusion: *E. camaldulensis* includes phytochemicals that may be utilised as pharmacological probes, and it also increases blood volume and may be used to treat anaemia in patients.

1. Introduction

Medicinal plants are those that have therapeutic effects or provide beneficial pharmacological impacts on the human or animal body when used [1]. Medicinal plants have always been crucial sources for drug lead compounds. Medicinal plants are utilized as crude drugs, which

include dried parts like roots, stems, wood, bark, leaves, flowers, fruits, seeds, extracts, or the entire plant. Only a small number of these plants have their active components isolated and used directly as medicines, either alone or in combination with other substances[1]. The World Health Organization states that over 30% of modern medicines are sourced directly or indirectly from medicinal plants. Examples include analgesics like aspirin, anticancer drugs such as vincristine, anti-hypertensive agents like reserpine, antimalarials such as quinine and artemisinin, and decongestants like ephedrine.Approximately 80% of the global population, particularly in Africa and other developing regions, continues to rely exclusively on traditional medicine for disease treatment [1] points out that in many regions of Nigeria and Africa, poverty and underdevelopment drive the predominant use of medicinal plants. Phytochemicals are plant-derived chemicals produced by plants through primary or secondary metabolic processes [2]. Phytochemicals, the compounds present in plants, come in various types, but the majority belong to four main biochemical categories: terpenes, alkaloids, glycosides, and polyphenols. These phytochemicals have the potential to be used as drugs. The known pharmacological activities and contents of these biochemical substances in medicinal plants form the scientific foundation for their applications in modern medicine, once scientifically validated. For example, daffodils (Narcissus) contain nine groups of alkaloids, including galantamine, which is approved for treating Alzheimer's disease. Alkaloids are often bitter-tasting and toxic, and are concentrated in parts of the plant, such as the stem, which are most likely to be eaten by herbivores; they can also provide protection against parasite [3]. These plants are preferred because they are readily available, affordable, and often as effective as expensive synthetic drugs. However, they are frequently used indiscriminately without proper clinical and laboratory diagnosis, monitoring of treatments, relapses, or potential toxicity. Despite the efficacy of medicinal plants the most frequent reason given by physicians for not accepting the use of medicinal plants is that they see such therapies as lacking rigorous scientific support and also limited inventories and documentation therefore difficult to know how they function which this research study is aimed at solving. Traditional medicines is based on the premise that medicinal plants contain natural substances that can promote health and alleviate illnessone of such plant is to be used in this research study, *Eucalyptus camaldulensis* [4] states that *Eucalyptus camaldulensis*, commonly known as the river red gum, is a species of flowering plant in the Myrtaceae family. This family comprises 140 genera and approximately 3800 species found in tropical and subtropical regions worldwide. The genus name originates from two Greek words: 'Eu' meaning 'well' and 'Kalyptos' meaning 'covered,' referring to the operculum that protects the flower before it opens. *Eucalyptus camaldulensis* has seven subspecies and typically grows to a height of 20 meters, though it can reach up to 45 meters. *Eucalyptus camaldulensis* is highly adaptable and can tolerate extreme conditions like drought and soil salinity, demonstrating rapid growth. These traits contribute to its potential to become invasive. Phytochemical studies reveal that extracts of *Eucalyptus camaldulensis* are rich in compounds such as flavonoids, phenol's, Saponins, terpenes, and tannin's[5]. In rural communities of Northern Nigeria, *Eucalyptus camaldulensis* plantations have boosted the local fuel wood supply, reducing the pressure on natural forests. Consequently, eucalyptus is recognized as the most suitable exotic tree species for afforestation in Northern Nigeria[6]. In Australia *E. camaldulensis* has been used for centuries, its leaves and essential oils have found various application in everyday life due to their antiseptic, anti-inflammatory and antipyretic properties. The Aborigines used *E. camaldulensis* medicinally to treat gastrointestinal issues (such as colic, diarrhea, and dysentery), respiratory diseases (including colds, coughs, asthma, laryngitis, pharyngitis, and sore throat), to stop bleeding, and to heal open wounds and cuts. They also used its decoctions to relieve muscle spasms, aches, and pains, as well as joint and tooth pain. Due to its effectiveness in treating various health conditions, *E. camaldulensis* and its traditional remedies spread to other parts of the world, including Africa. In Nigeria, it was used to prevent tooth decay and periodontitis by making teeth cleaning sticks from its tree, and its leaves containing eucalyptus oil were used in traditional medicine to heal wound infections [7].

In developing countries such as Nigeria there exists a continuous increase in the use of medicinal plants for therapeutic effects in the treatment of diseases, wounds, ailments etc. Most of these individuals lack adequate knowledge on the therapeutic and side effects of these medicinal plants hence this warrants an adequate investigation to confirm their suitability for the purpose they are used for which this study is designed to proffer solution to. This study is aimed at scientifically investigating and evaluating the effect of ethanol leaf extract of *Eucalyptus camaldulensison* hematology parameters of wistar rats.

2. Materials and Method

2.1. Materials

Albino wistar rats, weighing balance, feed, distilled water, rubber cages, water bath, chloroform, filter paper, dissecting kit, refrigerator, syringes, pair of scissors and forceps, blood sample, Ethanol, EDTA tube, distilled water, sieve cloth, conical flask, beakers, funnel, cotton wool, spatula, hand gloves, face masks and 3-part differential analyzer.

2.2. Plant Sample Collection

Fresh *Eucalyptus camaldulensis* leaves were obtained from Veritas university compound between the month of October to December.

2.3. Preparation of Plant Extract

The plant leaves were identified and authenticated in the Department of Biotechnology, Veritas University Abuja. The leaves of *Eucalyptus camaldulensis* collected were washed thrice, made free from sand and other impurities and air-dried under room temperature for one month. The fresh air-dried leaves were powdered in an electric kitchen blender. Using a weighing balance 641grams of the powdered leaves were soaked in 200ml of 90% ethanol in a storage container for 72 hours with daily vigorous shaking to facilitate the extraction process. After 72 hours the mixture were filtered using a cheese cloth, Whatman filter papers and a separating funnel, and were evaporated to dryness on a rotary evaporator and then the concentrate were dried in a water bath at a temperature of about 50°C for a period of two days until all the ethanol present in the sample has evaporated. The extract were collected in a beaker covered with an aluminium foil.

2.4. Experimental Design

Forty-two albino wistar rats weighing 100g-280g were used for the experiment. The thirty adult wistar rats were obtained from an animal farm in kaduna state, Nigeria. The rats were housed in the animal house of Veritas university Abuja to acclimatize for two weeks. While being acclimatized the rats were fed with standard rat feed and water ad libitum, and was maintained at standard laboratory conditions of 12 hours dark periodic alternations. The albino wistar rats were randomly assigned into six groups with seven animals in each group (n=7).

Table 1: Experimental Design

GROUPS	NUMBEROF RATS	GROUP TITLE	TREATMENT ADMINISTERED
GROUP A	7	Normal control (Nc)	Distilled water and feed.
GROUP B	7	Treatment group 1	150mg/kg body weight of Eucalyptus camaldulensis extract (0.2ml)
GROUP C	7	Treatment group 2	250mg/kg body weight of Eucalyptus camaldulensis extract (0.3ml)
GROUP D	7	Treatment group 3	350mg/kg body weight of Eucalyptus camaldulensis extract (0.4ml)
GROUP E	7	Treatment group 4	450mg/kg body weight of Eucalyptus camaldulensis extract (0.5ml)
GROUP F	7	Treatment group 5	550mg/kg body weight of Eucalyptus camaldulensis extract (0.6ml)

2.5. Animal Sacrifice and Collection of Samples for Analysis

All experimental animals were anaesthetized using chloroform fumes the next morning after food withdrawal and last administration of the extract. Blood samples were collected in tubes containing ethylene diamineteraacetic acid (EDTA) for haematological studies. Using an automated haematological analyzer which rely on impedance technology a full blood count was carried out.

2.6. Statsitical Analysis

The results obtained from the study was analyzed by Statistical Package for Social Science (SPSS) windows and will be presented as mean with standard deviation (SD). Differences at p value < 0.05 will be considered statistically significant.

3. Results

Table 2: Phytochemical constituent of *Eucalyptus camaldulensis*

Alkaloids	+
Saponins	+
Terpeniods	+
Catecholic Tannins	+
Carbohydrates	+
Phenols	-
Glycosides	+
Fats and oil	+

Key (+) = Present (-) = Absent

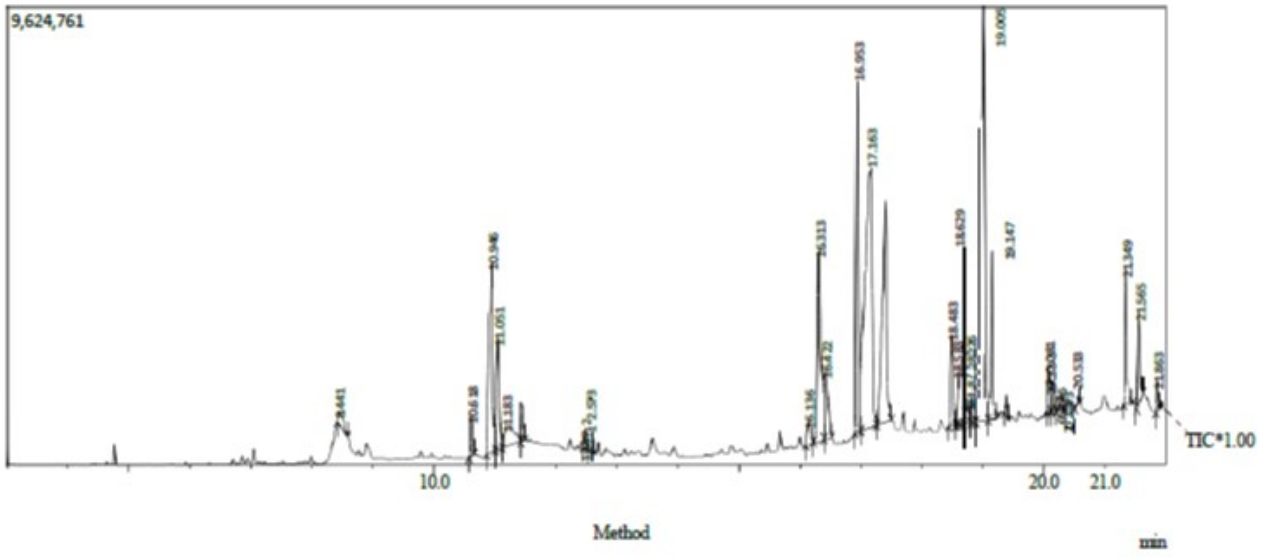

Figure 1: Chromatogram extract analysis of *E. camaldulensis* leaves

Table 3: GCMS analysis of extract of *E. camaldulensis* leaves

Peak#	R.Time	I.Time	F.Time	Area	Peak Report Area% TIC	Height	Height%	A/H	Mark	Name
1	8.441	8.408	8.583	1630165	0.72	275510	0.44	5.92		DiethylPhthalate
2	10.618	10.575	10.658	1537094	0.68	843931	1.36	1.82		1H-Cycloprop[e]azulene,decahydro
3	10.946	10.850	11.000	15365192	6.76	3992008	6.43	3.85		DiethylPhthalate
4	11.051	11.000	11.117	7597958	3.34	2354059	3.79	3.23	V	1,2-Benzenedicarboxylicacid,2-eth
5	11.183	11.117	11.417	5136357	2.26	458035	0.74	11.21	V	Phthalicacid,allylethylester
6	11.443	11.417	11.492	1550665	0.68	797889	1.29	1.94	V	Phenol,2,5-bis(1,1-dimethylethyl)-
7	12.472	12.442	12.517	818970	0.36	422160	0.68	1.94		(-)-Spathulenol
8	12.573	12.525	12.625	2408056	1.06	1159708	1.87	2.08		Globulol
9	16.136	16.083	16.217	2239083	0.99	510908	0.82	4.38		Phthalicacid,butyltetradecylester
10	16.313	16.217	16.392	15296304	6.73	4008339	6.46	3.82	V	1,2-Benzenedicarboxylicacid,bis(2
11	16.422	16.392	16.517	5423980	2.39	1434089	2.31	3.78	V	Phthalicacid,2-cyclohexylethylisob
12	16.953	16.892	16.992	17027944	7.50	7352769	11.84	2.32		Hexadecanoicacid,methylester
13	17.163	16.992	17.250	44117487	19.42	5449075	8.78	8.10	V	1,2-Benzenedicarboxylicacid,mono
14	17.406	17.250	17.492	21038355	9.26	4633947	7.46	4.54	V	n-Hexadecanoicacid
15	18.483	18.433	18.542	4324685	1.90	1896287	3.05	2.28		Behenicalcohol
16	18.583	18.542	18.600	2225889	0.98	1125555	1.81	1.98	V	Methyl5,13-docosadienoate
17	18.629	18.600	18.667	7715385	3.40	3906407	6.29	1.98	V	9-Octadecenoicacid,methylester
18	18.755	18.667	18.792	1969693	0.87	403478	0.65	4.88	V	7-Hexadecenal,(Z)-
19	18.826	18.792	18.867	1170038	0.52	559570	0.90	2.09	V	Methyl14-methyl-eicosanoate
20	19.005	18.867	19.075	41587940	18.31	8687943	13.99	4.79	V	6-Octadecenoicacid,(Z)-Octadecanoic acid
21	19.147	19.075	19.225	8436203	3.71	3506687	5.65	2.41	V	
22	19.379	19.350	19.417	705794	0.31	425969	0.69	1.66		Cholest-22-ene-21-ol,3,5-dehydro-6
23	20.081	20.033	20.108	2229190	0.98	867125	1.40	2.57		i-Propyl11,12-methylene-octadecan
24	20.133	20.108	20.192	1633296	0.72	638137	1.03	2.56	V	3',8,8'-Trimethoxy-3-
										piperidyl-2,2'-b

25	20.276	20.192	20.308	967184	0.43	341787	0.55	2.83	V	1-Heptatriacotanol
26	20.383	20.308	20.500	758695	0.33	246346	0.40	3.08	V	5.beta.,7.beta.H,10.alpha.-Eudesm-1
27	20.533	20.500	20.575	992729	0.44	525311	0.85	1.89		Ethyliso-allocholate
28	21.349	21.300	21.433	6255520	2.75	2814958	4.53	2.22		Phenol,3-pentadecyl-
29	21.565	21.517	21.617	3939182	1.73	1851898	2.98	2.13		Hexadecanoicacid, 2-hydroxy-1-(hy
30	21.863	21.825	21.892	1084950	0.48	595144	0.96	1.82		Di-n-octylphthalate
				227183983	100.00	62085029	100.00			

Table 4: Effects of *Eucalyptus camaldulensis* on Haematology Parameters

	Normal Control	150 mg/Kg	250 mg/Kg	350 mg/Kg	450 mg/Kg	550 mg/Kg
PCV	37.60±1.44	41.20±1.36	38.00±1.22	35.50±1.43	35.25±0.66	39.40±2.40
Haemoglobin	12.20±0.49	13.20±0.37	12.40±0.51	11.75±0.58	11.50±0.22	13.00±0.77
RBC	4.18±0.15	4.56±0.15	4.40±0.32	3.93±0.16	3.90±0.08	4.38±0.28
Neutrophils	52.00±2.24	50.60±5.03	58.60±4.97	52.50±3.71	56.75±4.22	67.60±5.76
WBC	5600.00±636.40	5960.00±743.37	7080.00±907.41*	6250.00±621.69	7000.00±788.04*	9360.00±1369.16*
Lymphocytes	39.20±2.31	40.20±4.47	33.60±4.46	38.00±3.44	34.50±3.67	25.00±4.43
MID	8.20±1.11	9.00±0.55	7.80±1.32	9.50±0.74	8.50±0.74	7.40±1.47
MCV	82.00±1.82	86.20±1.77	82.60±1.86	78.75±1.28	79.50±0.67	84.40±3.08
MCH	23.40±0.81	25.20±0.66	23.60±0.93	22.25±0.66	23.25±0.37	24.80±1.16
MCHC	280.40±5.22	286.40±4.96	281.20±9.49	278.25±5.30	265.00±4.79	294.00±13.66
RDW-CV	12.92±0.54	13.04±0.43	12.20±0.41	11.53±0.28	11.73±0.13	12.66±0.52
PLT	232.60±29.32	246.60±21.39	314.80±14.40	288.75±40.46	334.00±26.22	273.60±17.88

Data are represented as Mean±SEM. n=7.*p<0.05 significantly different from Normal Control.

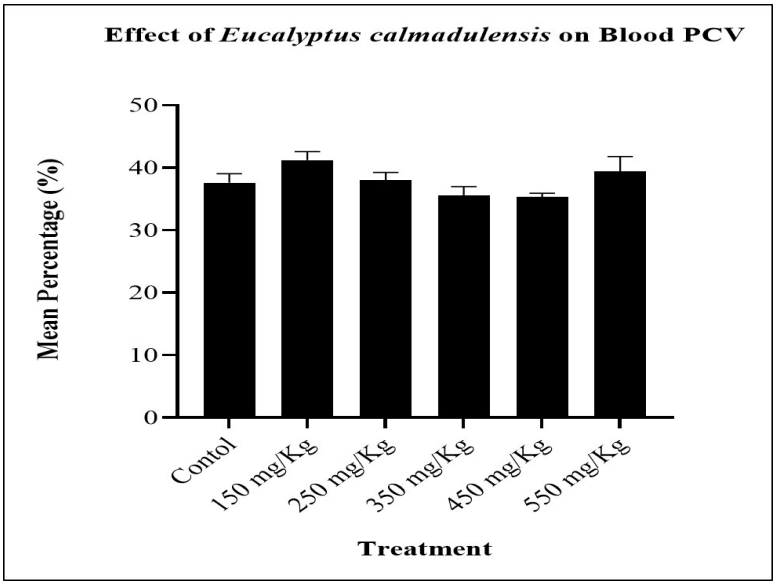

Figure 2: Effect of *Eucalyptus camaldulensis* on packed cell volume

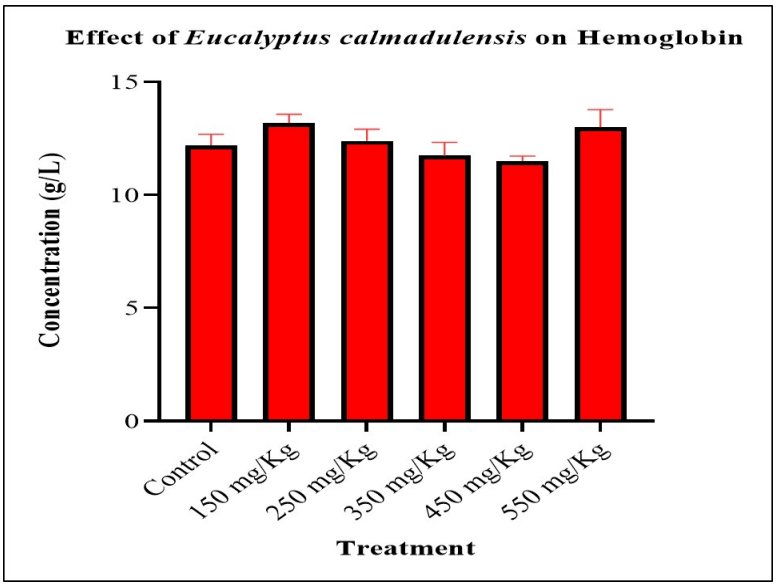

Figure 3: Effect of *Eucalyptus camaldulensis* on haemoglobin

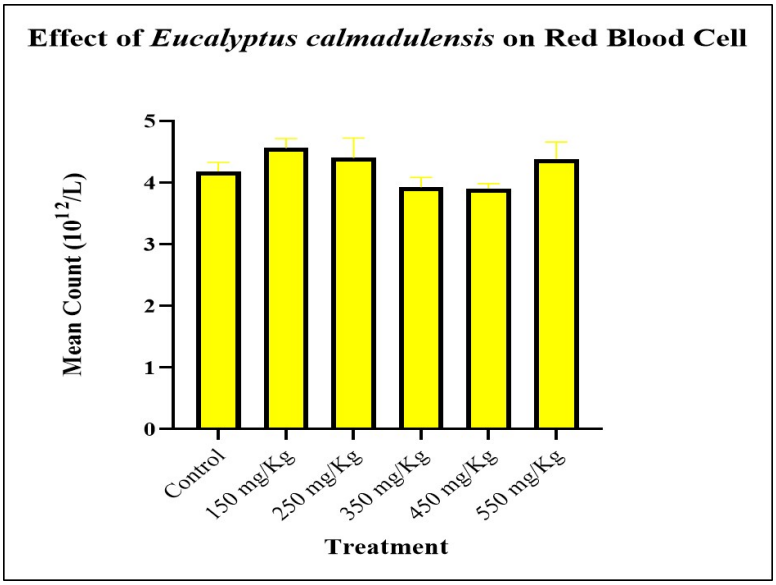

Figure 4: Effect of *Eucalyptus camaldulensis* on Red blood cell

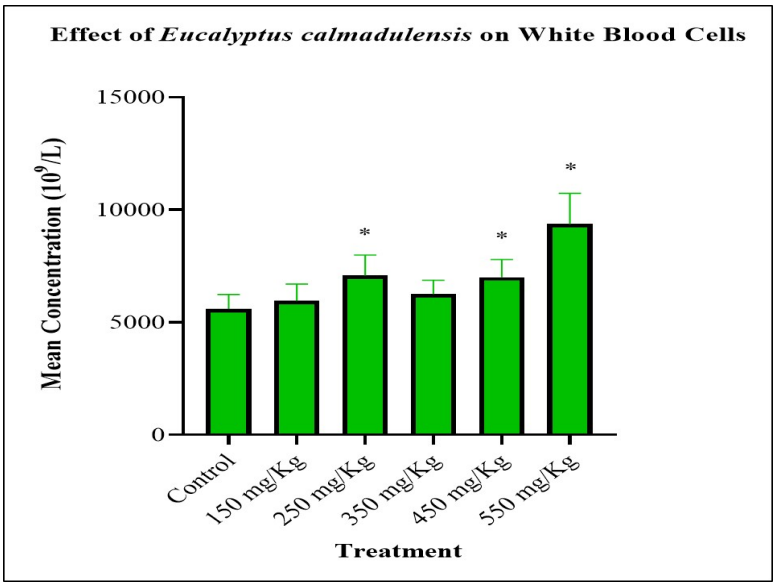

Figure 5: Effect of *Eucalyptus camaldulensis* on white blood cell

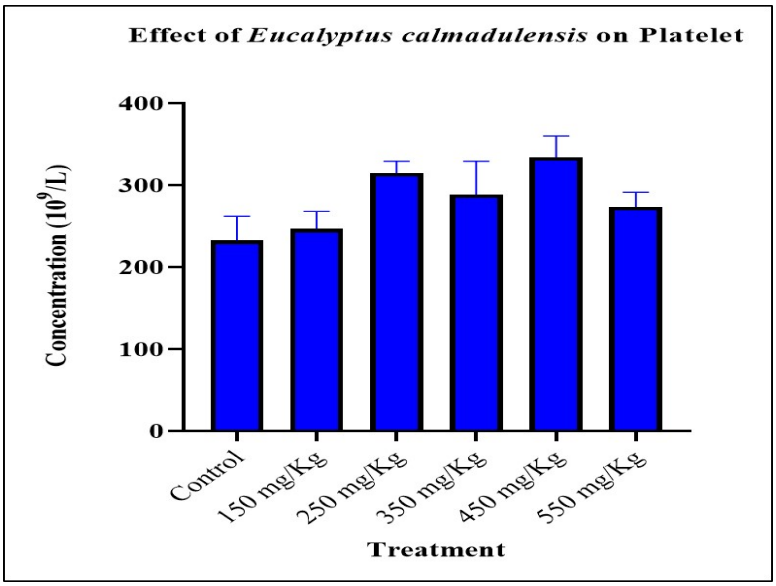

Figure 6: Effect of *Eucalyptus camaldulensis* on platelet

4. Discussion

Blood is the major transportation channel for food and foreign bodies, therefore, its components, such as haemoglobin, platelets, RBCs, and WBCs, are usually exposed to higher doses of toxic compounds thereby exposing these parameters to danger. Studying plant extracts for their effects on haematological parameters, provides insights into their potential impact on blood-related functions. Table 1 showed the experimental Design, animals were group from A to F. Normal control (group A) was administered (orally) distilled water, DMSO and feed, group B to F was administered (orally) 150mg/kg body weight, 250mg/kg body weight, 350mg/kg body weight, 450mg/kg body weight and 550mg/kg body weight respectively.

The evaluation of the effect of Eucalyptus camaldulensis ethanol leaf extract on haematological parameters following 21 days of administration in rats provides crucial insights into its safety and potential therapeutic applications. Figure 2 showed the effect of Eucalyptus camaldulensis on packed cell volume, figure 3 showed the effect of Eucalyptus camaldulensis on haemoglobin, figure 4 showed the effect of Eucalyptus camaldulensis on Red blood cell, figure 5 showed the effect of Eucalyptus camaldulensis on white blood cell and figure 6 showed the effect of Eucalyptus camaldulensis on platelet. These results obtained from this research further explains that haematological parameters, such as red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin concentration, hematocrit levels, and platelet count, are key indicators of the physiological and pathological state of the body [8]. Sub-chronic administration of Eucalyptus camaldulensis ethanol leaf extract could lead to significant changes in these parameters, reflecting the extract's impact on blood formation, immune response, and overall health. Any significant deviations from normal ranges could indicate potential haematotoxicity. Studies has shown that plant extracts can influence various haematological parameters, such as red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin levels, and platelet count. These effects are vital as they can indicate the effect of plant extracts on the hematopoietic system and overall health [9]. Thus, the toxicological profile of Eucalyptus camaldulensis on hematological parameters is a crucial aspect of understanding the potential effects on the hematopoietic system. While aqueous leaf extract of Eucalyptus camaldulensis has been studied for its biochemical and hematological effects [10], this study sought to evaluate the ethanolic extract effect on hematological parameters.

Red Blood Cells is produced in the bone marrow through a process called erythropoiesis [11]. It helps in transporting oxygen from the lungs to tissues and carbon dioxide from tissues to the lungs. Its haemoglobin content, an iron-rich protein that binds to oxygen, and also the biconcave shape to maximize surface area for gas exchange helps in the transport of oxygen. A decrease in the RBC count, haemoglobin, or hematocrit might suggest anemia or bone marrow suppression. Increase in the RBC and its indices following administration of the extracts is an indication of normal erythropoiesis. Additionally, haematological parameters are equally very important for assay because the hematopoetic system is a major target for toxic compounds. These parameters are also used to monitor the pathological and physiological status of animals and humans [12]. In this study, there was no change in the RBC and its component. Table 2 showed the presence of various phytochemicals like alkaloids,saponins,terpenoids, catecholic tanninsn, carbohyrades, glycosides, fats and oils in *E. camaldulensis* leaf extract This suggests that some of phytochemicals present in the plant extract has stabilizing effect and also did not compromise the red blood cells. In consonance with the findings of [13] the antioxidant activities of many plants have been linked to the presence of alkaloids, flavonoids, tannins and saponins compounds. Thus the extract could be said to be relatively safe. To further confirm this, the chromagram and GCMS in Figure 1 and Table 3 revealed the presence of compounds like 1H-Cycloprop[e]azulene,decahydro,1,2-Benzenedicarboxylicacid,mono,9-Octadecenoicacid,methylester, 7-Hexadecenal,(Z)-, 1,2-Benzenedicarboxylicacid,2-eth etc.

White Blood Cells are part of the immune system that originates from hematopoietic stem cells in the bone marrow. Originate from hematopoietic stem cells in the bone marrow. It include granulocytes (neutrophils, eosinophils, basophils) and agranulocytes (lymphocytes, monocytes). This cells increases in number to defend the body against infections and foreign materials during infections to fight pathogens[14].

In Table 4, Result obtained for WBC showed a significant increase ($p<0.05$) of 7080.00±907.41* at 250mg/kg b.wt, 7000.00±788.04* at 450mg/kg b.wt and 9360.00±136*9.16 at 550mg/kg b.wt. This suggests that this increase may be a normal reaction of rats to a foreign substance i.e the plant extract and this alters their normal physiological processes[15]. Also, increased WBC also suggests that the extracts may have immunological properties, which stimulated increased production of white blood cells, thus boosting the defence system of the animals, also that the extract did not compromise the immunity of the rats during the period of the treatment and this indicative of a normal cell-mediated immune response. In corroboration with the finding of[13] the increment in the white blood cells could be due to the stimulation of the bone marrow and lymphoid organs by the compounds such as alkaloids, glycosides, flavonoids that are found in the plant. These compounds may stimulate the lymphopoiesis process leading to the increased activity of the different cell lines and hence the observed increment in the various white blood cell types in the Wistar albino rats treated with the extract as compared to the control rats.

Other blood indices like MCV MCH, MCHC, RDW-CV and platelets showed no significant changes.

5. Conclusion and Recommendation

The 21-day administration of Eucalyptus camaldulensis ethanol leaf extract in rats has shown that this plant extract might be relatively safe for the red blood cell and its indices at the dose of 150-550mg/kg b.wt. Also serving as an immune booster at the dose of 250mg/kg b.wt, 450mg/kg b.wt and 550 mg/kg b.wt for the WBC. Though further studies should be carried out in the area of toxicity, histopathology to elucidate the mechanism of its effect and establish safe dosage guidelines for potential therapeutic use.

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