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Anti allergy activity of *Achyranthes aspera*

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ABSTRACT

The present study was aimed to evaluate the activity of *Achyranthes aspera* against allergy caused by heavy metal potassium dichromate in albino mice. The potassium dichloromate as induced at a concentration of 20 mg/kg. body weight dissolved in distilled water for 10 days continuously. On 11th day, blood was drawn from mice for serum analysis of IgM and IgG to confirm allergy induction in mice. After allergy induction the mice received petroleum ether, chloroform and methanolic extracts of *Achyranthes aspera* given 3 doses at concentration of – 200 (low), 400 (medium) and 600 (high) mg/kg to mice by oral route for 7 days after allergy induction. On the 8th day, blood was drawn from mice for serum analysis of IgM and IgG in determine the effect of extracts on serum Titre of mice blood.

***Keywords*:** *Achyranthes aspera*, potassium dichromate, mice

1. INTRODUCTION

Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species (Srivastav et al. 2011). According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care (Vijayan et al. 2007). *Achyranthes aspera* L (Amaranthaceae) is one of the medicinal plant, used for erect, it is annual herb distributed in the hilly district of inida (Vetericchelvan et al.,

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2003). The plant is indigenous system of medicine as emenagogue, anti arthritic, anti fertility, laxative, ecbolic, abentifacient, anti helminthic, anti coagulant, diruet and anti tumour (Ratraet al., 1970). *Achyranthes aspera* Lis reported to be purngent, astringent, pectoral and diuretic. It is also used as emaengogue and in piles and skin eruption. A decoction of the plant is useful in pneumonia and renal dropsy; in larger doses, decoction or juice acts as an ecbolic. The juice of the plant is reported to be used in opthalmia and dysentery too. Also, it is used in acute febrile illness and as an anti-inflammatory agent as well. The whole plant ,especially roots are of medicinal value. Active principle: Achyranthine, Betaine (Alkaloids) (Fikru, 2012; Gawande, 2015; Zheng, 2016; Bagavan, 2008; Banerji, 1970; Chakraborty, 2020; Girach, 1992; Gupta, 1972; Jayasingh, 2020; Valsaraj, 1997).

2. ALLERGEN

The allergen that is being used for our work is potassium dichromate. Potassium dichromate is also called as chromic acid, dipotassium salt and potassium bichromate. It is a bright yellowish crystalline substance, which is a strong potent allergen in metal and cement industries. It can act as contact allergen, respiratory allergen and also toxic to tissues and body. It is irritating to skin and mucous membrane, skin contact may cause skin irritation, rashes and burns, eye contact causes redness, swelling, tears, pairs and burns, even blindness. Inhalation may cause respiratory troubles, irritation of nose, throat, lungs, causing coughing, wheezing or shortness of breath. It is also very hazardous, can be fatal on ingestion can cause high levels of toxicity and damage to kidney, liver, inflammation of larynx and is also a potent carcinogen, shown to cause throat and lung cancer. (New Jersey Department of Health Sciences, July 2002). It cause Type II and Type IV hypersensitivity, respiratory challenge with dichromate is more likely to cause Type II hypersensitivity where as contact allergy is more likely to involve Type IV hypersensitivity.

3. MATERIALS AND METHODS

Collection of plant material

Whole plants of *Achyranthes aspera* were collected from Gulbarga University campus, Gulbarga during the months of October and November.

Methods of extraction

Hot extraction method using soxhlet apparatus was used for successive solvent extraction of the harvested plant materials. The whole plants collected were shade dried to complete dryness and then the material was ground to fine powder in a mixer-grinder.

The powder was weighed and 2 packets of approximately 35g of plant material was extracted successively using solvents ranging from polar to non-polar i.e. petroleum ether (40-60 °C), chloroform (60-70 °C), and methanol (80 °C) in a soxhlet. After complete extraction, the contents of each extraction were concentrated by distillation. The concentrated extracts were evaporated to dryness and weighed. Petroleum ether yields a greenish brown gummy extract, chloroform yields a dark green powder extract, where as methanol yields light greenish soft powder extract.

4. ANTIALLERGIC ACTIVITY

Albino Mice

Albino mice of wistar strain weighing 15-20g were procured from Animal House facility, Biogen Biotechnologies Pvt. Ltd., Bangalore and acclamatized and maintained at Animal House, Department of Biochemistry, Gulbarga University, Gulbarga, fed with standard diet and water adlibitum as described by CFTRI, Mysore.

Grouping of Animals for Experimentation

The animal were grouped as bellows:

i) **Group - 1:** This group is considered as negative control which received vehicle only.

ii) **Group - 2:** This group consists of allergen $K_2Cr_2O_7$ treated mice and considered as positive control for petroleum ether and chloroform extract of *Achyranthes aspera* treated group which received 4% Tween – 80 as vehicle for 7 days after induction of allergy.

iii) **Group - 3:** This group consists of allergen $K_2Cr_2O_7$ treated mice and considered as positive control for methanolic extract of *Achyranthes aspera* treated group which received distilled water as vehicle for 7 days after induction of allergy.

iv) **Group - 4:** This group was divided into 3 groups.

Group 4A: This group consists of allergen induced Mice which received petroleum ether extract at low dose of 200 mg / kg. body weight daily for 7 days.

Group 4B: This group consists of allergen induced mice which received petroleum ether extract at medium dose of 400 mg/ kg. body weight daily for 7 days.

Group 4C: This group consists of allergen induced mice which received petroleum ether extract at high dose of 600 mg/kg. body weight daily for 7 days.

v) **Group - 5:** This group was divided into 3 groups.

Group 5A: This group consists of allergen induced mice which received chloroform extract at low dose of 200 mg / kg. body weight daily for 7 days.

Group 5B: This group consists of allergen induced mice which received chloroform extract at medium dose of 400 mg/ kg. body weight daily for 7 days.

Group 5C: This group consists of allergen induced mice which received chloroform extract at high dose of 600 mg/kg. body weight daily for 7 days.

vi) **Group - 6:** This group was divided into 3 groups.

Group 6A: This group consists of allergen induced mice which received methanolic extract at low dose of 200 mg / kg. body weight daily for 7 days.

Group 6B: This group consists of allergen induced mice which received methanolic extract at medium dose of 400 mg/ kg. body weight daily for 7 days.

Group 6C: This group consists of allergen induced mice which received methanolic extract at high dose of 600 mg/kg. body weight daily for 7 days.

In all the above groups, minimum 9 animals were maintained. Induction of Allergy by $K_2Cr_2O_7$ (Potasiumdichloromate) and treatment with *Achyranthes aspera* extracts

Mice of group 2 and group 3 received potassium dichloromate at a concentration of 20 mg/kg. body weight dissolved in distilled water for 10 days continuously. The mice were given allergen by oral route using intragastric catheter tube once a day for the period of 10 days. On the 11^{th} day, blood was drawn from mice for serum analysis of IgM and IgG to confirm allergy induction in mice.

After allergy induction the mice of groups 4, 5 and 6 received petroleum ether, chloroform and methanolic extracts of *Achyrantheseaspera* given3 doses at concentration of – low, medium and high.

The petroleum and chloroform extracts were dissolved in 4% Tween 80 and methanolic extract was dissolved in distilled water and fed to mice by oral route for 7 days after allergy induction.

On the 8^{th} day, blood was drawn from mice for serum analysis of IgM and IgG in determine the effect of extracts on serum Titre of mice blood photographs showing loss of hairs due to induction of allergy by $k_2cr_2o_7$ (Image 1).

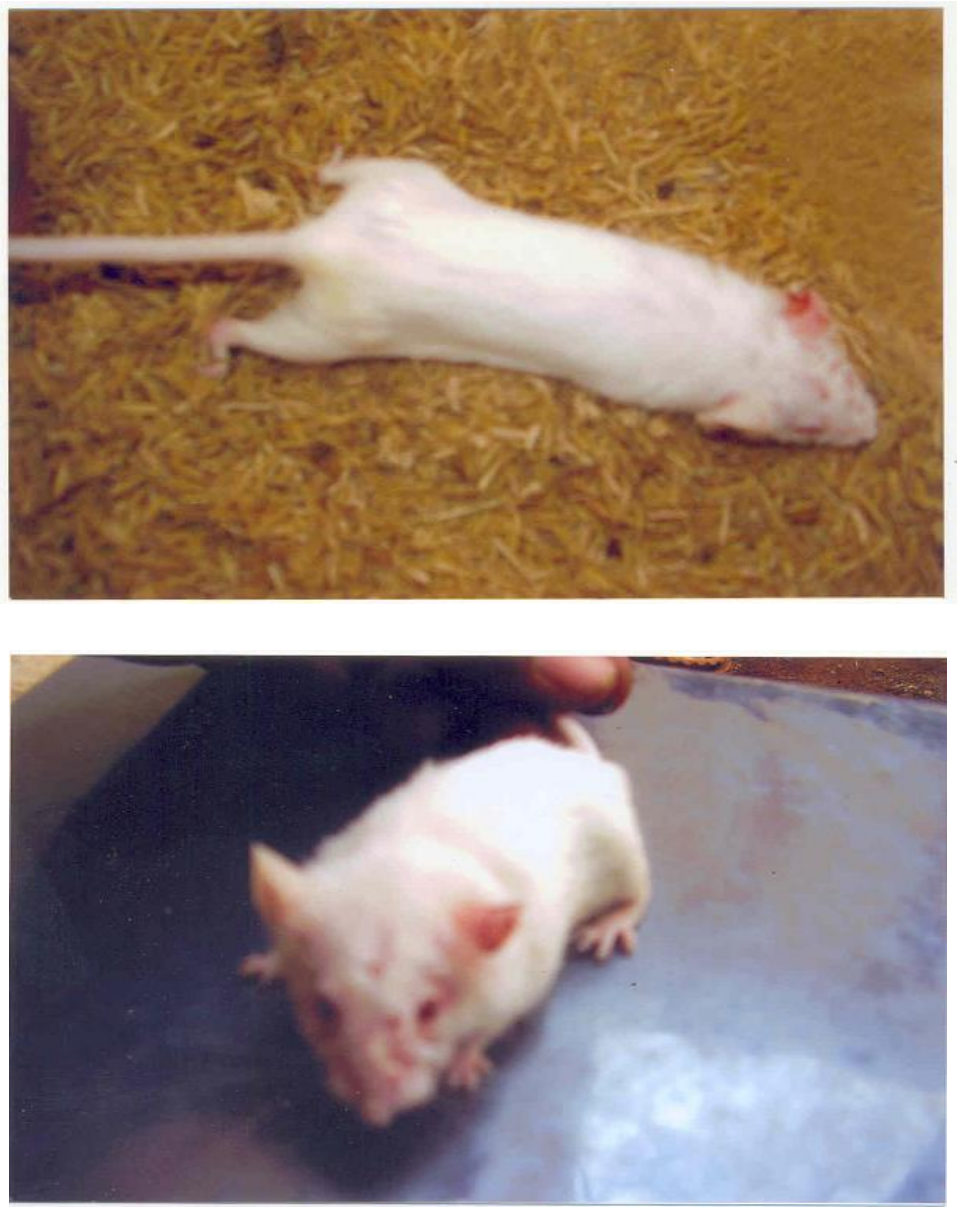


Image 1. Photographs showing loss of hairs due to induction of allergy by $K_2Cr_2O_7$

5. DIAGNOSIS OF ALLERGY

Total WBC count

Total WBC count is done to enumerate the total number of leucocytes in unit volume (1 mm^3) of blood. It can be used as an indicator for the presence of any pathological condition in the person, either pathogenic infection blood cancers or hypersensitivity conditions (Michael B et al., 2007).

Haemocytometer is a special glass slide with transverse bars whose surface is sunk 0.1 mm below the surface of the slide. The slide is divided in the centre into two counting

chambers called Neubauer's chambers. The ground area consists of 989 mm, each of the four squares are divided into 16 small chambers of squares. Each group is separated from each other by triple lines. The length of the smallest square is 1/20 mm. Hence, the actual volume of diluted blood in small square is $^{1}/_{4000}$ of mm^3.

6. DIFFERENTIAL CELL COUNT

Differential WBC count is due to enumerate the percentage of individual types of leucocytes i.e. Neutrophils, Basophils, Eosinophils, Lymphocytes, Monocytes etc. staining of cells reveals the nuclear arrangement of lobes and cytoplasmic granules which helps to differentiate among the cells (Foon et et, 1982).

This count is especially useful to reveal some diseased states like leukaemias, Burkitt's lymphoma, hypersensitivity conditions, pathogenic infections. White blood cells, or leukocytes, are classified into two main groups: granulocytes and non-granulocytes (also known as agranulocytes).

The granulocytes, which include neutrophils, eosinophils, and basophils, have granules in their cell cytoplasm. Neutrophils, eosinophils, and basophils also have a multilobed nucleus. As a result they are also called polymorphonuclear leukocytes or "polys." The nuclei of neutrophils also appear to be segmented, so they may also be called segmented neutrophils or "segs."

The non-granulocyte white blood cells, lymphocytes and monocytes, do not have granules and have non-globular nuclei. They are sometimes referred to as mononuclear leukocytes.

7. BLOOD COLLECTION FROM MICE

Blood was collected by cardiac puncture method from mice, whose serum Ig level was to be tested. The serum collected was stored in a clean sterilized eppendrof tubes sealed with para film and stored in freezer until it is further used (Wintrobe MM, 1981).

8. SERUM ANTIBODY DETECTION

ELISA (Enzyme linked immuno sorbent assay) is a useful immunological technique for rapid and accurate detection of specific Ab and Ag in biological samples and both qualitative and quantitative analysis can be done by this technique (Jeffery et al., 2001).

ELISA may be employed in several way, viz., direct, indirect, sandwich etc. depending on the type of analysis to be done and the biological component to be assayed.

ELISA has been employed for several applications including clinical diagnosis of pathogens, detection of several disease and pathological conditions etc.

9. RESULTS

Weight changes in Mice

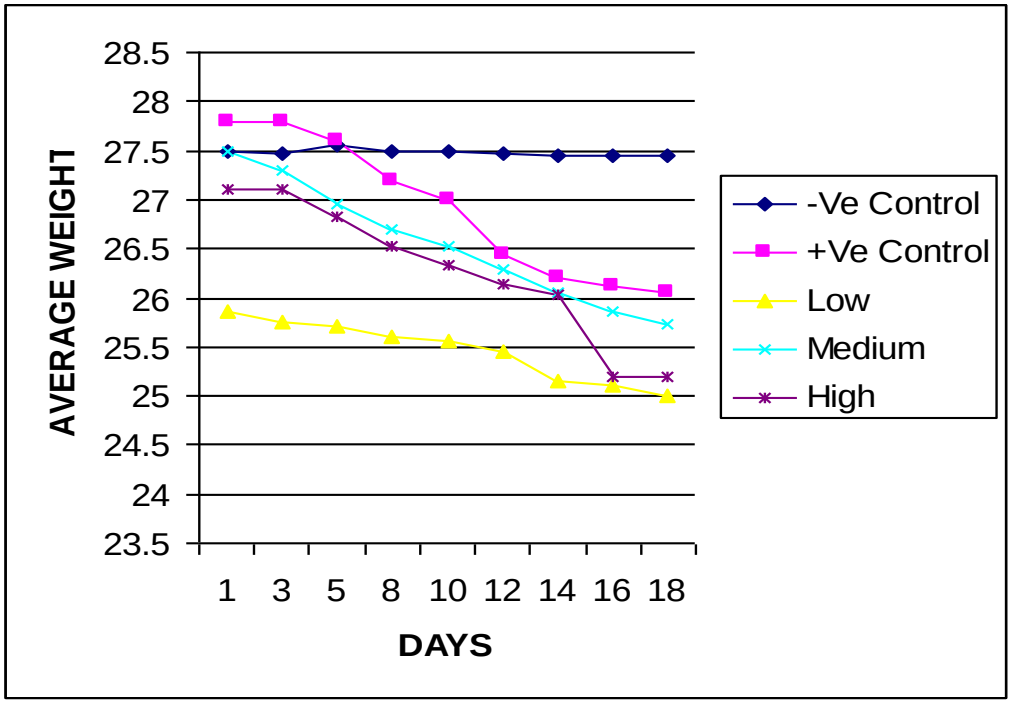
The mice showed steady decrease in weight from the beginning of allergy induction by gavage and then gradually gained weight when the allergy induction was stopped but weight gain was more pronounced in the case of mice which were fed with the plant extracts especially methanolic extract (Table 1 & Graph 1, 2, 3).

Table 1. Weight changes in Mice

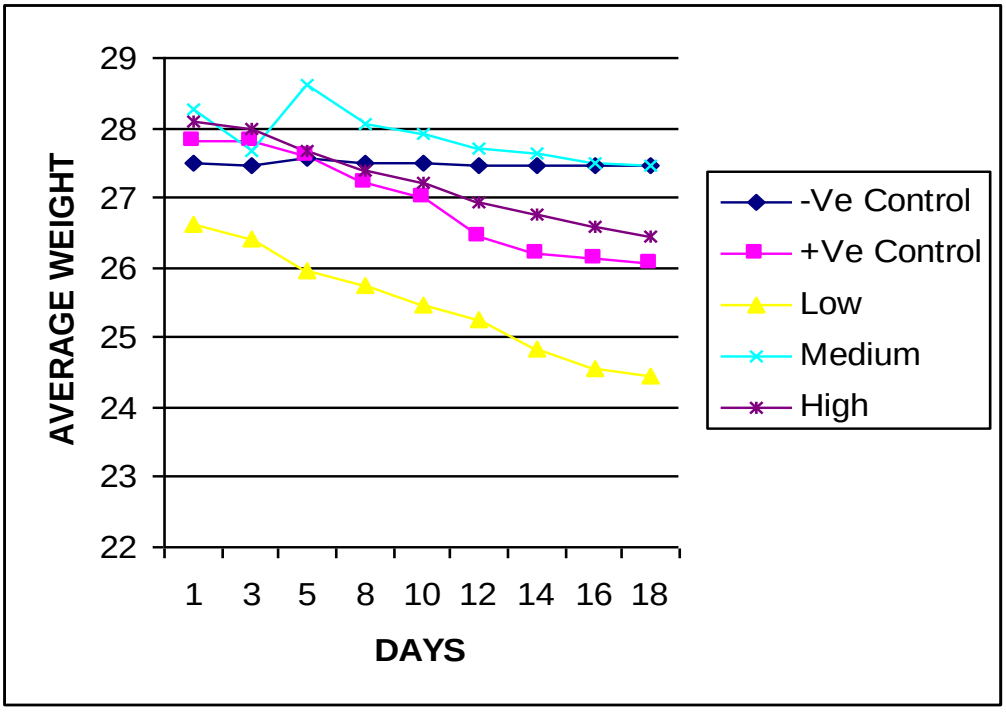
Sl. NO	MICE	WEIGHTS IN GRAMS								
		Day 1	Day 3	Day 5	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
Group I (Negative Control)										
1	M1m	26.2	26.2	26.3	26.2	26.2	26.3	26.2	26.2	26.2
2	M2m	25.9	25.86	25.9	25.9	25.8	25.8	25.8	25.8	25.8
3	M3m	24.6	24.5	24.4	24.5	24.5	24.5	24.3	24.5	24.5
4	M4 f	29.6	29.6	29.5	29.6	29.6	29.5	29.5	29.5	29.5
5	M5 f	28.3	28.3	28.3	28.4	28.4	28.3	28.3	28.3	28.3
6	M6 f	29.1	29.1	28.8	29.2	29.1	29.2	29.1	29.1	29.1
7	M7 f	25.6	25.3	25.4	25.4	25.4	25.4	25.5	25.5	25.4
8	M8 f	23.9	23.9	24	24	23.8	23.8	26.8	23.7	23.8
9	M9 f	27.8	27.8	27.8	27.7	27.8	27.7	27.7	27.7	27.7
Group II (Positive Control for Methanol)										
10	M1m	27.3	27.3	27.1	26.8	26.5	……			
11	M2m	29.8	29.7	29.7	29.4	29.3	……..			
12	M3m	27.8	27.8	27.1	26.6	26.5	26.3	25.9	25.8	25.8
13	M4 f	30.7	30.7	30.5	30.1	29.8	29.8	29.5	29.3	29.2
14	M5 f	24.4	24.7	24.7	24.2	24.1	23.9	23.8	23.8	23.7
15	M6 f	26.9	26.6	26.4	26.1	25.9	25.8	25.6	25.6	25.5
16	M7 f	26.3	26.3	25.6	25.2	24.8	24.9	24.2	24.3	24.2
17	M8 f	29.7	29.7	29.5	29.2	29.0	28.4	28.6	28.5	28.4

18	M9 f	27.3	26.9	26.5	25.9	25.6	25.1	24.8	24.7	24.5
Group III (Positive control for Petroleum ether and chloroform)										
19	M1m	26.1	26.3	25.8	25.5	25.2	25.0	24.8	24.5	24.3
20	M2m	27.8	27.6	27.4	27.1	26.8	26.9	26.5	26.4	26.1
21	M3m	29.2	28.7	28.2	27.6	27.1	27.1	26.8	26.5	26.5
22	M4 f	25.9	25.7	25.8	25.6	25.5	25.5	25.3	24.8	24.3
23	M5 f	26.3	26.1	25.7	25.3	25.3	25.1	24.8	24.5	24.7
24	M6 f	28.2	28.2	27.8	27.7	27.5	27.2	27.2	26.8	26.5
25	M7 f	26.2	26.3	26.1	25.7	25.5	25.2	24.8	24.7	24.8
26	M8 f	27.5	27.6	27.6	27.1	26.7	26.5	26.4	26.2	25.0
27	M9 f	29.8	29.7	29.5	29.5	29.2	29.0	28.7	28.2	28.0
Sl.NO	MICE	WEIGHTS IN GRAMS								
		Day 1	Day 3	Day 5	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
Group IV (Petroleum Ether Extract)										
28	LOW	M1f	24.7	24.6	24.6	24.3	……..……..……			
29		M2f	25.1	25.1	25	24.8	24.2	24	23.8	23.8	23.7
30		M3m	27.8	27.6	27.5	27.2	26.9	26.9	26.5	26.4	26.3
31	MED UUMM	M4f	27.2	27.1	26.8	26.5	26.4	26.3	25.9	25.8	25.7
32		M5f	28	27.7	27.3	27.1	26.8	26.5	26.5	26.1	25.9
33		M6m	27.3	27.1	26.8	26.5	26.4	26.1	25.8	25.7	25.6
34	HIGH	M7f	28.5	28.5	28.3	27.8	27.6	27.6	27.5	……..……	
35		M8f	26.1	26.1	25.8	25.6	25.5	25.2	25.1	25	25
36		M9m	26.7	26.7	26.4	26.2	25.9	25.6	25.5	25.4	25.4
Group V (Chloroform Extract)										
37	LOW	M1f	25.7	25.6	25.1	24.9	24.5	24.3	24.1	23.8	23.7
38		M2f	26.8	26.5	26.4	26.1	25.8	25.7	25.6	25.3	25.2
39		M3m	27.4	27.1	26.4	26.2	26.1	25.8	……..		
40	MED	M4f	28	27.9	27.8	27.6	27.5	27.2	27.2	26.9	26.9
41		M5f	27.6	26	……..……						

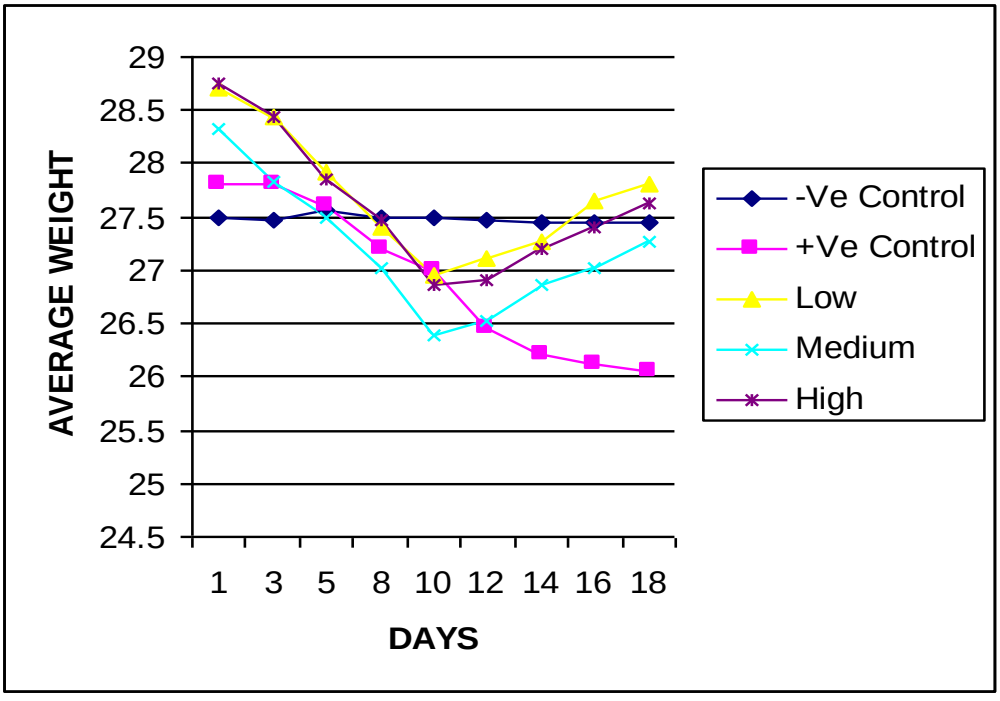
42		M6m	29.2	29.1	28.8	28.5	28.3	28.2	28.1	28.1	28
43	HIGH	M7f	29.1	29	28.7	28.4	28.1	27.6	27.4	27.3	27.2
44		M8f	27.1	27.1	26.8	26.5	26.5	26.3	26.2	26	25.9
45		M9m	28.1	27.9	27.5	27.3	27	26.9	26.7	26.5	26.2
Group VI (Methanolic extract)											
46	LOW	M1f	29.1	28.7	28.2	27.6	27.1	27.2	27.3	27.6	27.9
47		M2f	27.2	27.1	26.5	26.1	25.5	25.7	25.9	26.3	26.3
48		M3m	29.8	29.5	29.1	28.5	28.3	28.4	28.6	29.1	29.2
49	MED	M4f	28.1	27.6	27.3	26.9	26.4	26.6	26.8	27.1	27.3
50		M5f	27.1	26.8	26.5	26.1	25.4	25.6	25.9	26.1	26.4
51		M6m	29.8	29.1	28.7	28.1	27.4	27.4	27.9	27.9	28.1
52	HIGH	M7f	26.4	26.1	25.6	25.4	25.1	25.2	25.2	25.4	25.7
53		M8f	30.1	29.7	29.3	28.9	28.1	28.1	28.5	28.9	29.1
54		M9m	29.8	29.5	28.7	28.1	27.4	27.4	27.9	27.9	28.1



Graph 1. Average Weights of Mice Treated with Petroleum ether Extract



Graph 2. Average Weights of Mice Treated with Chloroform Extract



Graph 3. Average Weights of Mice Treated with Methanolic Extract

Total WBC Count of Mice

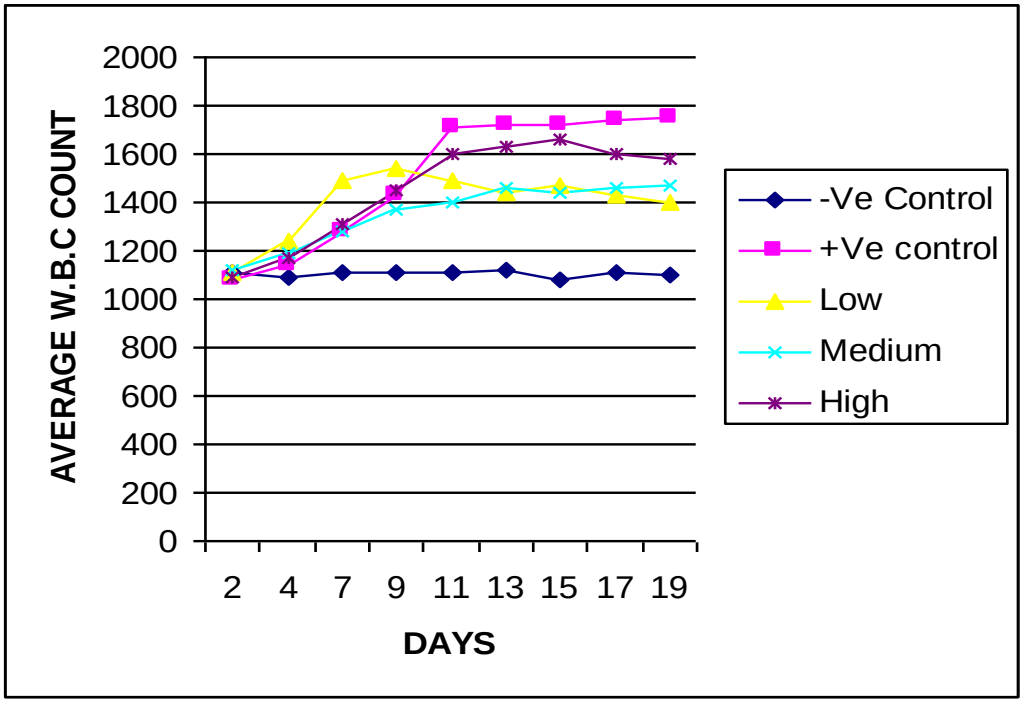
Total WBC count of mice was done during the period of the project work. WBC was counted regularly on alternate days and the mice uniformly showed an increase in WBC count during allergy induction. As allergy induction was stopped, gradual decrease of WBC count was seen in the mice being treated with plant extracts compared to untreated mice, more so in the case of methanolic extract where the decrease was more prominent (Table 2, Graph 4, 5, 6 & Image 2).

Table 2. Total WBC Count of Mice

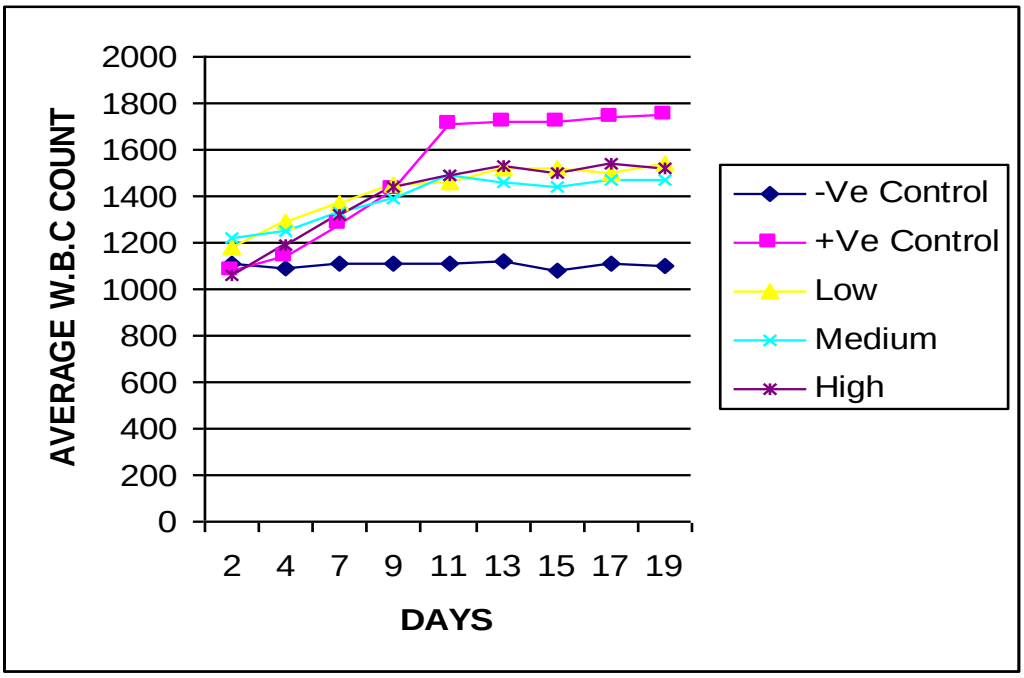
Sl. NO	MICE	Cells / mm^3								
		Day 2	Day 4	Day7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19
Group I (Negative Control)										
1	M1 m	865	850	890	865	875	885	890	865	875
2	M2 m	965	985	950	1010	980	975	895	955	990
3	M3 m	780	810	800	820	800	710	815	825	810
4	M4 f	1350	1290	1275	1280	1275	1295	1265	1315	1280
5	M5 f	1275	1250	1325	1295	1310	1325	1285	1315	1265
6	M6 f	1280	1290	1270	1255	1260	1280	1275	12958	1280
7	M7 f	1475	1390	1550	1490	1480	1510	1530	1490	1485
8	M8 f	1390	1410	1380	1425	1400	1420	1415	1400	1410
9	M9 f	1250	1265	1250	1245	1265	1255	1270	1265	1260
Group II (Positive Control Methanol)										
10	M1 m	865	890	970	955	1005	……..			
11	M2 m	850	965	1150	1190	1375	……			
12	M3 m	825	965	985	1101	1350	1390	1410	1400	1390
13	M4 f	1360	1425	1550	1925	2050	2135	2175	2150	2055
14	M5 f	1310	1325	1450	1455	1625	1875	1835	1975	2090
15	M6 f	1250	1275	1550	1925	2150	2145	2160	2175	2195
16	M7 f	1150	1215	1275	1450	1515	1490	1525	1495	1535
17	M8 f	1260	1375	1435	1460	1430	1450	1460	1465	1465
18	M9 f	1310	1325	1345	1360	1390	1405	1445	1425	1435

Group III (Positive control for petroleum ether and chloroform)										
19	M1 m	980	1015	1140	1255	1305	1320	1350	1390	1410
20	M2 m	810	800	830	855	870	910	935	950	965
21	M3 m	780	820	810	850	865	895	910	935	940
22	M4 f	1330	1355	1345	1395	1425	1430	1495	1510	1525
23	M5 f	1180	1195	1210	1235	1250	1270	1295	1305	1325
24	M6 f	1080	1210	1315	1455	1455	1490	1490	1495	1510
25	M7 f	1205	1290	1365	1565	1575	1670	1710	1720	1735
26	M8 f	1150	1250	1290	1380	1460	1510	1530	1535	1590
27	M9 f	1310	1340	1450	1520	1610	1630	1640	1680	1680
Sl.NO	MICE	Cells / mm3								
		Day 2	Day 4	Day7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19
Group IV (Petroleum Ether Extract)										
28	LOW	M1f	1050	1335	1670	1810	……..…….…….			
29		M2f	1290	1410	1790	1780	1795	1750	1770	1680	1705
30		M3m	985	990	1015	1030	1190	1130	1165	1175	1095
31	MED UUUMM	M4f	1150	1225	1445	1550	1565	1595	1475	1555	1505
32		M5f	1350	1375	1420	1495	1535	1610	1620	1595	1605
33		M6m	850	965	980	1055	1090	1190	1225	1245	1290
34	HIGH	M7f	1225	1325	1465	1525	1650	1780	1790	…….Died…….	
35		M8f	1075	1190	1365	1575	1785	1715	1810	1805	1790
36		M9m	965	1010	1090	1250	1355	1410	1380	1395	1365
Group V (Chloroform Extract)										
37	LOW	M1f	1315	1495	1555	1650	1610	1680	1550	1575	1580
38		M2f	1250	1365	1425	1455	1480	1565	1490	1435	1500
39		M3m	970	1005	1130	1245	1295	1310	……Died……		
40	MED	M4f	1275	1365	1410	1495	1590	1565	1495	1510	1525
41		M5f	1335	1250	……..……..Died……...…….						
42		M6m	1050	1135	1255	1295	1390	1360	1385	1425	1410

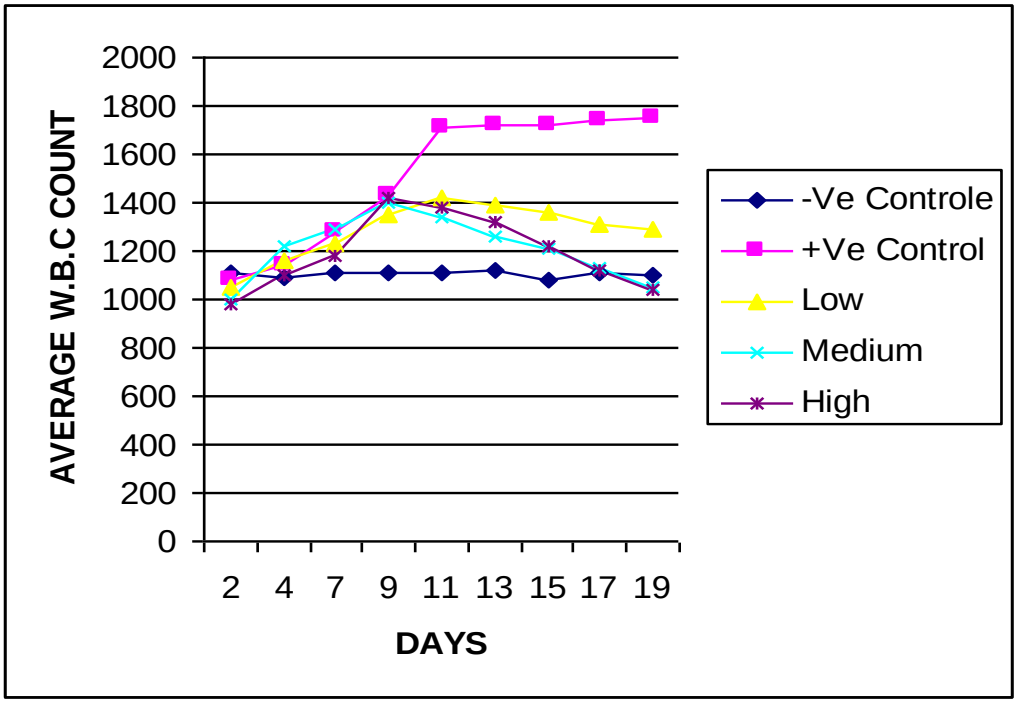
43	HIGH	M7f	1150	1215	1275	1450	1515	1490	1525	1495	1535
44		M8f	1050	1225	1365	1485	1480	1590	1480	1565	1515
45		M9m	975	1130	1310	1395	1475	1510	1495	1550	1525
Group VI (Methanolic extract)											
46	LOW	M1f	1150	1250	1365	1475	1525	1465	1340	1350	1310
47		M2f	1010	1190	1210	1390	1490	1455	1490	1395	1375
48		M3m	980	1050	1110	1180	1250	1255	1260	1200	1180
49	MED	M4f	1090	1275	1370	1485	1465	1400	1360	1210	1125
50		M5f	1080	1210	1310	1450	1390	1280	1215	1190	1105
51		M6m	840	1170	1205	1280	1155	1105	1070	980	910
52	HIGH	M7f	900	1070	1130	1390	1310	1265	1190	1090	980
53		M8f	1090	1175	1280	1490	1475	1395	1275	1160	1115
54		M9m	950	1050	1120	1390	1365	1290	1185	1125	1015



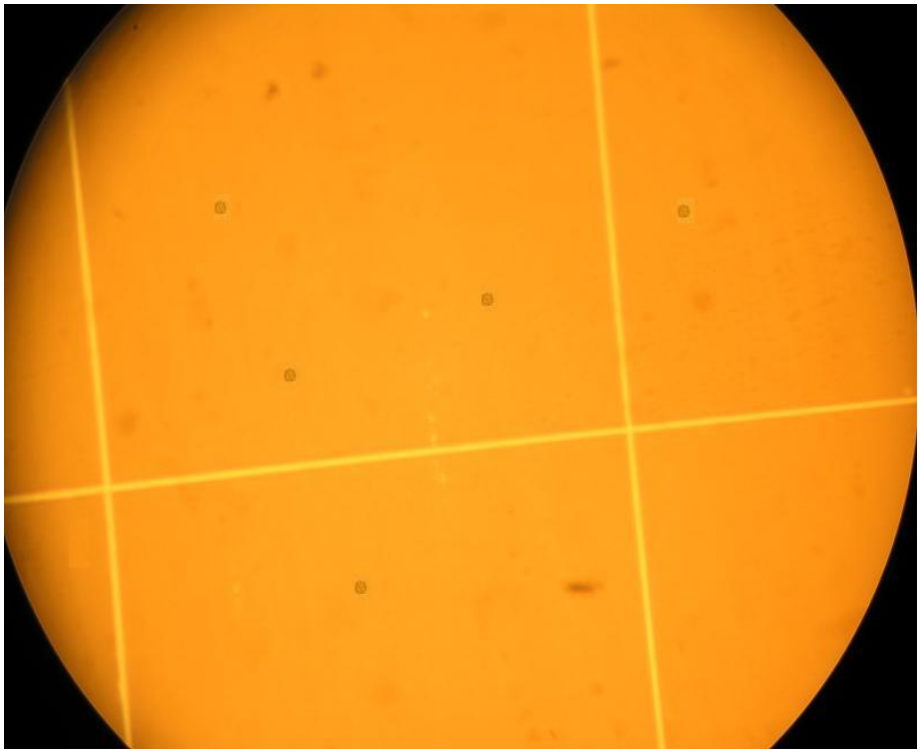
Graph 4. Effect of Petroleum ether Extract on Total W.B.C Count



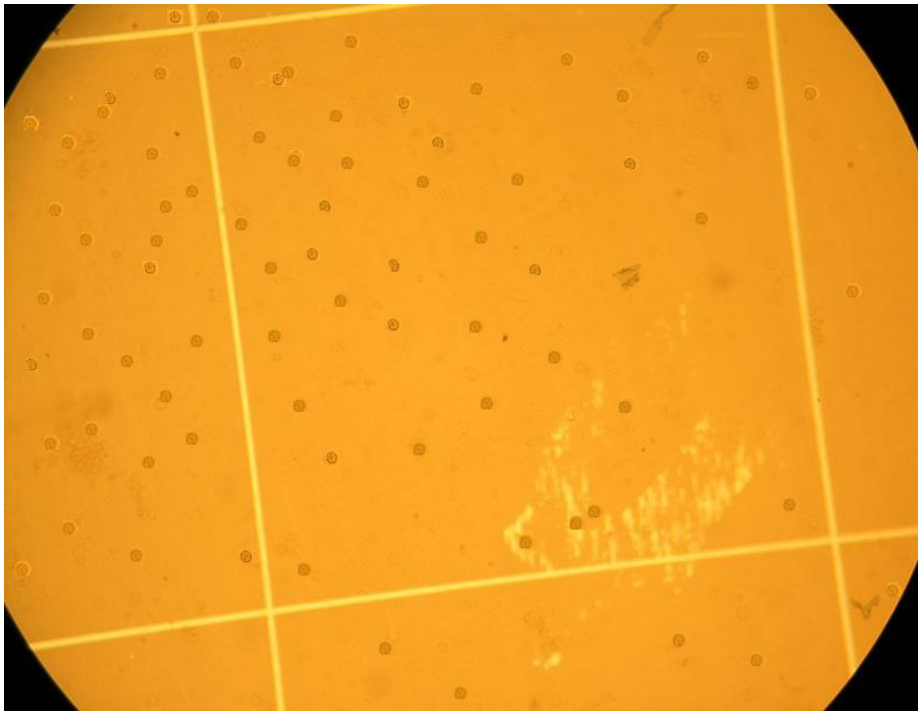
Graph 5. Effect of Chloroform Extract on Total W.B.C Count



Graph 6. Effect of Methanolic Extract on Total W.B.C Count



Microphotograph of Neubauer's chamber showing W.B.C's of Normal Mice



Microphotograph of Neubauer's chamber showing W.B.C's of $K_2Cr_2O_7$ Induced Mice

Image 2.

Differential WBC count of Mice

Differential WBC counting revealed the percentage of WBC in the blood of mice at various stages of induction and treatment. Increase in the eosinophils, monocytes and lymphocytes were observed during allergy induction in mice. On treatment of allergy induced mice with plant extracts of *A. aspera*, the number of the differential cells was reduced, more so in the case of treatment with methanolic extract.

The averages of the different WBC of mice blood computed and tabulated and shown here (Table 3 & Image 3).

Table 3. Average Differential WBC count of different groups

	CELLS	PERCENTAGE (%) OF CELLS											
		NORMAL	DAY 5			DAY 10			DAY 15			DAY 18	
			L	M	H	L	M	H	L	M	H	L	M	H
PETROLEUM ETHER	Neutrophil	58	55	56	52	38	49.8	50	55	50	57	57.2	62	55
	Eosinophil	1	3	2	6	5.8	5.2	5	6	6	5	5.6	6	5
	Basophil	2	1	1.2	1.2	1.8	1	1.2	2	1	1.2	2.4	1.2	1
	Monocyte	6	8	6.8	6.1	7.2	9.2	8.9	7	6.3	4.8	7.1	6.5	5
	Lymphocyte	31	33	34	35.4	27.2	34.8	34.9	34	33.4	33.3	27.7	35.4	33
CHLOROFORM	Neutrophil	58	56	55	54	50	50	49.8	54	52	60.2	49.8	58	55
	Eosinophil	1	2	3	4	5	6	5.2	6	6	5	5.2	5.8	4
	Basophil	2	1.2	1	1	1.2	2	1	1	1.2	1	0.5	1.8	0.8
	Monocyte	6	6.8	8	7	8.9	7	8.8	6.3	6.1	7	5.5	7.2	6.2
	Lymphocyte	31	34	33	34	34.9	35	35	33.4	35.4	38	39	21.2	34
METHANOL	Neutrophil	58	57	55	57	51	50	49	52	54	49	54	60.2	61
	Eosinophil	1	2	3	1	6	5	6	4	5.2	6	1.2	1	1.8
	Basophil	2	1.2	1	2	1	1.2	1	5	2	1	2	1	2
	Monocyte	6	7.8	7	7	7	8.9	9	6	6	6	6	5.2	4
	Lymphocyte	31	33.3	34	33	35	34.9	35	28	32.8	32.8	32.8	29.8	29.9

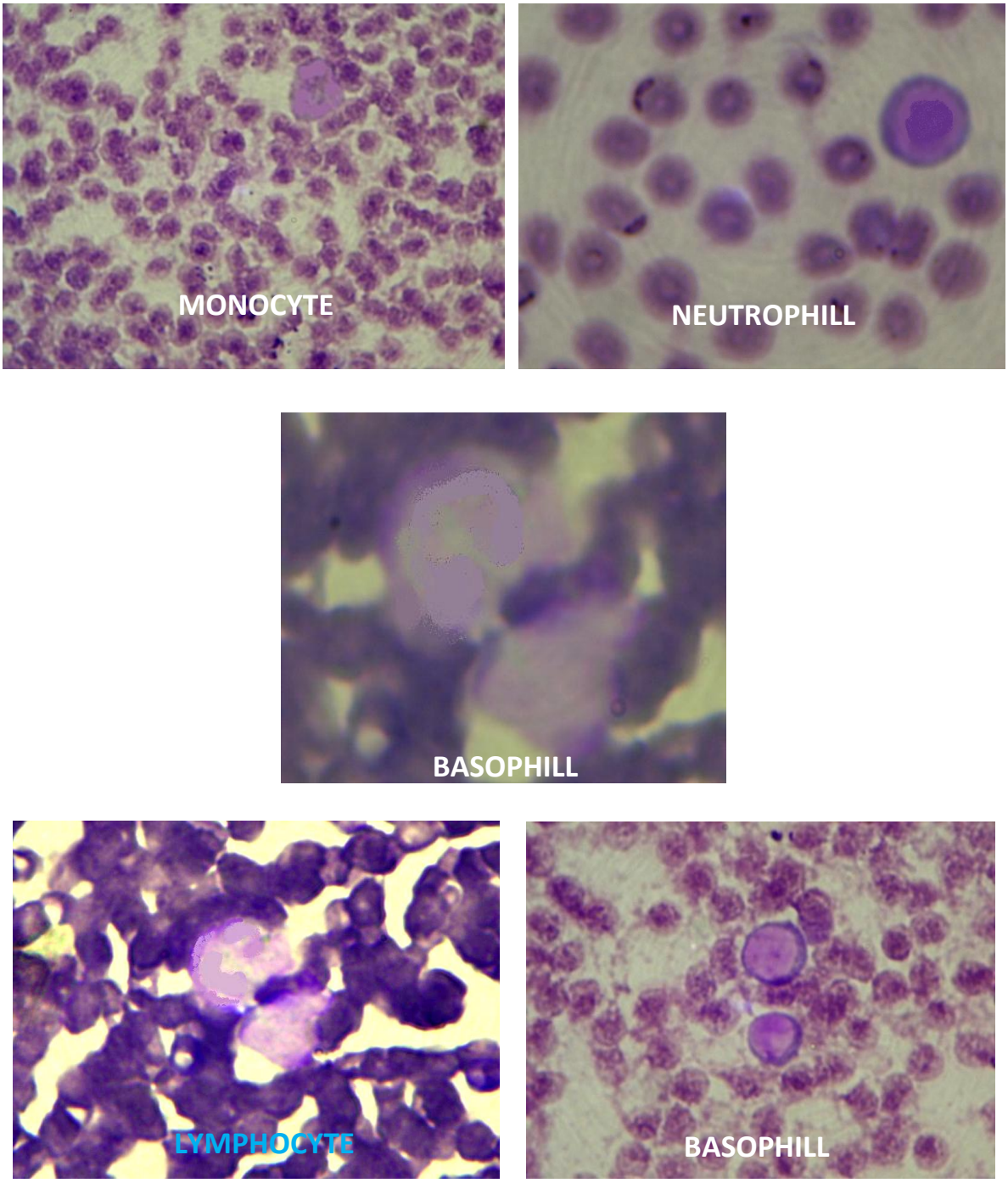


Image 3. Microphotographs of differential white blood cells

Serum Antibody Analysis

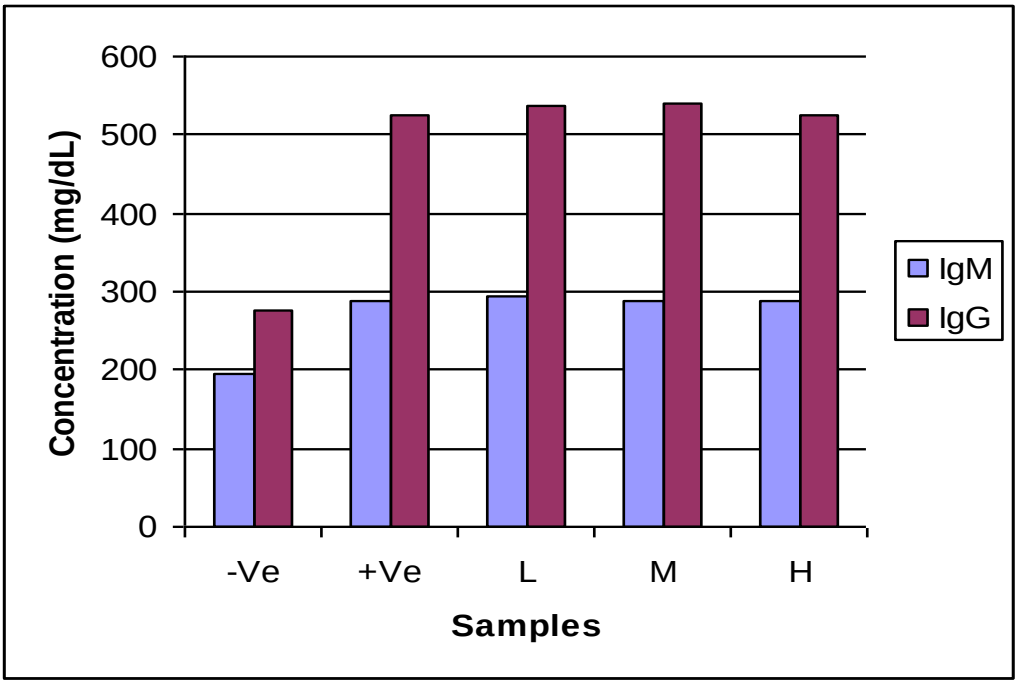
Immunoglobulin titres of IgM and IgG in the serum were quantitatively estimated by sandwich ELISA. The normal range of mice IgM is 60-250 mg/dL and that for mice IgG is 90-450 mg/dL. Serum titres were significantly raised during the course of allergic induction

as indicated by the serum Ab titre values. Thereafter there was a reduction in the serum Ab when the mice were treated with plant extracts. The decrease in the Ab titre was more prominent in the mice that were treated with methanolic extracts.

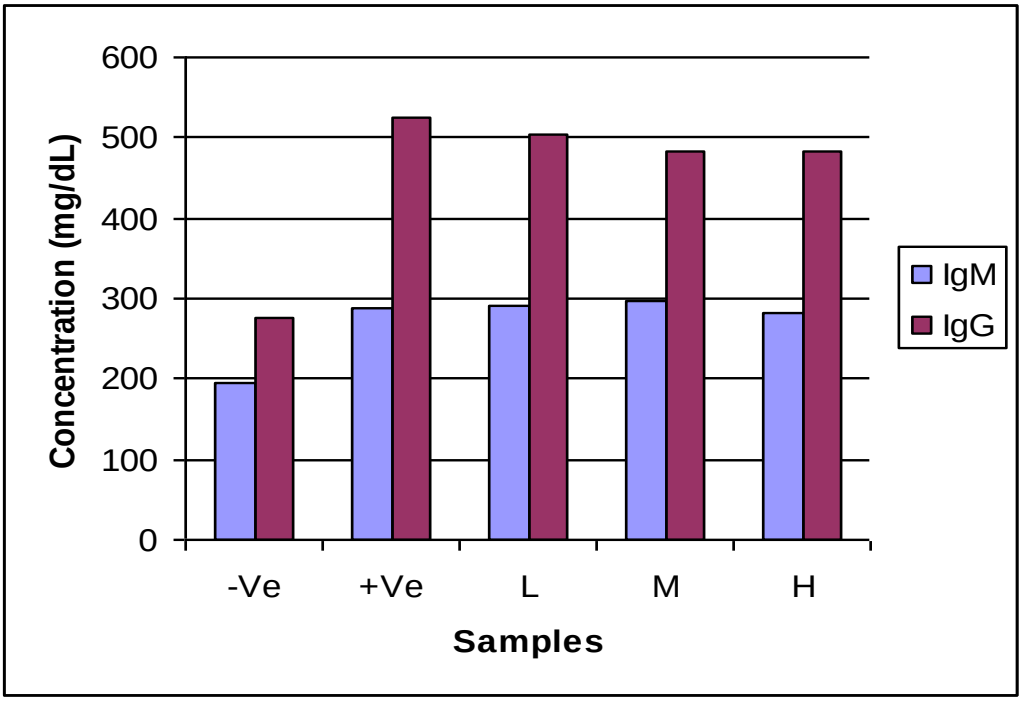
The following are the IgM and IgG titres of normal untreated mice, allergy induced mice and plant extract treated mice (Table 4 & Graph 7, 8, 9).

Table 4. Serum Ab titres of Mice on Allergy induction and treatment

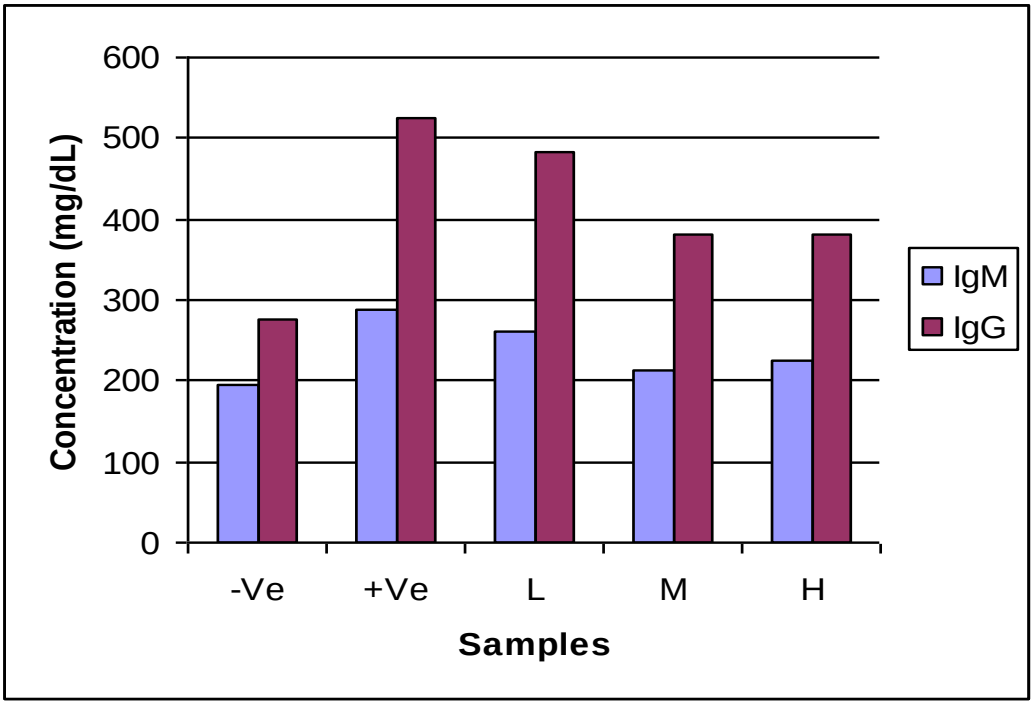
SL. NO.	SAMPLE	CONCENTRATION (mg/dL)	
		IgM	IgG
1	-Ve Control	196.4	276.6
2	+Ve Control	297.3	545.3
Petroleum ether extract treated Mice			
3	Low	293.5	537.5
4	Medium	288.4	539.1
5	High	287.6	525.3
Chloroform extract treated Mice			
6	Low	290.3	505.3
7	Medium	295.7	482.1
8	High	281.0	483.7
Methanolic extract treated Mice			
9	Low	260.2	483.8
10	Medium	213.6	380.4
11	High	223.6	382.1



Graph 7. Effect of Petroleum ether Extract on serum IgM and IgG titre



Graph 8. Effect of Chloroform Extract on serum IgM and IgG titre



Graph 9. Effect of Methanolic Extract on serum IgM and IgG titre.

10. DISCUSSION

It's study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity, to characterize the active compounds and to elucidate the exact mechanism of action by which they exert their antiallergic effects.

The plant is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases etc. (Jain 1991). Traditional healers claim that addition of A. aspera would enhance the efficacy of any drug of plant origin.

Allergy induction by dichromate may be classified as type II hypersensitivity due to the increase in cells associated i.e. neutrophils, lymphocytes, eosinophils and macrophages and increase in IgM and IgG titre after allergy induction (Abdul Ghaffar et al, 2002, and Cohen. S.R., Davis D.M, Zelokoffet al. and Kramkowski P E, 1974.)

Weight reduction, loss of appetite and hair loss can be seen as external symptoms after induction.An increase in total W.B.C count was observed on allergy induction. This count was reduced at the end of the treatment with extracts of which methanolic extract showed the greater lowering the W.B.C count to near normal.

A lowering in the differential W.B.C cells involved in Type II allergy was typically greater by the methanolic extract. Moreover the higher IgM titre seen on allergy induction was reduced to greater extent by the methanolic extract. Also in the case of weight reduction, there was greater weight increase in case of methanolic extract treated mice

Hence, the methanolic extract has greater efficiency in reducing the effects of dichromate induction.

Reported that the petroleum ether extract (200 mg/kg, i.p.) of the plant shows significant antiallergic activity in both milk induced leukocytosis and milk induced eosinophilia in mice. Thus the antiallergic activity of *A. aspera* may be due to the presence of steroids. Thus these steroids present in the plant may be responsible for the antiallergic activity. Edwin et al. (2008) investigated the ethanolic and aqueous extracts of leaves of *Achyranthes aspera* for wound healing activity.

12. CONCLUSION

The present study tries to evaluate the utility of *Achyranthes aspera* in treating allergies caused due to heavy metals, particularly dichromate. *Achyranthes aspera* is a useful plant found throughout the Indian landmass with many curative properties including anti-allergic effects, but it is considered as a common weed. $K_2Cr_2O_7$ can cause intense hypersensitive reaction on contact or if inhaled into respiratory system. The allergic reactions induced by $K_2Cr_2O_7$ in mice were to some extent reduced by *A. aspera* extracts especially methanolic extracts.

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