5.1 REVIEW OF MOMORDICA DIOICA

Momordica dioica Roxb. belongs to the family Cucurbitaceae (and under the genus *Momordica*, a genus of annual or perennial climbers that contains about 80 species (Raj *et al.*, 1993) *Momordica dioica* climber plant commonly known as Teasle Gourd, Kakrol, Kankro, Kartoli, Kantoli, Kantola, Kantroli, Ban karola or Small bitter-gourd is a relatively small oval to ovoid vegetable. It is also called as janglee karela (Harish, 2008). It is often cultivated for its fruits, which are used as vegetable (Sastri, 1962). Teasle gourd is a cucurbitaceous popular summer vegetable. The fruits, young twigs and leaves of this crop are used as vegetable or cooked as a vegetable (Bandyopadhyay and Mukherjee, 2009). It has two different types of varieties male & female as well as fruited variety & fruitless variety. *Momordica dioica* Roxb. (chormosome number 28) dioecious and propagated vegetatively through tuberous root (Rashid, 1993). It is dioecious, perennial in nature having tuberous roots. The green fruit is extensively used as vegetable by cooking or frying. Leaves 1.5-4 inches long, cordate, acute more or less 3-5 lobed; Flowers large, dioecious and yellow in colour; Fruit 1-3 inches long, shortly beaked, densely covered with soft spines (Rasul *et al.*, 2004).

This is climbing creeper generally found throughout India, Pakistan, Bangladesh, Himalayas to Ceylon. Reported up to an altitude of 1500 m in Assam and Garo hills of Meghalaya (Ram *et al.*, 2002). Kakrol is a Cucurbitaceous crop originated in the Indo –Malayan region (Rashid, 1976 and Singh, 1990).

Classification

The Plant Database (1996)

Kingdom	-	Plantae
Subkingdom	-	Tracheobionata
Super division	-	Spermatophyta
Division	-	Magnoliphyta
Class	-	Magnoliopsida

Subclass	-	Dilleniidae
Order	-	Violales
Family	-	Cucurbitaceae
Genus	-	*Momordica*
Species	-	*dioica*

Synonyms

Bengoli	-	Kartoli
English	-	Small bittergourd, spine gourd
Hindi	-	Kakora, Parora, Golbandra
Malyalam	-	Venpaval, Erima pasel
Marathi	-	Kartoli
Tamil	-	Aegaravalli, Tholoopavai, Paluppakkay
Telagu	-	Karkotaki, Agakara
Cannad	-	Madahagala –Kayi
Sanskrit	-	Vahisi
Panjabi	-	Dharkarela
Assam	-	Batkarila

Phytochemical Studies

It contains Lectins, proteins, triterpenes and vitamins (Naik, 1951). The fruit contains a high amount of vitamin C (Bhuiya, 1977).The fruit is rich in ascorbic acid and contain iodine (Rao, 2001). The fruit also contain alkaloid, flavonoids, glycosides and amino acids (Kushwaha *et al.*, 2005) *Momordica dioica* also contains an alkaloid, a fragrant extractive matter and ash 3 to 4 p.c. Ash contains a trace of manganese (Data, 2010).

Momordica dioica as the average nutritional value per 100 g edible fruit was found to contain 84.1% moisture, 7.7 g carbohydrate, 3.1 g protein, 3.1 g fat, 3.0 g fiber and 1.1 g minerals. It also contained small quantities of essential vitamins like ascorbic acid, carotene, thiamin, riboflavin and niacin (Singh, 2006) It also content protein in the leaves and dry weight of aerial plant parts remained higher in male as compared to female defruited, and monoecious plants (Ghosh,2005)From

Momordica dioica fruit isolated 6-methyl tritriacont-50on-28-of and 8-methyl hentracont-3-ene along with the known sterol pleuchiol. Momodicaursenol, an unknown pentacylic triterpene isolated from the seeds, had been identified as urs-12, 18(19)-dien-3 beta-ol on. Phytochemical investigations have revealed the presence of traces of alkaloids and ascorbic acid in fruits. Lectins, b-sitosterol, saponins, glycosides, triterpenes of ursolic acid, hederagenin, oleanolic acid, a-spiranosterol, stearic acid, gypsogenin, two novel aliphatic constituents (Ali and Srivastava, 1998, Sadyojatha and Vaidya, 1996, Ghosh *et al.*, 1981, Luo *et al.*, 1998). From the dry root of *Momordica dioica* isolated three triterpenes and two steroidal compounds. These were alphaspinasterol octadecanonate(I), alphaspinasterol-3-O-beta-D-glucopyranoside(II), 3-O-beta-D-glucuronopyranosyl gypsogenin(III), 3-O-beta-D-glucopyranosyl gypsogenin(IV) and 3-O-beta-D-glucopyranosyl hederagenin(V). Constituent III was a new compound.

Pharmacological activities

The plant is used, for the treatment of eye diseases, against fever, snake bite, inflammation caused by lizard; is also used as medicine for diabetes (Khare, 2004, Kirtikar, 1999, Nadkarni, 2004). While investigating the spermatogenic properties of the ethanolic extract of the fruit extract of M. dioica on the animal, the behavioral observations led us for sedative activity of the extract. Not much work is available about the pharmacological and other activities of the plant. Passiflora actinia possesses anxiolytic and sedative activity (Santos *et al.*, 2006). Likewise, many plants have been reported to have anxiolytic and sedative activity such as Aloysia polystachya, Euphorbia hirta, Kigelia Africana and Coriandrum sativum (Lanhers *et al.*, 1990, Owalabi *et al.*, 2008, Emamghoreishi and Hamedani, 2006).

Juice of root of ***Momordica dioica Roxb.ex.*** is immunostimulant and antiseptic. Its fruit are used as vegetable (Sadyojatha and Vaidya, 1996). The fruit leaves, and tuberous roots of ***Momordica dioica*** have

long been used in India as a folk remedy for diabetes and other health problems (Chakravarty, 1959). The juices appear toxic and abortificant. The whole plant is used for treatment of eye disease and against fever (Satyavati *et al.*, 1987). Mishra (1991) and Gupta (1993) reported antimalerial and antiallergic activity, respectively, in ***M. dioica Roxb.ex.*** Thirupathi *et al.* (2006) reported protective effect of ***Momordica dioica Roxb.ex.*** against hepatic damage caused by carbon tetrachloride in rats. Ali and Deokul have found some important nutrient in different plants and provided the concentration of important nutrients .*Momordica dioica* also possess many essential nutrient compound which are essential for proper functioning of the body .It contains Calcium -0.5 mg/g, Sodium -1.5 mg/g, Potassium -8.3 mg/g, Iron -0.14mg/g ,Zinc-1.34 mg/g, Protein-19.38%, Fat-4.7%,Total phenolic compound 3.7 mg/g, Phytic acid -2.8 mg/g , 4.1calories and ash value was 6.7% . Jain *et al* reported the antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica*. From both the extracts, ethanolic extract was found to be more potent hepatoprotective. The antioxidant and free radical scavenging activities were positive for both ethanolic and aqueous extract. This activity may be due to free radical scavenging and antioxidant activities which may be due to presence of flavonoids in extracts. Mishra *et al.* reported that the solvent extracted from vegetable seed oil of Small bitter gourd (*Momordica dioica*), evaluated as grain protectant against Callosobruchus chinensis on the stored legume-pulse grains. *Momordica dioica* roots shows antiallergic activity for alcoholic extract.

5.2 RESULT OF MOMORDICA DIOICA

5.2.1 Qualitative chemical tests

The aqueous extracts of *Momordica dioica* showed the presence of protein, carbohydrate, glycosides, saponin, flavonoids, steroid and terpenoids and the ethanolic extract showed presence of protein, carbohydrates, alkaloid, tanin, flavonoids, saponin, and Glycosides.

5.2.2 TLC Studies

Thin layer chromatographic studies were performed for aqueous and ethanolic extracts of *Momordica dioica*. Aqueous extracts of *Momordica dioica* have shown best separation in Acetone+ Water (90+10) and give 9 spots. (R_f value- 0.3, 0.6, 0.7, 0.8, 0.9, 0.11, 0.14, 0.17, 0.20). Spots were visualized by Conc. H_2SO_4.

Ethanolic extract of the drug best separates in Toluene: Chloroform: Ethanol (26+24+9.5) and give sixteen spots (R_f value-0.02, 0.07, 0.12, 0.23, 0.30, 0.35, 0.40, 0.44, 0.50, 0.52, 0.59, 0.63, 0.69, 0.73, 0.81). Spots were visualized by Anisaldehyde-sulphuric acid reagent.

5.2.3 Toxicity Studies

In toxicity test with *Momordica dioica*, no mortality was recorded in both the extracts.

5.2.4 Carbon Clearance Test

Carbon Clearance depends on time and it was calculated as phagocytic index of time interval between the treated groups of animals compared with the control group. The mean phagocytic index of control (Group I) was found to be 1.007 ± 0.020. The crude aqueous extract of *Momordica dioica* treated groups II, III and IV were elevated as 1.264 ± 0.037 ($P<0.025$), 1.361 ± 0.042 ($P<0.025$) and 1.632 ± 0.064 ($P<0.001$) when animals treated with 100, 150 and 50mg/kg b.wt intraperitoneally for seven days. (Table 5.2.1 and Fig 5.2.1)

The crude ethanolic extract had given significantly increased phagocytic index as 1.438 ± 0.069 (P<0.001), 1.563 ± 0.045 (P<0.001) and 1.521 ± 0.058 (P<0.001) respectively with 50, 100 and 150mg/kg b.wt intraperitoneally for seven days (Table 5.2.2 and Fig 5.2.2).

5.2.5 Delayed Type Hypersensitivity Test

Delayed Type Hypersensitivity response to SRBC was calculated as a measure of paw volume (in mm) for each animal and compared with control group I which was injected 2ml of 5% Normal saline intraperitoneally for seven. Paw volume was calculated after 24, 48, 72 and 96 hrs. The increased value for group II, III and IV after 24 hrs were found to be 1.76 ± 0.021 ml (P<0.05), 1.79 ± 0.028 ml (P<0.05) and 1.77 ± 0.013 ml (P<0.05) and after 48 hrs there were 1.25 ± 0.017 ml (P<0.025), 1.20 ± 0.023 (P<0.05) ml and 1.26 ± 0.019 ml (P<0.025) and for 72 and 96 hrs paw volume was reduced as after 72 hrs it was 0.61 ± 0.018 ml, 0.57 ± 0.021 ml and 0.56 ± 0.011 for 96 hrs it was found as 0.25 ± 0.013 ml, 0.23 ± 0.029 ml and 0.21 ± 0.017 ml (P<0.05) respectively (Table 5.2.3 and Fig 5.2.3).

Animal treated with crude ethanolic extract showed reduced paw volume after 48, 72 and 96 hrs. The only increase in paw volume for group II, III and IV after 24 hrs were found to be 1.64 ± 0.022 ml, 1.69 ± 0.014 ml (P<0.05) and 1.64 ± 0.020 ml and after 48 hrs there were 0.95 ± 0.018 ml, 0.92 ± 0.019 ml and 0.89 ± 0.024 ml (P<0.05) after 72 hrs it was 0.59 ± 0.016 ml, 0.57 ± 0.021 ml and 0.58 ± 0.017 ml and after 96 hrs it was found to be 0.28 ± 0.024 ml, 0.26 ± 0.016 ml and 0.27 ± 0.023 ml respectively (Table 5.2.4 and Fig 5.2.4).

5.2.6 SRBC Agglutination Test

Agglutination titer to sheep red blood erythrocyte was calculated and compared with Group I (control). Group II, III and IV were treated with crude aqueous extract orally for ten days (50, 100, 150mg/kg b.wt.) and on 10^{th} day agglutination titer were observed in various serum dilution (X: 20, X: 40, X: 80, X: 160, X: 320). An increased was observed

at the dose of 100 and 150mg/kg b.wt. while for the 50mg/kg b.wt. it was found stable (Table 5.2.5 and Fig 5.2.5).

Group I was given 5% normal saline and agglutination titer was compared with treated one. Group II, III and IV that received the crude ethanolic extract at the dose of 50, 100 and 150mg/kg b.wt. respectively, no change was observed in the animals of group II and III which received 50 and 100mg crude ethanolic extract /kg b.wt. while group IV showed an increase in the activity (Table 5.2.6 and Fig 5.2.6).

5.2.7 Drug Induced Myelosuppression Using Cyclophosphamide

The Group I was control and received as usual 2 ml of 5% of normal saline and various hematological observations were taken. In them the mean haemoglobin was 13.11 ± 0.12 gms/dl, mean RBC count was 4.65 ± 0.154 million/mm^3 and mean WBC count was 13.27 ± 0.425 thousand/mm^3. Neutrophils count was 53.10 ± 1.51%, Lymphocytes count was 40.82 ± 1.08%, Monocytes count was 2.45 ± 0.38%, Eosinophil count was 2.35 ± 0.51% and platelets count was 3.23 ± 0.243 lacs/mm^3. In Group II cyclophosphamide (3mg/kg b.wt) was administered and there was a significant decrease in all hematological parameters studied except Neutrophils and Monocytes count which were slightly increased. Mean haemoglobin was 8.25 ± 0.29 gms/dl ($P<0.025$), mean RBC count was 3.72 ± 0.121 million/mm^3 ($P<0.05$) and WBC count was 11.16 ± 0.241 thousand/mm^3 ($P<0.05$). Mean Neutrophils count was 59.84 ± 0.89%, Lymphocytes count was 33.59 ± 0.85% ($P<0.05$), Monocytes count was 3.01 ± 0.26%, Eosinophil count was 2.41 ± 0.24% and platelets count was 2.40 ± 0.456 lacs/mm^3 ($P<0.05$).

Group III, IV and V were administered crude aqueous extract of (50, 100 and 150mg/kg b.wt.) with cyclophosphamide intraperitoneally, in them the mean haemoglobin was found to be 11.89 ± 0.24 ($P<0.05$), 12.64 ± 0.27 ($P<0.05$), 13.02 ± 0.18 gms/dl ($P<0.025$) respectively. Mean RBC count was 4.02 ± 0.122, 4.18 ± 0.151 ($P<0.05$), 4.39 ± 0.125 million/mm^3 ($P<0.05$), mean WBC count was 11.47 ± 0.109, 11.89 ±

0.126, 12.23 ± 0.217 thousand/mm^3, Neutrophils count was 56.48 ± 2.41%, 55.33 ± 2.04%, 54.26 ± 1.58%, Lymphocytes count was 34.83 ± 1.27%, 36.61 ± 1.73%, 38.06 ± 1.18% (P<0.05), Monocytes count was 2.82 ± 0.16%, 2.77 ± 0.39%, 2.54 ± 0.40%, Eosinophils count was 2.26 ± 0.42%, 2.29 ± 0.23%, 2.32 ± 0.51% and platelets count was 2.74 ± 0.304, 2.92 ± 0.419, 3.11 ± 0.428 lacs/mm^3 (P<0.05) (Table 5.2.7 and Fig 5.2.7 to 5.2.14).

In another sets of experiments Group III, IV and V were administered crude ethanolic extract of (50, 100 and 150mg/kg b.wt.) with cyclophosphamide intraperitoneally, in them the mean haemoglobin was found to be 10.56 ± 0.29, 10.95 ± 0.43, 11.76 ± 0.39gms/dl (P<0.05) respectively. Mean RBC count was 3.85 ± 0.187, 3.99 ± 0.202, 4.13 ± 0.154 million/mm^3, mean WBC count was 11.84 ± 0.255, 12.51 ± 0.323, 12.83 ± 0.307 thousand/mm^3 (P<0.05), Neutrophils count was 56.89 ± 1.20%, 56.24 ± 1.63%, 55.96 ± 1.37%, Lymphocytes count was 34.69 ± 0.38%, 36.47 ± 0.44%, 37.10 ± 0.61%, Monocytes count was 2.90 ± 0.47%, 2.76 ± 0.38%, 2.61 ± 0.44%, Eosinophils count was 2.39 ± 0.56%, 2.46 ± 0.49%, 2.52 ± 0.32% and platelets count was 2.66 ± 0.514, 2.71 ± 0.437, 2.84 ± 0.281 lacs/mm^3 (Table 5.2.8 and Fig 5.2.15 to 5.2.22).

5.2.8 Cytokines (IL-2 and IL-6) Assay

Cytokines (IL-2 and IL-6) level was observed in all Groups (I, II, III and IV) and treated Groups were compared with control (Group I). The IL-2 and IL-6 levels were observed as 24.21 ± 1.352 and 30.58 ± 2.846pg/ml respectively for control. Crude aqueous extract had given relatively higher IL-2 level as 45.59 ± 5.381 (P<0.05), 48.94 ± 4.817 (P<0.05), 55.18 ± 6.279 pg/ml (P<0.05) and IL-6 level were found stable as 32.72 ± 2.811, 39.65 ± 2.106, 42.19 ± 3.352pg/ml respectively with 50, 100, 150mg/kg b.wt. (Table 5.2.9 and Fig 5.2.23).

Crude ethanolic extract had also given higher IL-2 level as 38.22 ± 6.217, 42.52 ± 5.391, 45.37 ± 6.254pg/ml (P<0.05) and IL-6 level were

found stable as 31.29 ± 3.020, 33.99 ± 4.519, 35.25 ± 2.982pg/ml respectively with 50, 100, 150mg/kg b.wt. (Table 5.2.10 and Fig 5.2.24).

5.2.9 Electrophoresis Study of Serum Protein Profile

For analysis of potential protein complexes, serum from mice was used. Standard protein molecular markers were also run with mice serum to estimate molecular weight of separated protein bands. Results demonstrate that a total of 8 major gel bands could be clearly distinguished after Coomassie blue staining in control and treated groups. Bands 3 and 8 were observed thicker and darker in all treated groups for crude aqueous and ethanolic extract with comparison to control. Many other bands were present in the gel, but they were not distinguishable. So along with the original gel photograph, a sketch representation was also made of that gel (Fig 5.2.25 and 5.2.26).

Table: 5.2.1 Effect of Crude Aqueous Extract of *Momordica dioica* on Phagocytic Activity in Carbon Clearance Test.

	Groups	Mean absorbance SD		Phagocytic Index(k) ± SD
		0 min	15 min	
I.	Control	0.3361 ± 0.008	0.2413 ± 0.019	1.007 ± 0.020
II.	Crude Aqueous extract (50mg/kg body wt.)	0.3198 ± 0.016	0.2104 ± 0.025	1.264 ± 0.037**
III.	Crude Aqueous extract (100 mg/kg body wt.)	0.3009 ± 0.028	0.1915 ± 0.021	1.361 ± 0.042**
IV.	Crude Aqueous extract (150 mg/kg body wt.)	0.2914 ± 0.023	0.1694 ± 0.015	1.632 ± 0.064***

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.1 Effect of Crude Aqueous Extract of *Momordica dioica* on Phagocytic Activity.

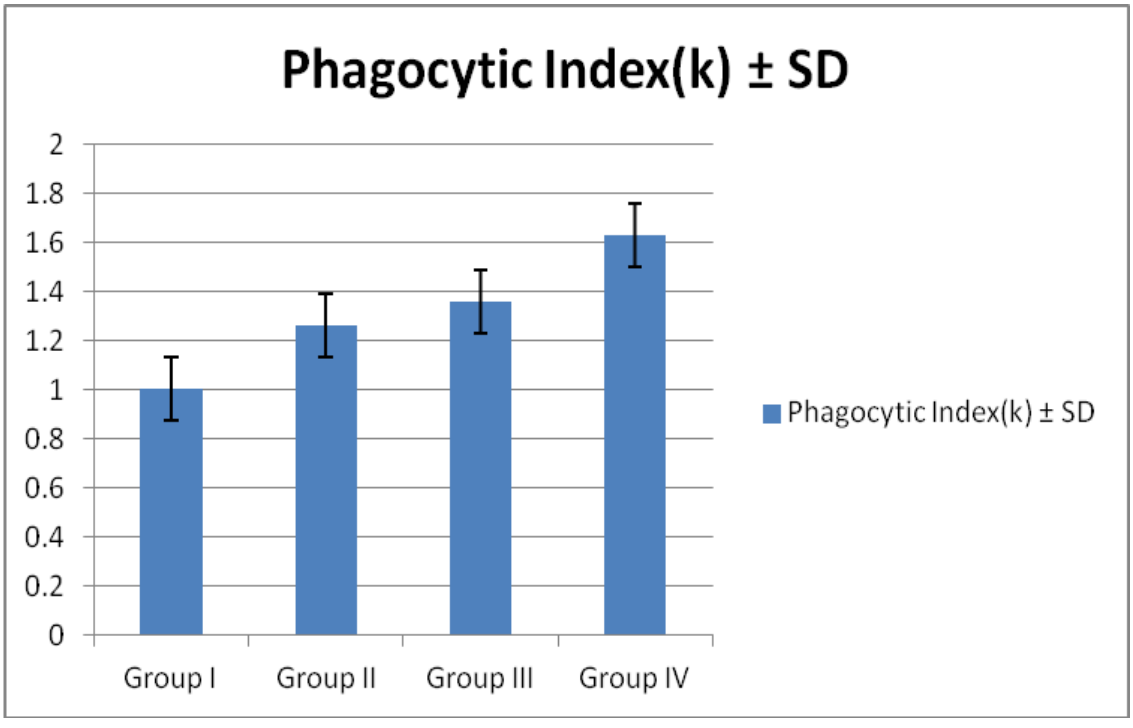


Table: 5.2.2 Effect of Ethanolic Extract of *Momordica dioica* on Phagocytic Activity in Carbon Clearance Test.

	Groups	Mean absorbance SD		Phagocytic Index(k) ± SD
		0 min	15 min	
I.	Control	0.3361 ± 0.008	0.2413 ± 0.019	1.007 ± 0.020
II.	Crude Ethanolic extract (50mg/kg body wt.)	0.2953 ± 0.024	0.1834 ± 0.026	1.438 ± 0.069***
III.	Crude Ethanolic extract (100 mg/kg body wt.)	0.2769 ± 0.022	0.1649 ± 0.019	1.563 ± 0.045***
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	0.2491 ± 0.027	0.1505 ± 0.023	1.521 ± 0.058***

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.2 Effect of Crude Ethanolic Extract of *Momordica dioica* on Phagocytic Activity.

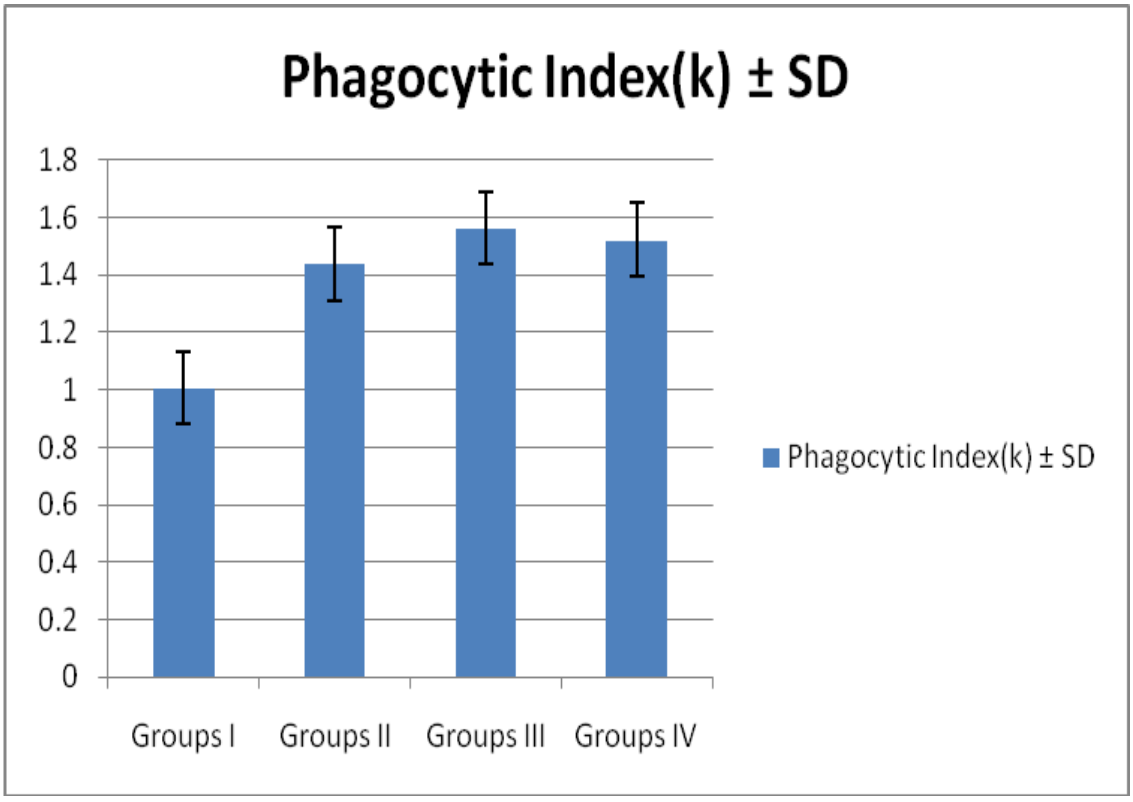


Table: 5.2.3 Effect of Crude Aqueous Extract of *Momordica dioica* on Delayed Type of Hypersensitivity

	Groups	Paw Volume (ml) ± S.D.			
		24 Hrs	48 Hrs	72 Hrs	96 Hrs
I.	Control	1.59 ± 0.010	1.01 ± 0.029	0.64 ± 0.021	0.31 ± 0.016
II.	Crude Aqueous extract (50mg/kg body wt.)	1.76 ± 0.021*	1.25 ± 0.017**	0.61 ± 0.018	0.25 ± 0.013
III.	Crude Aqueous extract (100 mg/kg body wt.)	1.79 ± 0.028*	1.20 ± 0.023*	0.57 ± 0.021	0.23 ± 0.029
IV.	Crude Aqueous extract (150 mg/kg body wt.)	1.77 ± 0.013*	1.26 ± 0.019**	0.56 ± 0.011	0.21 ± 0.017*

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.3 Effect of Crude Aqueous Extract of *Momordica dioica* on Delayed Type of Hypersensitivity.

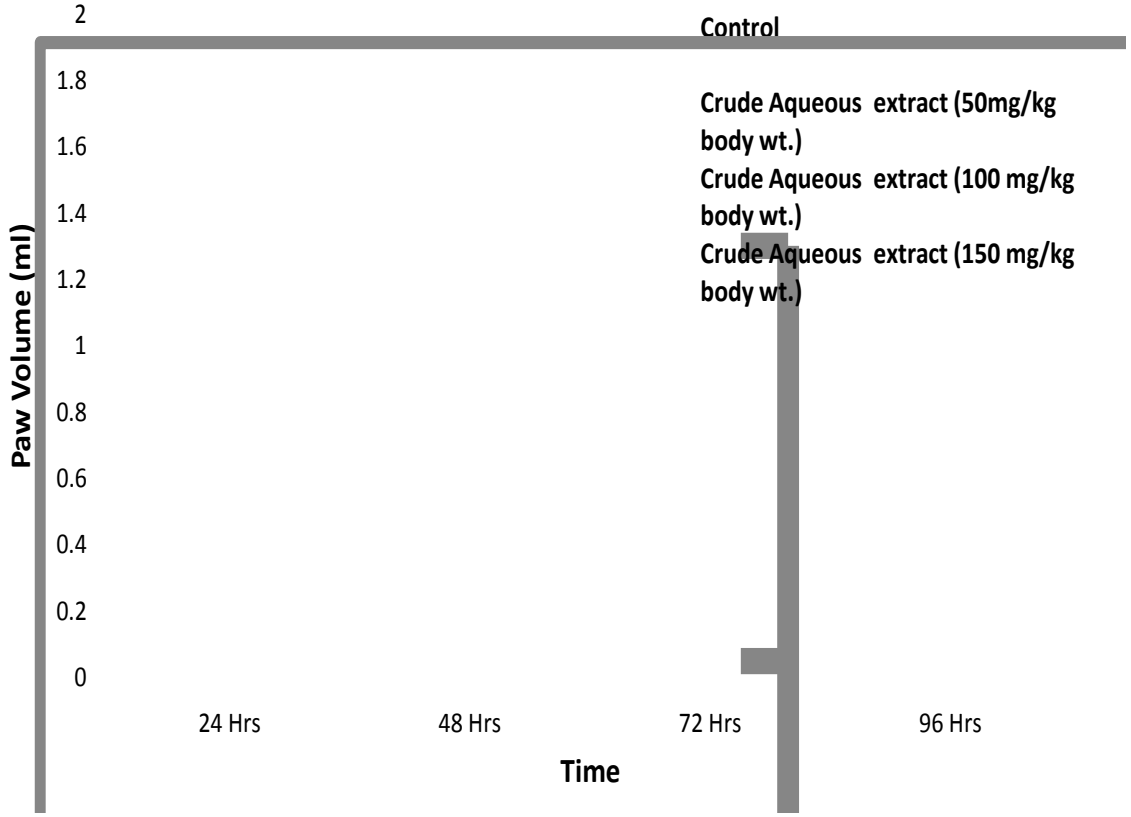


Table: 5.2.4 Effect of Ethanolic Extract of *Momordica dioica* on Delayed Type of Hypersensitivity.

	Groups	Paw Volume (ml) ± S.D.			
		24 Hrs	48 Hrs	72 Hrs	96 Hrs
I.	Control	1.59 ± 0.010	1.01 ± 0.029	0.64 ± 0.021	0.31 ± 0.016
II.	Crude Ethanolic extract (50mg/kg body wt.)	1.64 ± 0.022	0.95 ± 0.018	0.59 ± 0.016	0.28 ± 0.024
III.	Crude Ethanolic extract (100 mg/kg body wt.)	1.69 ± 0.014*	0.92 ± 0.019	0.57 ± 0.021	0.26 ± 0.016
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	1.64 ± 0.020	0.89 ± 0.024*	0.58 ± 0.017	0.27 ± 0.023

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.4 Effect of Crude Ethanolic Extract of *Momordica dioica* on Delayed Type of Hypersensitivity.

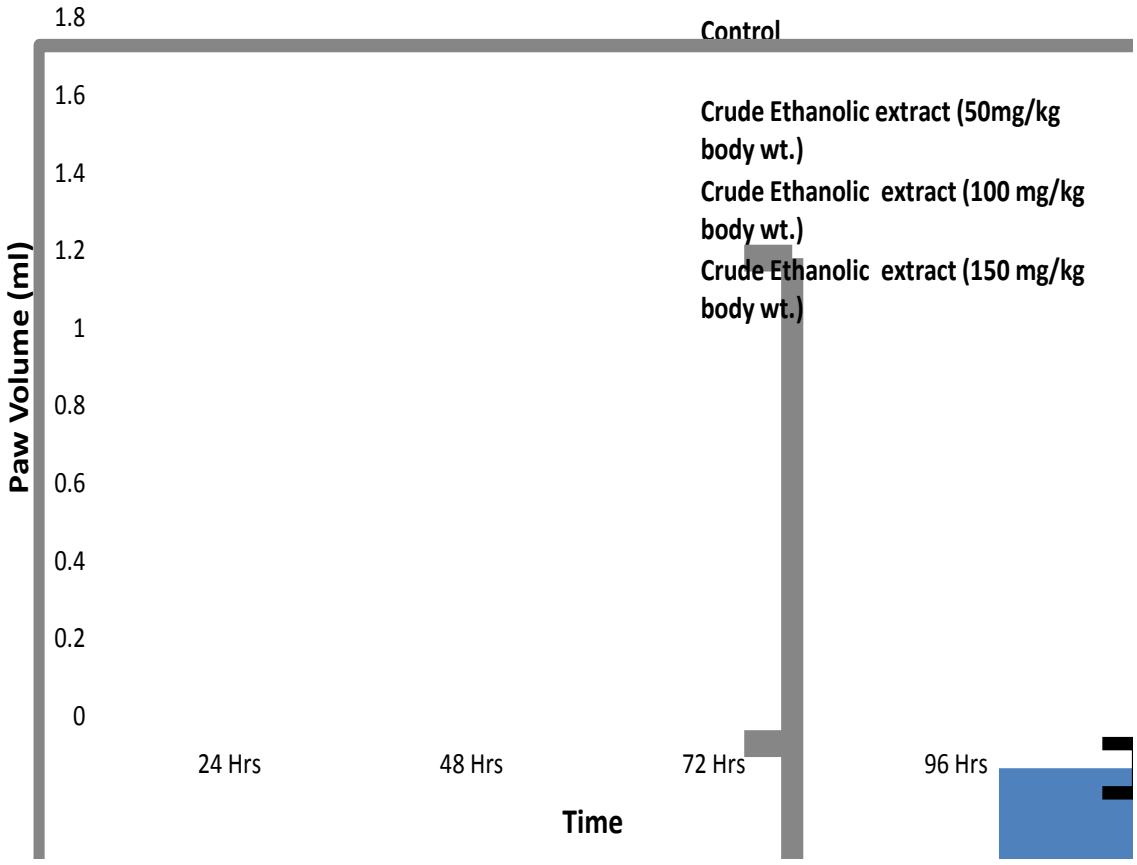


Table: 5.2.5 Effect of Crude Aqueous Extract of *Momordica dioica* on Agglutination Titre to SRBC

	Groups	Serum Dilution in Normal Saline ± 50µl antigen				
		X: 20	X: 40	X: 80	X: 160	X: 320
I.	Control	+	+	+	-	-
II.	Crude Aqueous extract (50mg/kg body wt.)	+	+	+	-	-
III.	Crude Aqueous extract (100 mg/kg body wt.)	+	+	+	+	-
IV.	Crude Aqueous extract (150 mg/kg body wt.)	+	+	+	+	+

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.5 Effect of Crude Aqueous Extract of *Momordica dioica* on Agglutination Titre to SRBC.

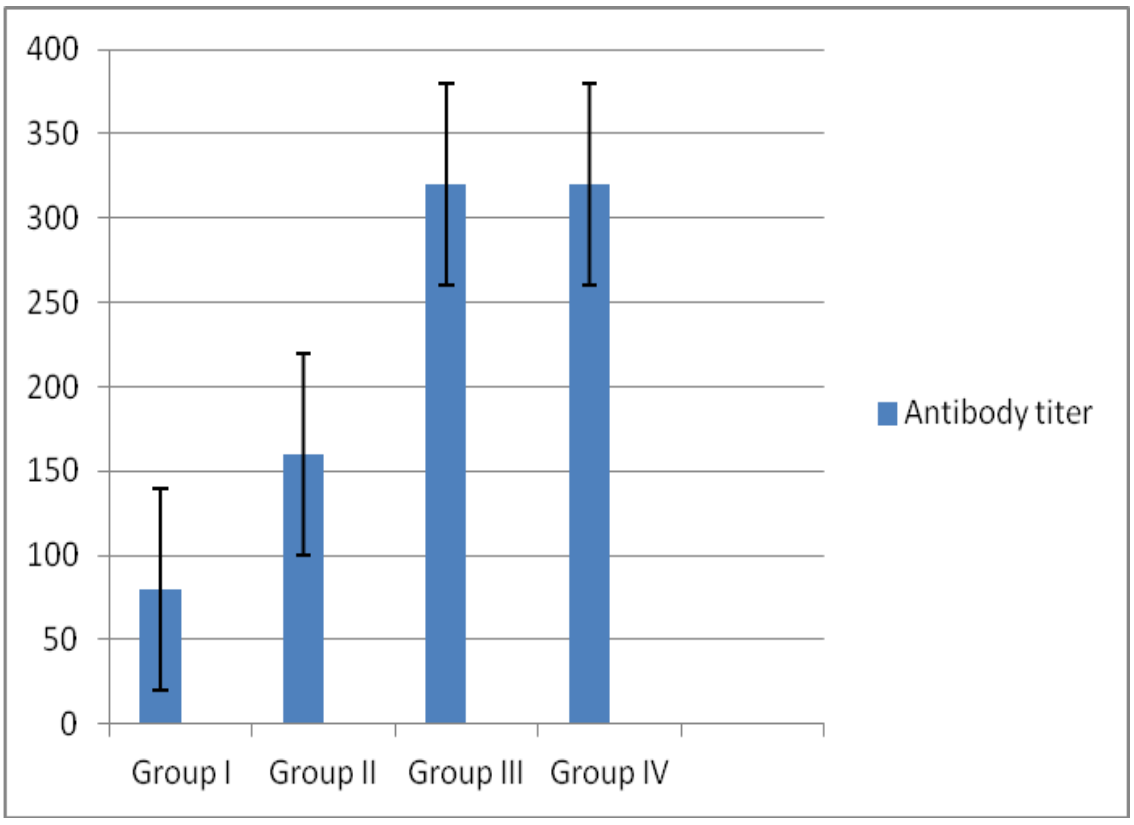


Table: 5.2.6 Effect of Ethanolic Extract of *Momordica dioica* on Agglutination Titre to SRBC

	Groups	Serum Dilution in Normal Saline ± 50µl antigen				
		X: 20	X: 40	X: 80	X: 160	X: 320
I.	Control	+	+	+	-	-
II.	Crude Ethanolic extract (50mg/kg body wt.)	+	+	+	-	-
III.	Crude Ethanolic extract (100 mg/kg body wt.)	+	+	+	-	-
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	+	+	+	+	-

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.6 Effect of Crude Ethanolic Extract of *Momordica dioica* on Agglutination Titre to SRBC

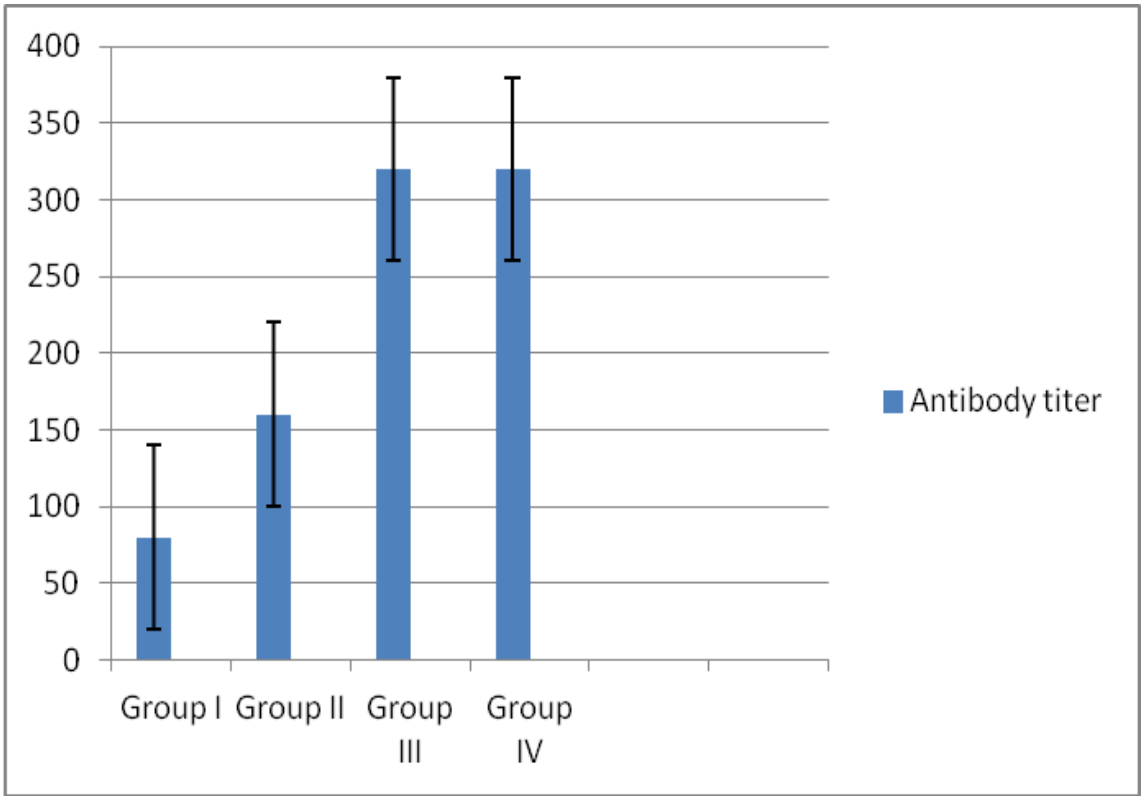


Figure: 5.2.7 Effect of Crude Aqueous Extract of *Momordica dioica* on Haemoglobin concentration.

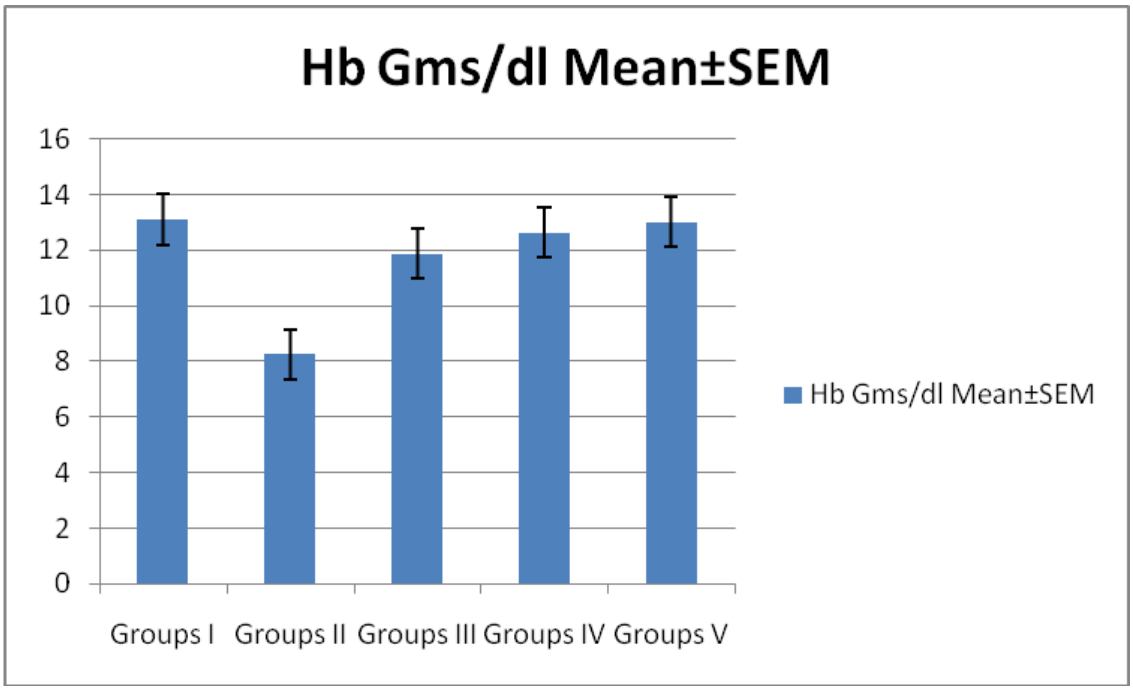


Figure: 5.2.8 Effect of Crude Aqueous Extract of *Momordica dioica* on RBCs Count.

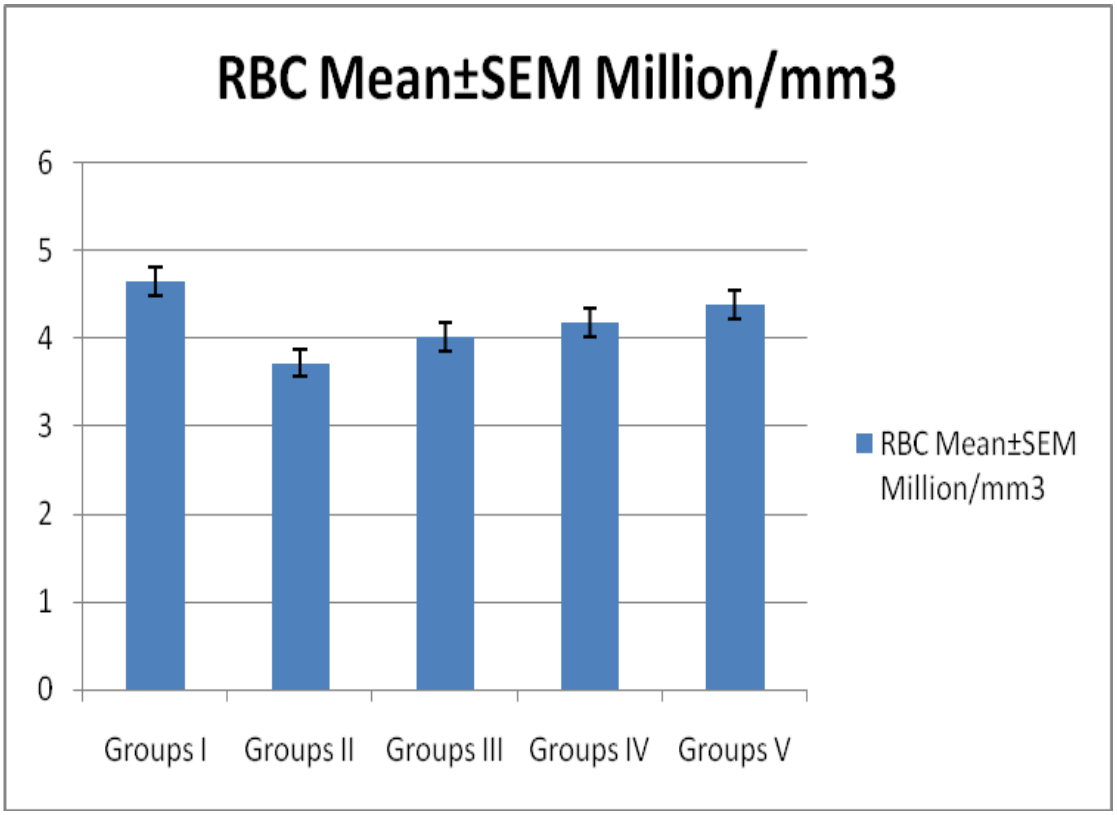


Figure: 5.2.9 Effect of Crude Aqueous Extract of *Momordica dioica* on WBCs count.

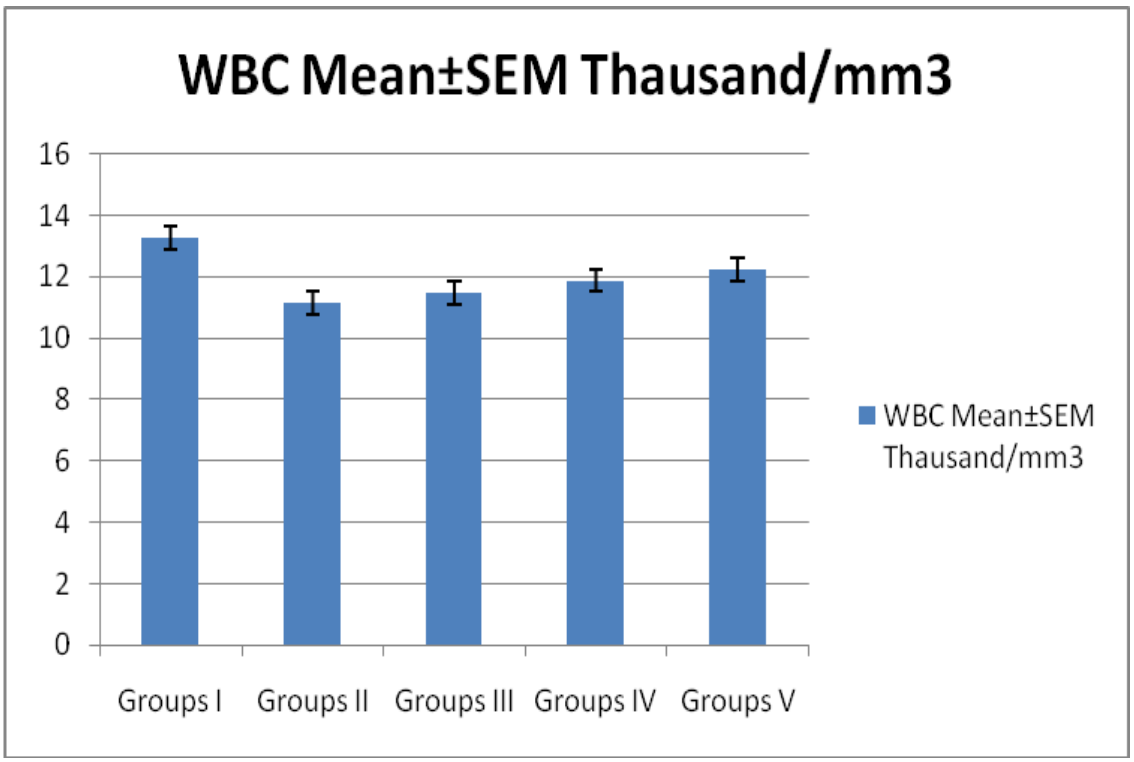


Figure: 5.2.10 Effect of Crude Aqueous Extract of *Momordica dioica* on Neutrophills Percentage.

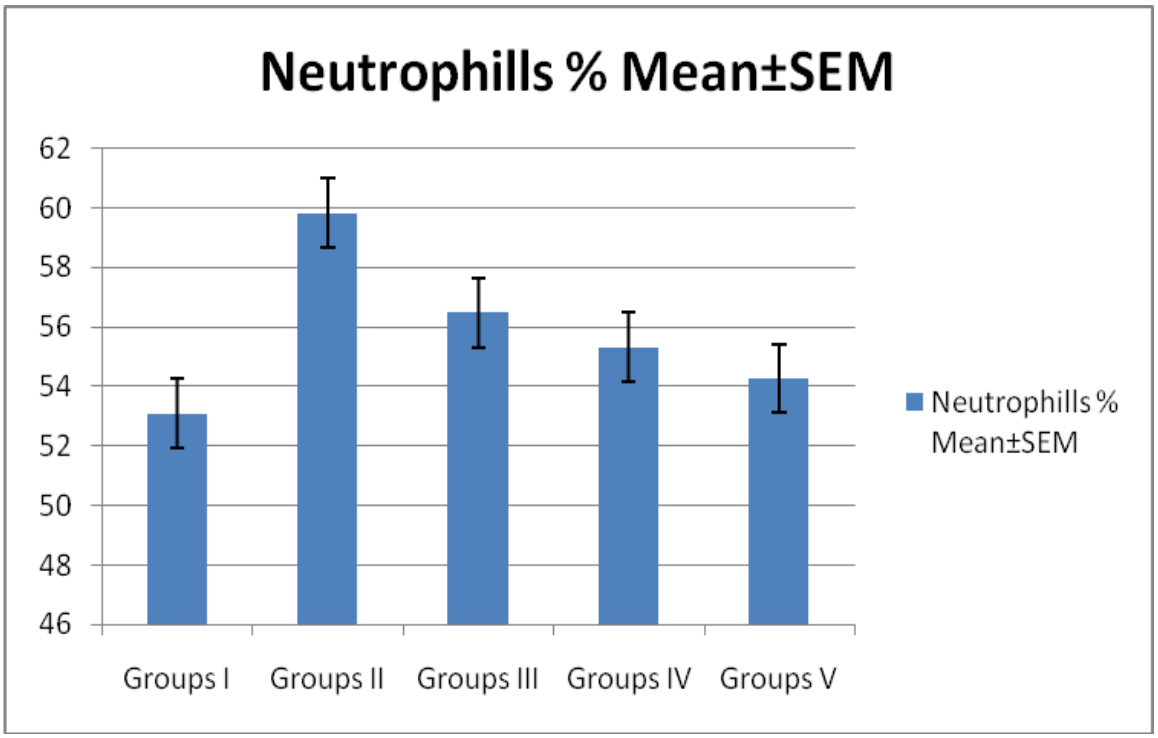


Figure: 5.2.11 Effect of Crude Aqueous Extract of *Momordica dioica* on Lymphocytes Percentage.

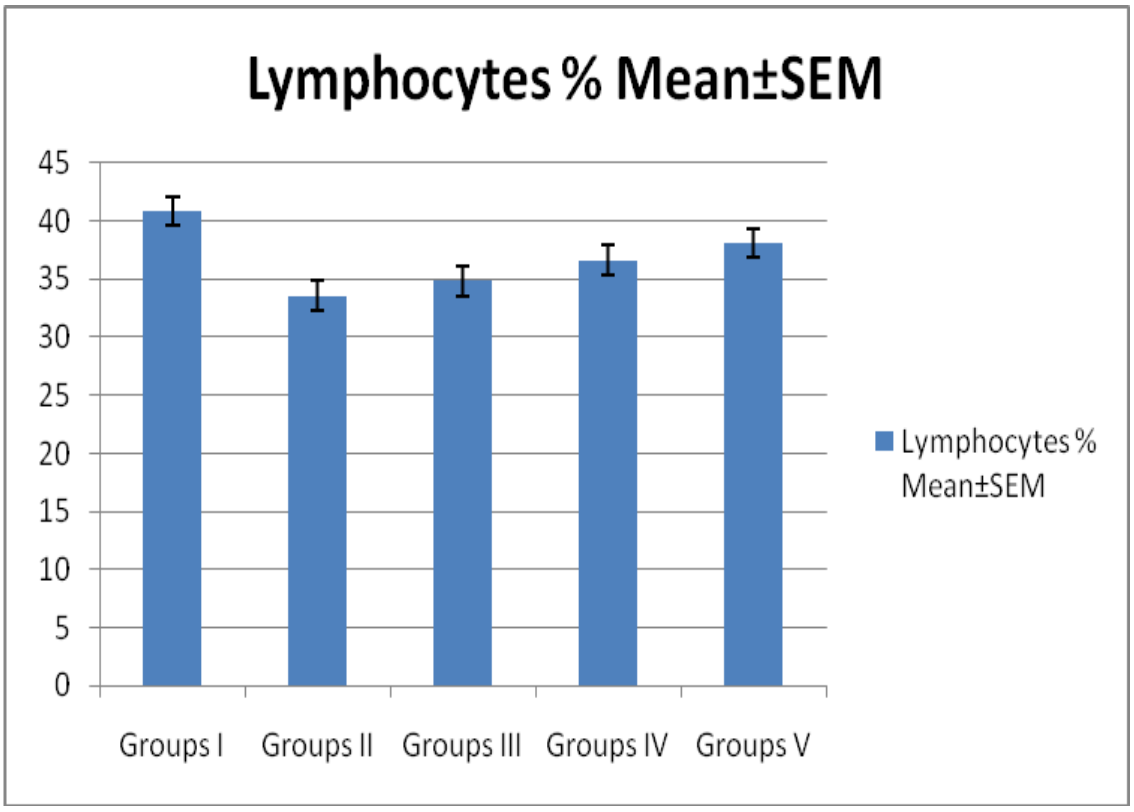


Figure: 5.2.12 Effect of Crude Aqueous Extract of *Momordica dioica* on Monocytes Percentage.

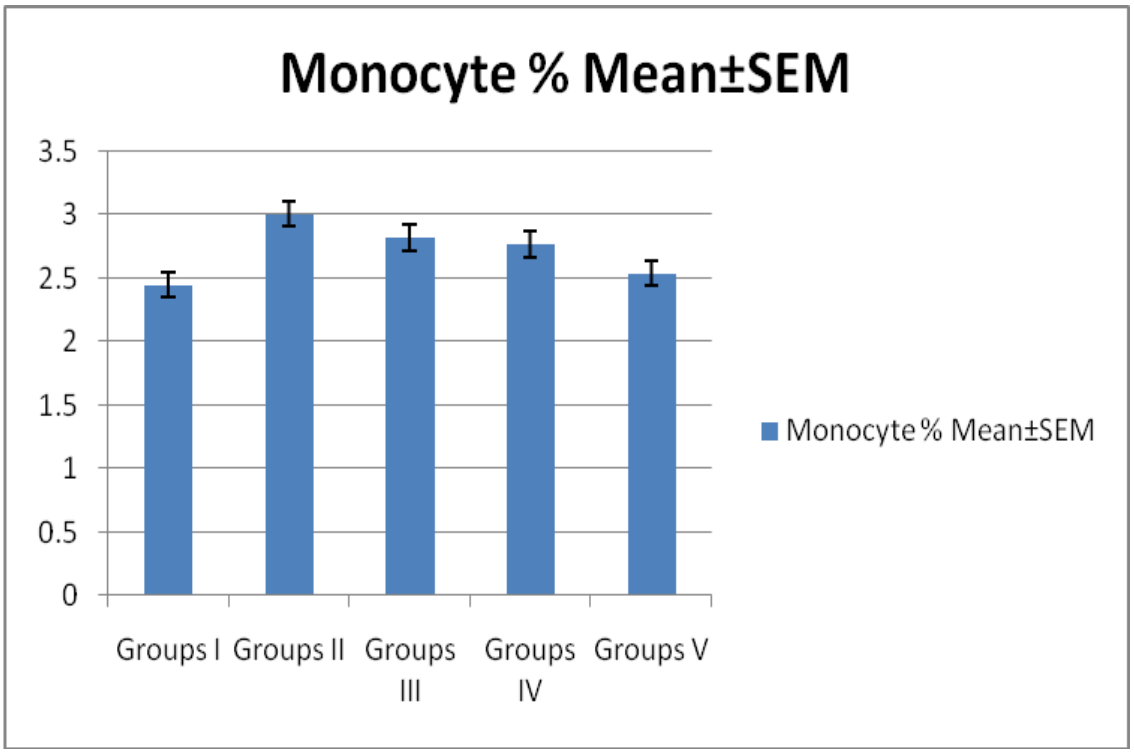


Figure: 5.2.13 Effect of Crude Aqueous Extract of *Momordica dioica* on Eosinophills Percentage.

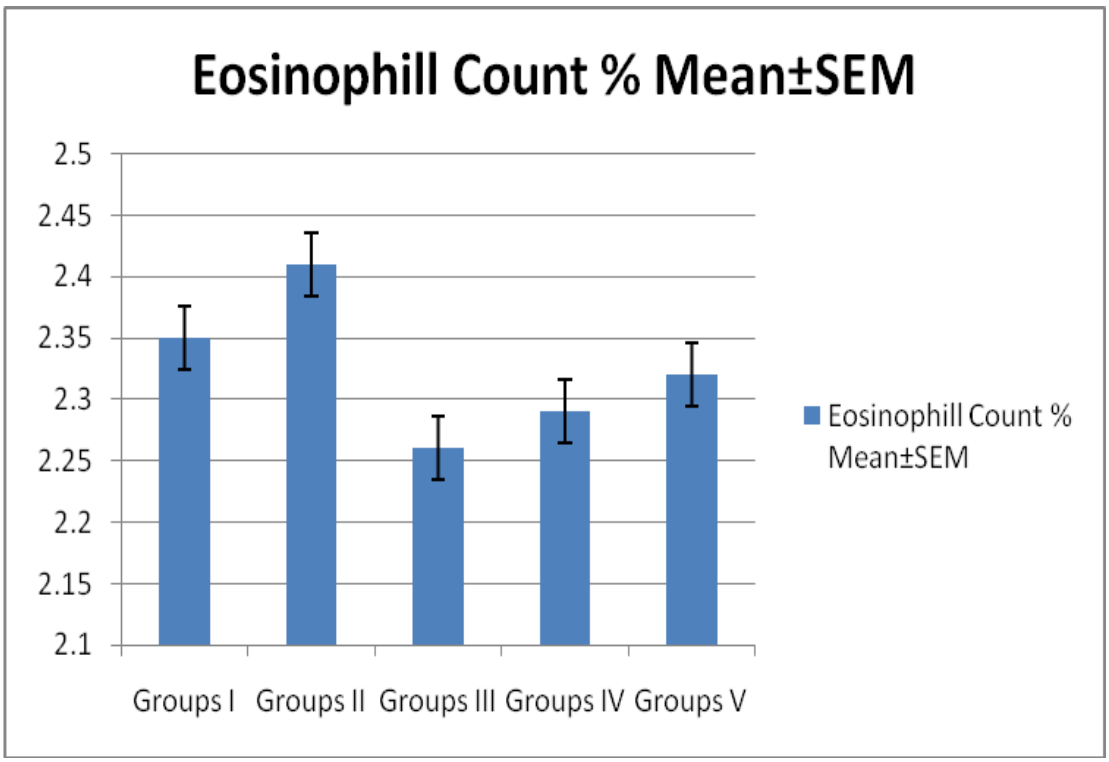


Figure: 5.2.14 Effect of Crude Aqueous Extract of *Momordica dioica* on Platelets count.

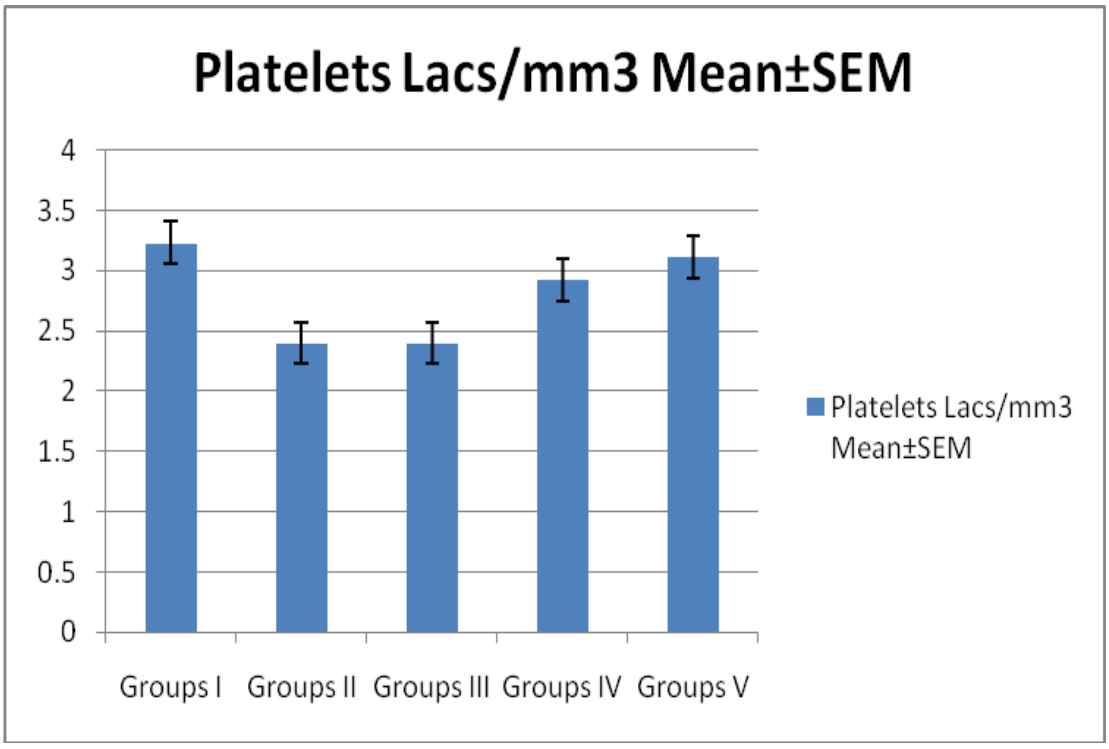


Figure: 5.2.15 Effect of Crude Ethanolic Extract of *Momordica dioica* on Haemoglobin concentration.

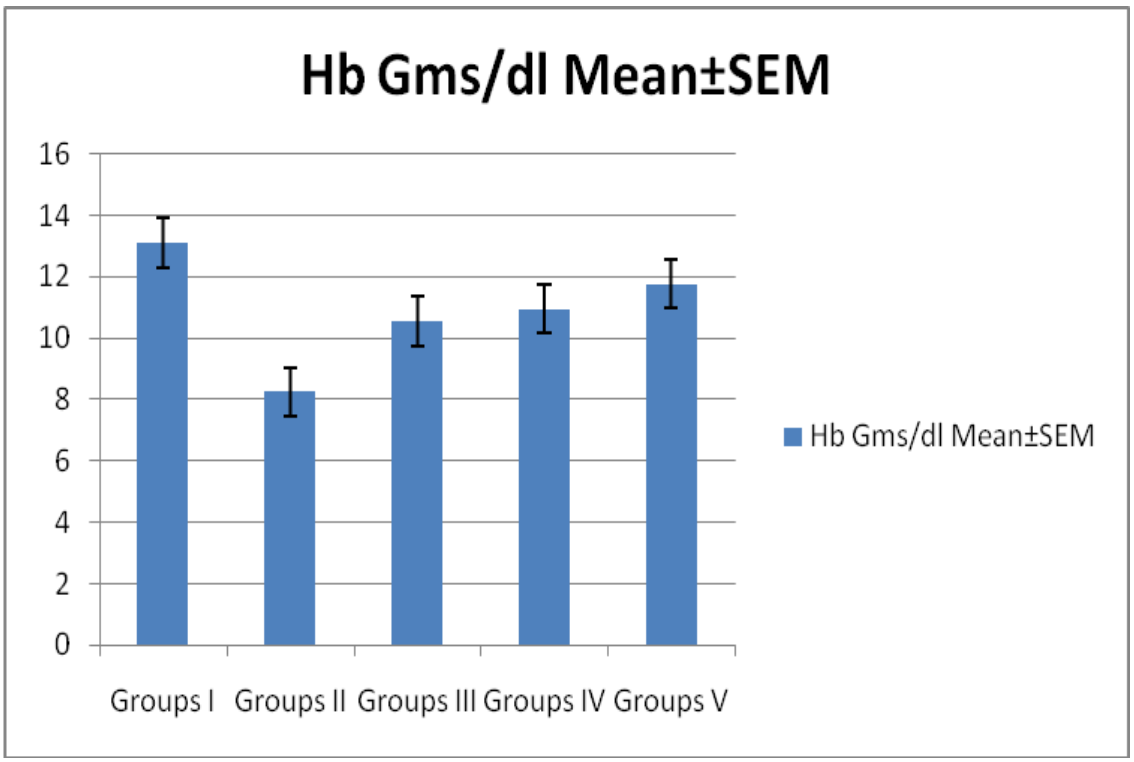


Figure: 5.2.16 Effect of Crude Ethanolic Extract of *Momordica dioica* on RBCs count.

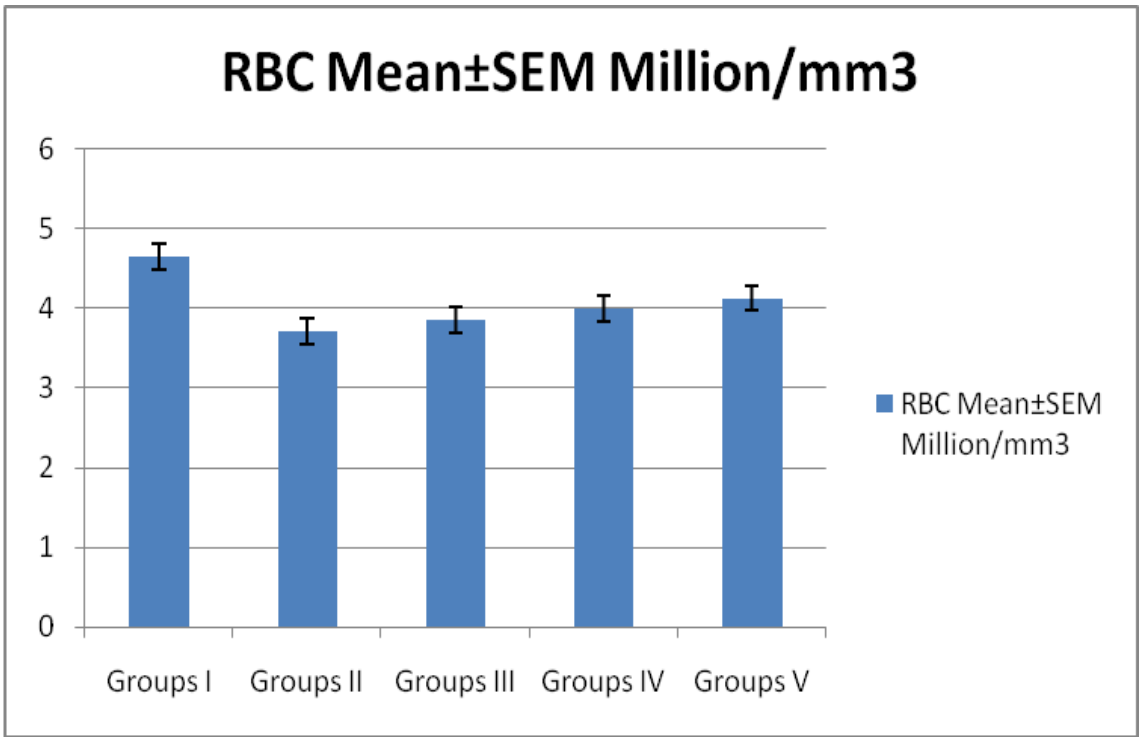


Figure: 5.2.17 Effect of Crude Ethanolic Extract of *Momordica dioica* on WBCs count.

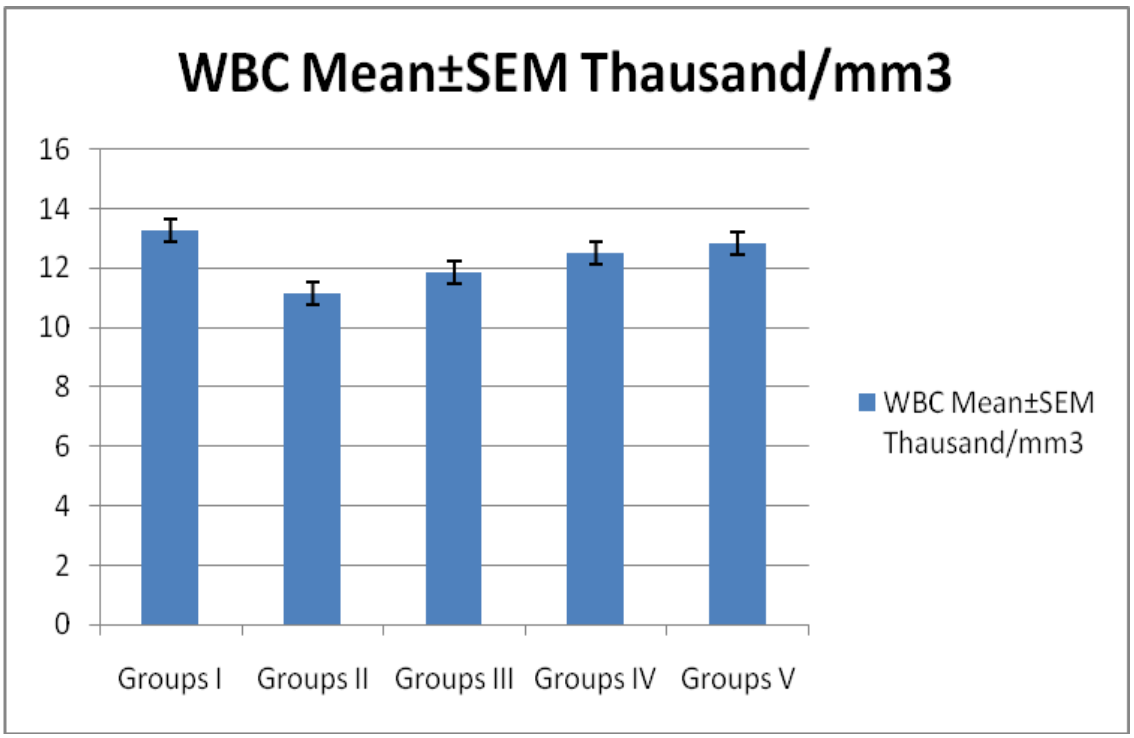


Figure: 5.2.18 Effect of Crude Ethanolic Extract of *Momordica dioica* on Neutrophills Percentage.

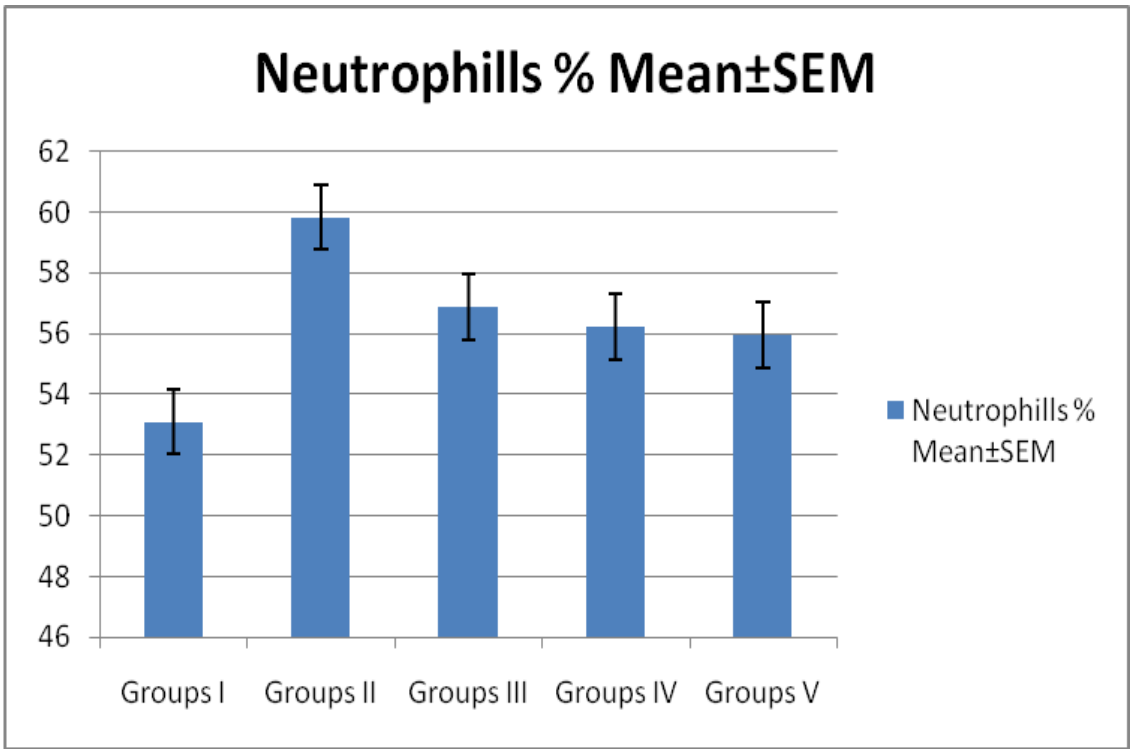


Figure: 5.2.19 Effect of Crude Ethanolic Extract of *Momordica dioica* on Lymphocytes Percentage.

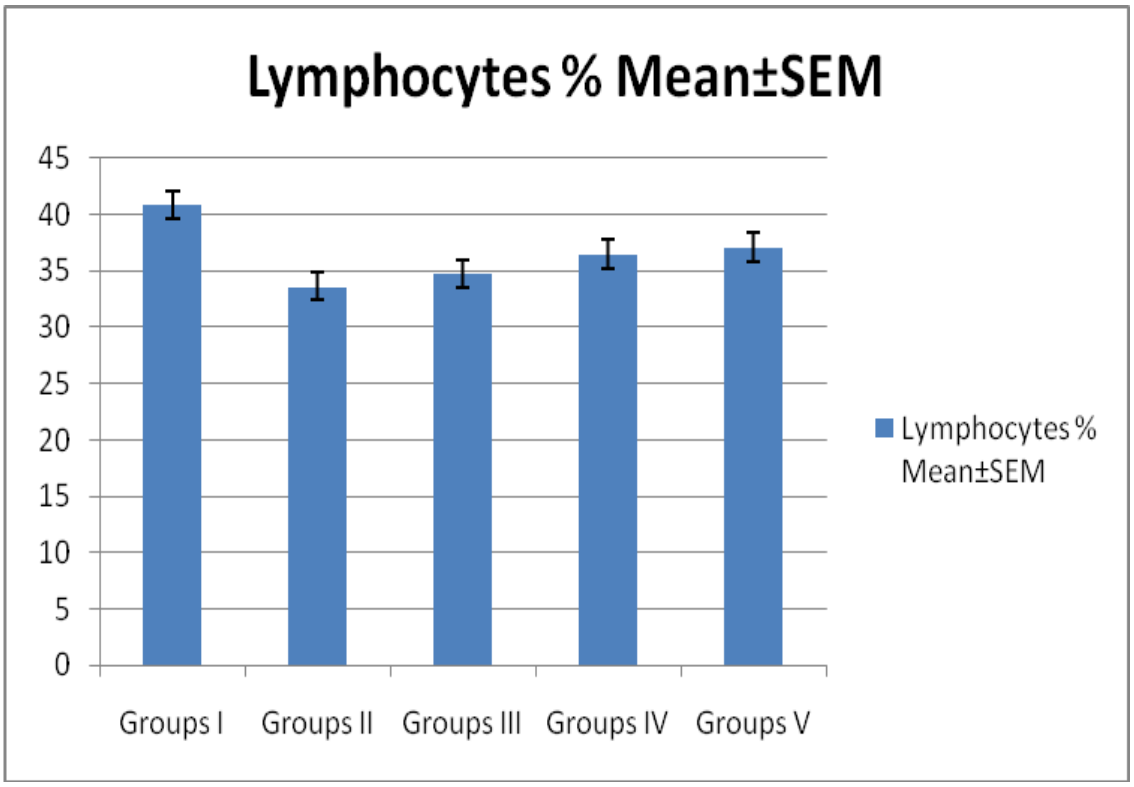


Figure: 5.2.20 Effect of Crude Ethanolic Extract of *Momordica dioica* on Monocytes Percentage.

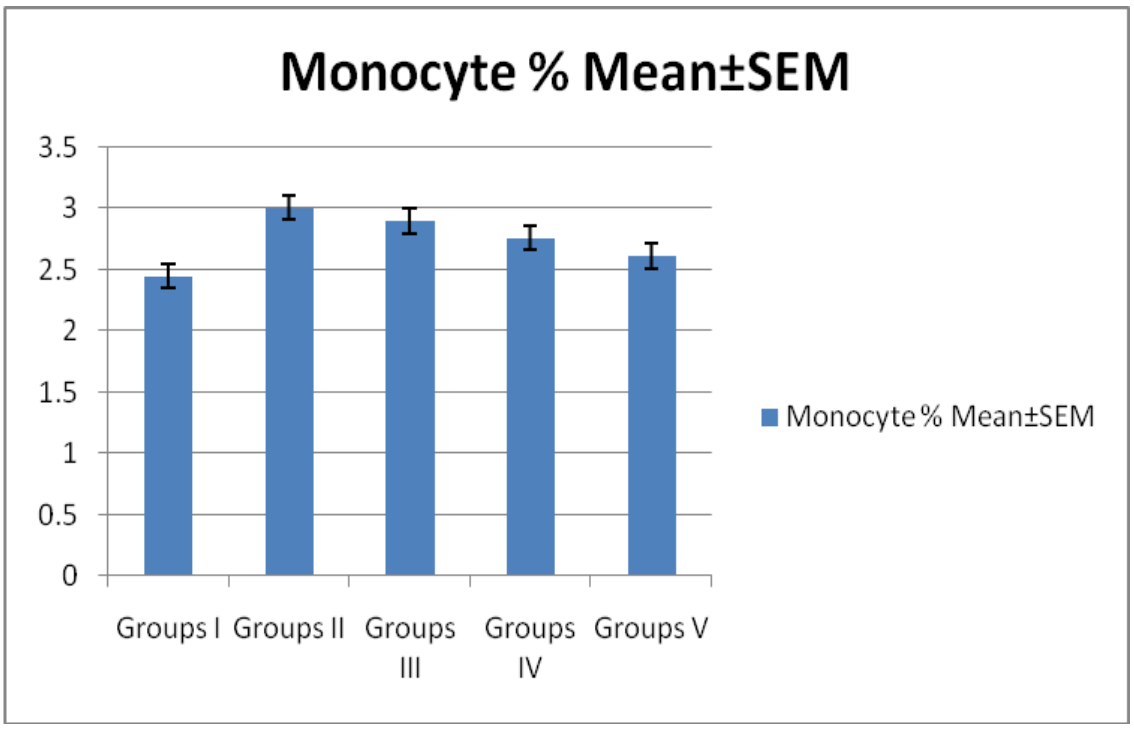


Figure: 5.2.21 Effect of Crude Ethanolic Extract of *Momordica dioica* on Eosinophill Percentage.

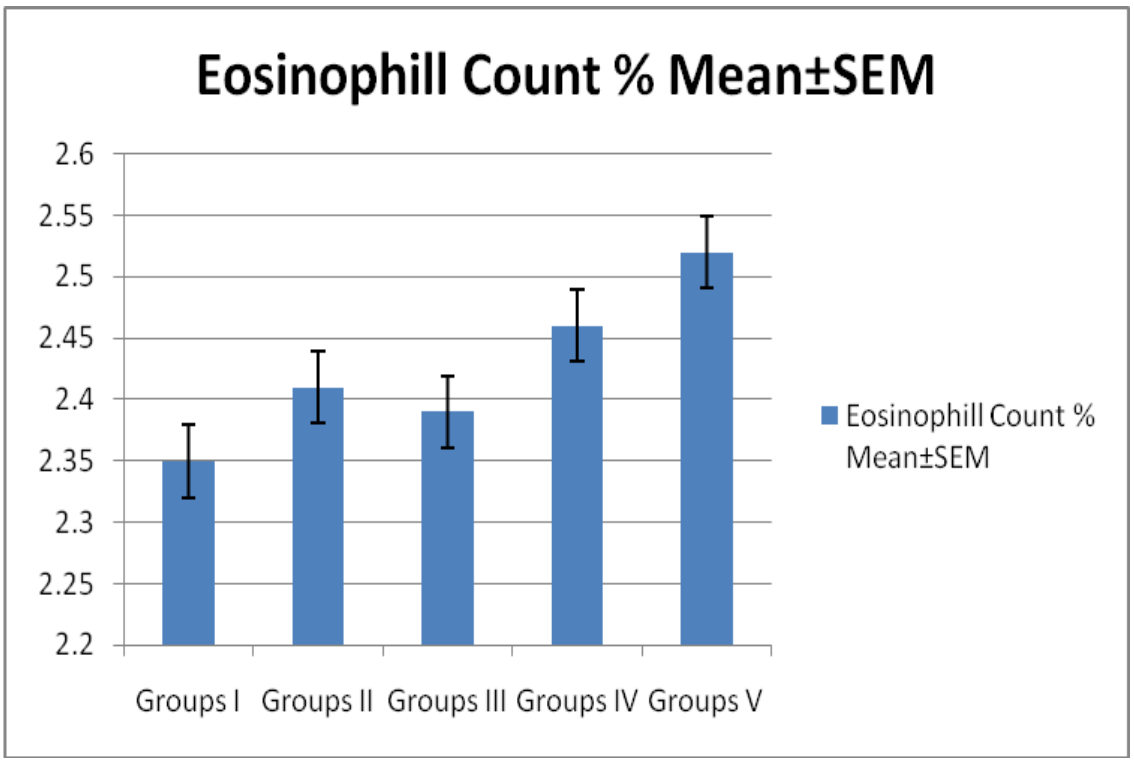


Figure: 5.2.22 Effect of Crude Ethanolic Extract of *Momordica dioica* on Platelets count.

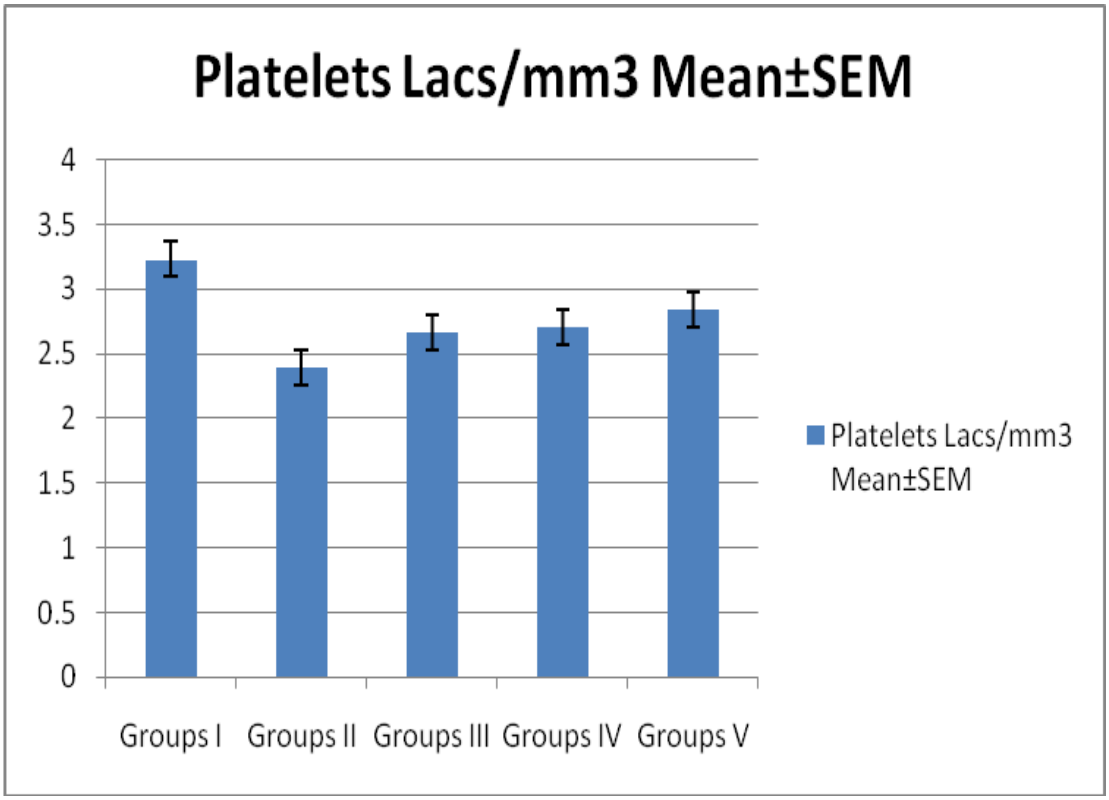


Table: 5.2.9 Effect of Crude Aqueous Extract of *Momordica dioica* on cytokines (IL-2 and IL-6)

	Groups	IL-2 concentration in mice serum (pg/ml) Mean ± S.D.	IL-6 concentration in mice serum (pg/ml) Mean ± S.D.
I.	Control	24.21 ± 1.352	30.58 ± 2.846
II.	Crude Aqueous extract (50mg/kg body wt.)	45.59 ± 5.381*	32.72 ± 2.811
III.	Crude Aqueous extract (100 mg/kg body wt.)	48.94 ± 4.817*	39.65 ± 2.106
IV.	Crude Aqueous extract (150 mg/kg body wt.)	55.18 ± 6.279*	42.19 ± 3.352

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.23 Effect of Crude Aqueous Extract of *Momordica dioica* on cytokines (IL-2 and IL-6)

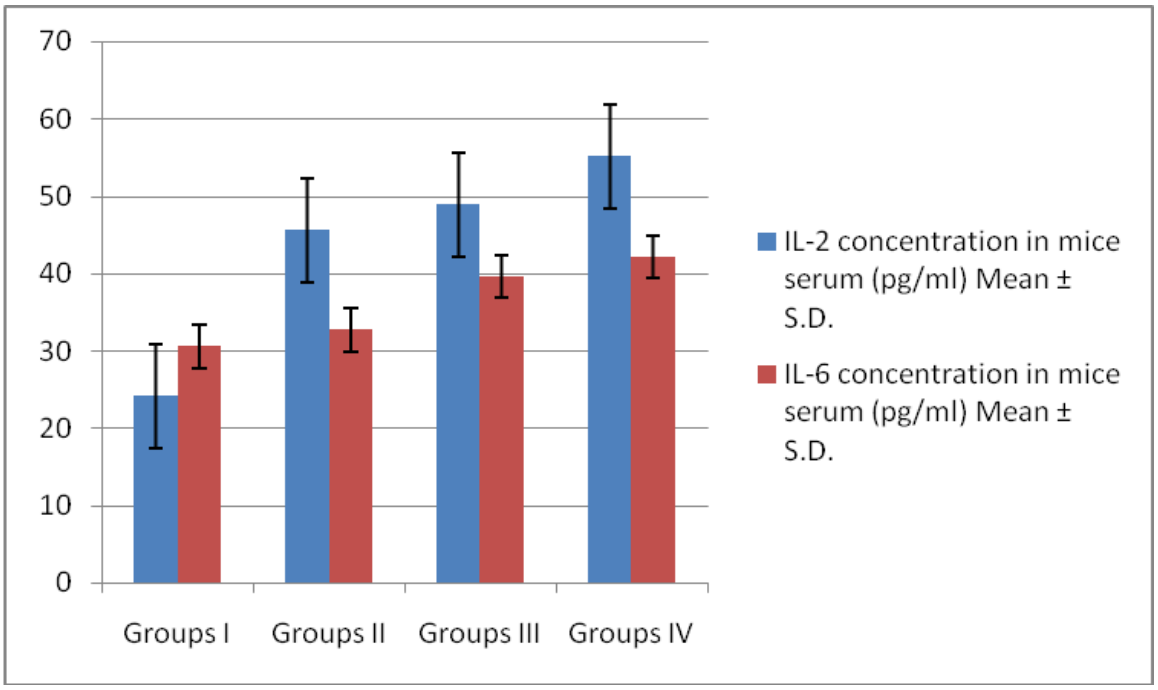
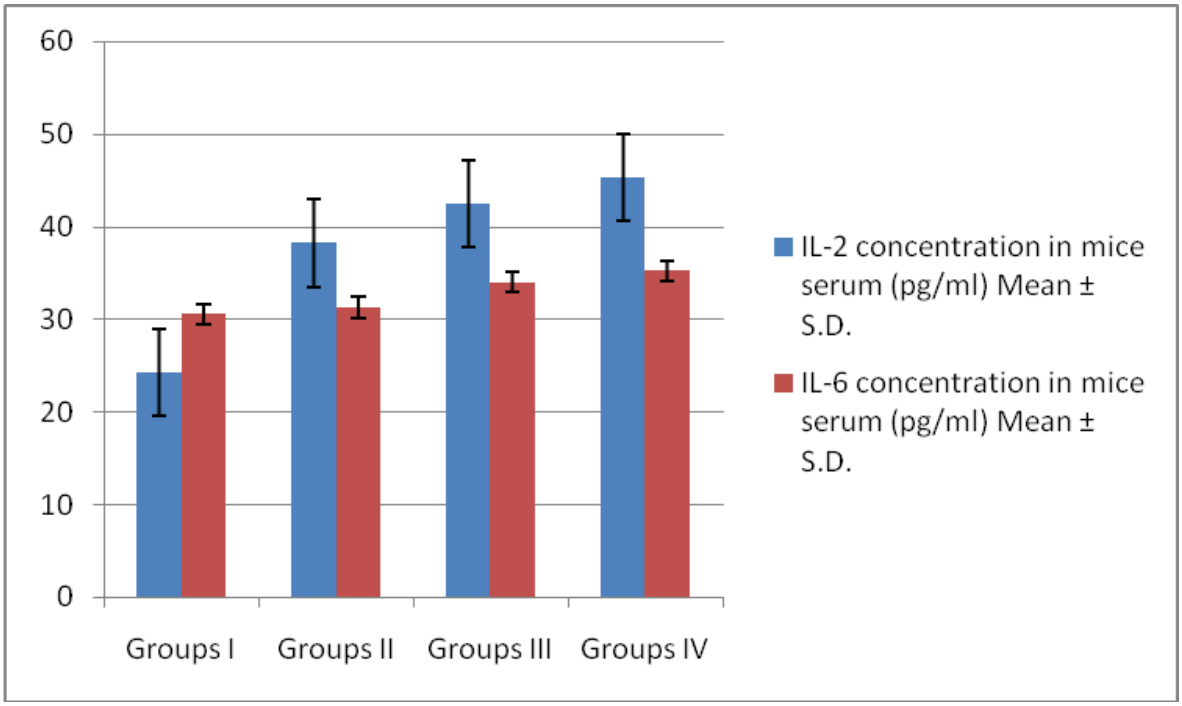


Table: 5.2.10 Effect of Ethanolic Extract of *Momordica dioica* on Cytokines (IL-2 and IL-6)

	Groups	IL-2 concentration in mice serum (pg/ml) Mean ± S.D.	IL-6 concentration in mice serum (pg/ml) Mean ± S.D.
I.	Control	24.21 ± 1.352	30.58 ± 2.846
II.	Crude Ethanolic extract (50mg/kg body wt.)	38.22 ± 6.217	31.29 ± 3.020
III.	Crude Ethanolic extract (100 mg/kg body wt.)	42.52 ± 5.391	33.99 ± 4.519
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	45.37 ± 6.254*	35.25 ± 2.982

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 5.2.24 Effect of Crude Ethanolic Extract of *Momordica dioica* on Cytokines (IL-2 and IL-6)



5.3 DISSCUSSION OF MOMORDICA DIOICA

Momordica dioica Roxb. (Cucurbitaceae) is commonly known as a bitter gourd. It is traditionally used as astringent, febrifuge, antiseptic, antihelminthic and spermicidal. It is also used in bleeding piles, urinary infection and as a sedative. Studies indicate that it possesses antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anti-lipid peroxidative, hypoglycemic and analgesic properties.

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor (Makare *et al.*, 2001).

The role of phagocytosis is the removal of microorganisms and foreign bodies, dead or injured cells. The increase in the carbon clearance index reflects the enhancement of the phagocytic function of mononuclear macrophage and non specific immunity. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by the opsonisation of parasites with antibodies and complementing C3b, leading to a more rapid clearance of parasites from the blood. AI appeared to enhance the phagocytic function by exhibiting a clearance rate of carbon by the cells of the reticuloendothelium system (Pallabi *et al.*, 1998).

The carbon clearance test was done to evaluate the effect of drugs on the reticuloendothelial system. The reticuloendothelial system (RES) is a diffuse system consisting of phagocytic cells. Cells of the RES play important role in the clearance of particles from the bloodstream. When colloidal carbon particles in the form of ink are injected directly into the

systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation (Gokhale *et al.*, 2003).

Momordica dioica increases the rate of phagocytic index with respect to control. It was observed that crude aqueous extract and crude ethanolic extract enhance the phagocytic index significantly. Increase in phagocytic index is suggestive of activation of WBC. Increase was dependent on the dose in the case of crude aqueous extract, as the dose increases phagocytic index also increases. Results of these studies clearly indicate that *Momordica dioica* activates the process of phagocytosis. The extract influences the role of neutrophils, digestive enzymes in phagocytic vesicle, and the synthetic processes in the cytoplasm. In treated animal, hyper granulation of WBC is the evidence of it. The secretary material appeared in the cytoplasm is to meet the necessity of the cell to phagocytose and digest the antigen. Stimulation of phagocytosis was influenced by the activation of macrophages, these activated macrophages secrete a number of cytokines such as IL-1, IL-2, etc. (Sonada *et al.*, 1998) and that in turn mobilize the immune cell. Aqueous as well as ethanolic extract significantly influenced and activated microphages. Gao *et al.*, 1989 also reported aggregation and activation of neutrophils when expressed to the extract of different plants. *Momordica dioica* has saponins, flavones and other compounds as it is mentioned earlier the compound in this plants have different R_f values hence, their chemical composition would be slightly different and so also their effects on the body. The comparative effects of the extract on phagocytosis vary. The mode of action of the compounds on FCRIII, CRb and CRBi may vary in intensity and the same may be in case antibody and the complement proteins. It is obvious that some of the constituents have definite effect on myeloid tissue directly or through interleukins.

Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). DTH requires the specific recognition of a given antigen by activated T lymphocytes, which

subsequently proliferate and release cytokines (Miller *et al.*, 1991). These in turn increase vascular permeability, induce vasodilatation, macrophage accumulation, and activation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. When activated TH1 cells encounter certain antigens, viz. SRBCs. They secrete cytokines that induce a localized inflammatory reaction called delayed type hypersensitivity. DTH comprises of two phases, an initial sensitization phase after the primary contact with SRBCs antigen (Bafna and Mishra, 2004).

During this period TH1 cells are activated and clonally expanded by APC (antigen presenting cells) with class II MHC molecule (e.g. langerhans cells and macrophages are APC involved in DTH response). A subsequent exposure to the SRBCs antigen induces the effector phase of the DTH response, where TH1 cells secrete a variety of cytokines that recruits and activates macrophages and other non specific inflammatory mediators. The delay in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages (Rao, 2006).

Both the extracts influenced T-Cell activity significantly which in turn increased vascular permeability induced vasodilatation, macrophage accumulation and activation, and which finally resulted in the increase in the paw volume which promoted phagocytic activity and also increased the concentration of lytic enzymes for more effective killing, this ultimately resulted in reducing the paw volume after 72 and 96 hrs.

The proteoglycons of *Momordica dioica* present in ethanol insoluble fraction when suspended in a solution and injected to the animal strongly behaved as chemo attractant especially to monocytes and other lymphocytes. This behavior itself is suggestive of activation of immune system at cellular level with lymphocytes. Cytokines, prostaglandulin E etc. are also liberating form the neighboring cells and the cumulative effect it termed as DTH (Steven, 2002).

Crude ethanolic extract did not make any significant increase. Since it may contain lesser amount of immunostimulating agents or having some other compounds, which may be partially antagonizing the stimulator. Increase in DTH reaction in mice in response to SRBC revealed the stimulatory effect to aqueous and ethanolic extract on T-lymphocytes and accessory cell types (Luster *et al.,* 1982, Elgert, 1996 and Descotes, 1999). The results reported here also in conformation. It can be opined that the contents present in *Momordica dioica* are much more effective and efficient enough to attract CD4 population of I lymphocytes, monocytes and other lymphocytes.

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody secreting cells. Antibody functions as the effectors of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross linking to form latex that is more readily ingested by phagocytic cells (Gokhale *et al.*, 2003). Aqueous and ethanolic extracts of *Momordica dioica* increased the agglutination titer to SRBC (antigen). In crude aqueous extract agglutination titer increased, as increase in the dose. Crude ethanolic extract did not show any significant increase in the agglutination titer as compared to control. On the other hand crude aqueous extract showed maximum increase the agglutination titer at the doses of 150 mg/ kg. b. wt. with these doses the agglutination was observed up to serum dilution of X: 320.

The crude ethanolic extract showed increase of agglutination titer at the dose of 150 mg/ kg b.wt. The titer was observed up to the serum dilution of X: 160. This indicated the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibodies production and gave higher agglutination titer with sheep red blood cells (Eisen, 1980).

Momordica dioica antagonizes the myelosuppressive effect induced by cyclophosphamide, which produces significant myelosuppression in experimental animal. By the administration of cyclophosphamide

haemoglobin, RBC count, WBC count, lymphocyte, monocytes, eosinophil count and platelet count decrease significantly (Doherty, 1981, Gill and Liw, 1978). But with the treatment of aqueous and ethanolic extract many of the above parameters increase. This indicates the protection produced by the drug against cyclophosphamide. In the case of crude ethanolic extract increase was observed in a dose dependent manner but it was not significant in each case. Natural product like polypeptides, oligosaccharides, proteoglycons, saponins, flavonoids etc are some of the compounds which are reported to be responsible for the genesis of antibodies still few of them are also to negate the toxic effect of chemicals on haemopoietic tissue, myeloid and lymphoid tissue even some them have both qualities observed with *Momordica dioica*. The compound(s) through the path way ultimately activate(s) B- lymphocytes to form plasma cell, which in turn release particular type of antibodies. Appearance of some thicker protein bands during electrophoratic separation, increase in serum protein concentration and high titer for SRBC strongly indicate about humoral immunity stimulation. Cyclophosphamide is a known myelosuppressant agent causes to decrease immunological parameters but some compounds of the extract reduce the toxic effect or the components of the extract prevent the entry of cyclophosphamide or bind with this compound to make is insoluble or unite to form a molecule to bind receptor site to wash away the effect of the compound in an short period, *Momordica dioica* thus effective in both ways, to stimulate the immune system and to protect it from immunosuppressants.

The cyclophosphamide induced neutropenia model concentrates on the effect of drugs on the haemopoeitic system. *Morus alba* extract at low dose and high dose caused decrease in the cyclophosphamide induced neutropenia suggesting that it attenuates the effect of cyclophosphamide on the haemopoetic system (Diwanay *et al.*, 2004).

In vivo and *in vitro* studies on some herbs do suggest that the immunomodulating effects of the botanical medicines, at least in part, to

cytokine modulation. Furthermore, the broad-spectrum effect of cytokines on cell-to-cell communication, it seems likely some of the other organ systems and tissue effects of the herbal immunomodulators are due to modulation of cytokine expression. Both crude aqueous and ethanolic extracts enhanced IL-2 levels in a dose dependent manner while the IL-6 showed almost insignificant increase in its levels.

The qualitative analysis of IL-2 in both control and experimental animals was assessed and correlated with significant increase in WBCs count/lymphocyte count in experimental animals. So for IL-6 is concerned its increase can be correlated with increase paw volume. The cytokines whenever increase in very low concentration a definite effect is produced. IL-2 is formed to increase with many plants extracts as reported by Ganguly *et al.*, 2001 and Bone, 1996. These low molecular weight proteins activate the receptors on lymphocytes to cure these sensitivity a growth and activity. IL-2 also stimulates other cellular effectors like GCFs and GMCSF although these factors are not estimated but an indirect conclusion about their increase can be made which inhibits WBCs count and paw edema test. Probably the glucosteroids and flavones which are present in good quantity in the extracts, they are directly or indirectly responsible to elevate the cytokines.

The effects of cytokines on their target cells and tissues may be inhibited or enhanced by other cytokines, hormones, and cytokine-receptor antagonists and circulating receptors. Just as pharmacological activity by specific plant constituents is suggested to be affected by combinations of constituents (Spelman *et al.*, 2006, Gilbert and Alves, 2003, Wills, 2000) combinations of cytokines have been found to have additive, inhibitory, or synergic effects (Gabay and Kushner, 1999). Further research may find that the herbal immunomodulators affecting multiple cytokines which can each generate a unique signature of immune perturbation, dependent on the concerted effect on arrays of cytokines.

A. membranaceus, in an *in vitro* human model, has been shown to lower IL-6 (Shon, 2002). IL-6 is implicated in a number of inflammatory disorders and as a global marker of impending deterioration (Kiecolt-Glaser *et al.*, 2002). The decrease of IL-6 activity provides a possible rationale for thousands of years of use of this plant in deficiency and wasting diseases. In addition, Astragalus is also indicated in shortness of breath and edema, symptoms that could be suggestive of cardiovascular effects. Notably, increased levels of IL-6 and C-reactive protein are associated with a significant increase in cardiovascular-related death (Kiecolt-Glaser *et al.*, 2002, Rader, 2000). Thus, a possible mechanism for the cardiovascular effects of *A. membranaceus* could be due to its reduction of IL-6.

A polysaccharide, angelan, isolated from roots of *Angelica gigas*, was shown to trigger the release of cytokines IL-2, -4, -6 and INF-γ from macrophages. Cytokine-release was found to occur in a sequential manner, with IL-6 presenting an almost immediate increase, followed by IL-4, with IL-2 having the slowest rate of increase (Sang *et al.*, 1998).

The electrophoretic pattern of drug treated animal is almost similar to that of serum proteins electrophoretic pattern of control. Both the extracts of *Momordica dioica* did not elicit any response for the formation of new serum protein. Possibly the technique may not be show much sensitive to detect the new protein which appeared in very little concentration in blood as the constituents of the extracts did not produced new protein. This cannot be ignored that the constituents of both extracts of the plant exhibited a definite immunostimulatory effect as evidence by various parameters for immunostimulation increased phagocytic index and SRBC test go in favour of the drugs for immunostimulation. The increased concentration of serum protein (The reading is not incorporated here) also points to the fact that the liver and probably the plasma cells also produced to increase the concentration of serum protein. Serum of the drug treated animal showed a more darkened spots in comparison to that of control. This shows that the

constituents of the drug having effects either on liver are on B lymphocytes and plasma cells.

Finding of these studies suggest that the both the extracts are capable to strengthen the immune system. Both the extract modulate immune responses significantly as they increase the phagocytic index, modulate the phagocytic functions of macrophages and phagocytes, which means they have a profound effect over the innate immunity. They also modulate the function of cytotoxic T-cell that produces delayed type hypersensitivity immune response, which gives a better protection against viruses and tumors. They also increase the antibody titer, which means modulation of humoral immunity.