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"A COMPARATIVE STUDY OF IMMUNOMODULATORY ACTIVITY OF *ACTIDINIA DELICOSA* CV. HAYWARD FRUIT AND *BRASSICA OLEARACEAE* VAR.CAPITATA LEAF IN EXPERIMENTAL ANIMALS"

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Abstract:

Aim: Comparative study of Immunomodulatory activity of *Actidinia delicosa* cv. Hayward fruit and *Brassica olearaceae* var. Capitata leaf in experimental animals.

Material and Method: The assessment of immunomodulatory activity of ethanolic extract of *Actidinia delicosa* cv. Hayward fruit (200 and 400 mg/kg p.o.) and methanolic extract of *Brassica olearaceae* var. Capitata leaf (250 and 500 mg/kg, p.o.) were done by Carbon clearance test and Neutrophil adhesion test for Non-specific immunity, Delayed Type Of Hypersensitivity Reaction for Cell mediated immune response, and T-Cell population test.

Result: The present studies suggest that the methanolic extract of *Brassica olearaceae* var. Capitata leaf was found to possess significant Immunostimulatory activity on Immune system than the ethanolic extract of *Actidinia delicosa* cv. Hayward fruit in dose dependent manner when compare with control group.

Keywords: Immunostimulant action, kiwi, red cabbage, Levamisole, etc

Index Terms - Immunostimulant action, kiwi, red cabbage, Levamisole, etc

I. INTRODUCTION

The tendency of human body to fight against several infections is complex biological systems of our body. It plays an important role in protecting the body from the harmful effects of microbial pathogens. Firstly, Immune response is to recognize pathogen or foreign substances and produce response to eliminate it. Therefore, most disease caused by an increase or decrease activity of the body's immune system.[1]

Immunomodulators referred to as substance, synthetic/biological, involves innate and adaptive branches of the immune response, which can enhance, suppress or regulate all parts of the body's resistance system.[2]

Immunopharmacology is a new and evolving branch of pharmacology whose goal is to find Immunomodulators. The advantages of Immunomodulators in clinical remedy involves the restoring of immune deficiency (such as treating AIDS) and the suppressing normal or excessive immune function (such as graft rejection or autoimmune disease).[2]

Actidinia is a genus, commonly known as kiwifruit, which belong to the family actidinaceae. It is common all over the world, especially in East Asia. It is used to cure various types of cancers, including digestive system, cancer and breast cancer. Since kiwifruit as a health food and traditional medicine has a wide range of pharmacological and biological properties, people have renewed interest in its chemical components and biological activities. Biologically active plant ingredients, includes polysaccharides, alkaloids, saponins and organic acid. A number of studies has shown that kiwi juice can inhibit the growth of cancer cells.[3]

Cabbage (*Brassica olearaceae* L.var. Capitata) is an important vegetable grown in the world. It belongs to the cruciferous family, including broccoli, kale and cauliflower. Brassica family is rich in polyphenols, especially flavonoids. It also contains ascorbic acid, Vitamin C & E, Amino acid, flavonols, quercetin and kaempferol, lutein, and the glucosinolates. Red cabbage suffers from serious diseases, including cancers, neurodegenerative disorders, diabetes etc. Some studies have shown that cabbage may be an important source of antioxidant and anti-inflammatory associated with the prevention of continual illnesses associated with oxidative stress.[4,5]

Hence in present research work we have planned to compare immunomodulatory activity of *Actidinia delicosa* cv. Hayward fruit and *Brassica olearaceae* var. capitata leaf.

II. MATERIAL AND METHODS

Collection and extraction process of plant material

Preparation of Ethanolic extract of *Actidinia delicosa* cv. Hayward fruit:[6,7]

Fresh and ripe fruits were bought from local market in Sangli. The samples were washed with fresh water to remove impurities, and the samples were shade dried, pulverized and extracted with 99.9% ethanol in cold over a period of 5 days with occasional shaking. The extract was filtered & then concentrated by vacuum drying, the resulting concentrated crude extract was used for the experiments.

Preparation of Methanolic extract of *Brassica olearaceae* var. Capitata:[8]

Brassica olearaceae var. Capitata f. rubra (Red cabbage) were bought from the Satara local market. Methanolic extraction is a classical method for extracting anthocynins from plant materials, and also this method is a rapid, simple, and effective method for extracting anthocynins. This process involves soaking or agitation of the plant material in methanol containing low concentration (0.01%) of inorganic acid (HCL).

500 g of fresh plant *Brassica olearaceae* var. Capitata f. rubra (red cabbage) was homogenized (precisely weighed and recorded) in 2 volume (w/v) acidified methanol and allowed to extract overnight under refrigerated conditions. Slurry was filtered through a Whattman no.1 filter paper by vaccum suction using a buchnar funnel. The plant material was re-extracted with acidified methanol until a faint-colored extract was obtained. The filtrates were pooled and the plant material was discarded.(Three consecutive extractions are sufficient). Pooled filtrate was poured in a boiling flask and methanol was evaporated in a evaporator at 40^{o}C under vaccum. The dried material contains anthocynins obtained from *Brassica olearaceae* var. Capitata f. rubra were used for the present study. If the sample is to be analyzed in 2 days, stored the extract at 4^{o}C. For long-term (up to 2 year or even longer), store at -18^{o}C. Note: To avoid repeated freezing and thawing.

SELECTION OF DOSE:

In order to decide the dose of plant extract, it is essential to go through the toxicity study of the extracts according to OECD guidelines (IAEC/ABCP/01/2020-21). The drug did not show any sign of toxicity up to oral dose of 400 mg/kg and 500 mg/kg which is mentioned in already reported research papers.

Therefore, in the present study the dose of kiwi and red cabbage is 200, 400 mg/kg and 250,500 mg/kg has been fixed. They were given by oral route using oral gavage.[6,7]

STANDARD DRUG:

Levamisole is a synthetic derivative of tetramisole belongs to the group imidazole and is used extensively as a veterinary anthelmintic drug. It is available in market as levamisole hydrochloride. It fails to hyper stimulate the normal functioning immune system but can restore impaired cell-mediated immune responses to normal levels. Thus, it shows the primary mechanism of action of levamisole is suggested to facilitate the participation of monocytes in the cellular immune response apparently by enhancing monocytes chemotaxis. In addition it also claimed to increase DNA synthesis of T lymphocytes and to augment their proliferative responses to mitogens, as well as their production of mediators of cellular immunity in vitro.[9] When used as an anthelmintic, it acts as autonomic ganglionic stimulant and causes activation of parasympathetic and sympathetic nervous system. This cause continuous muscular contraction in the susceptible nematodes result in muscular paralysis. These effects are considered to be nicotinic like action. At high dosages, the levamisole also interferes with the parasites carbohydrates metabolites by preventing or blocking fumarate reductase and succinate oxidase enzymes.

EXPERIMENTAL ANIMAL:

All experiments were performed using male albino rat of wistar strain weighing in between 160 to 210 grams. The animals have free access to food and water, and maintain a natural (12hr each) light-dark cycle. Animals were acclimatized for atleast 5 day under laboratory conditions before the experiment. The experimental protocol was approved by the institutional animal ethics committee (IAEC/ABCP/01/2020-2021).

DRUGS AND CHEMICALS:

All the drugs and Chemical were of analytical grade while the other drugs were procured - Levamisole (Johnson & Johnson Ltd.), colloidal carbon (Indian Ink, camel India Pvt. Ltd.) kiwi and red cabbage (Indian Market)

DRUG TREATMENT:

In the present research work, the rats grouped into seven different groups consisting six rats in each group.
Screening method for Immunomodulatory activity as follows;[10]

- IN-VIVO METHODS:
  1. Delayed type of hypersensitivity reaction
  2. Carbon clearance test
  3. Neutrophil adhesion test
- IN-VITRO METHODS:
  1. T-Cell Population test

IN-VIVO SCREENING METHOD

DELAYED TYPE HYPERSENSITIVITY REACTION (21 DAYS MODEL)

- **Purpose and Rationale:**

DTH is a reaction of cell-mediated immunity and become visible only after 16-24 hours.

- **Procedure:**[11,12,13]

1. The animals were divided into seven groups comprising six animals in each.
2. Group-I (control group) received vehicle (water) only with a dose of 10 ml/kg b.w.p.o.
3. Group– II (standard group) received the std. drug Levamisole with a dose of 50 mg/kg b.w.p.o.
4. Group-III (Test 1) received the *Actidinia delicosa* extract with dose 400 mg/kg b.w.p.o.
5. Group-IV (Test 2) received the *Brassica olearaceae* extract with dose 500 mg/kg b.w.p.o.
6. Group V, VI and VII (Test 1 + Test 2) received the combination of extract of *Actidinia delicosa* and *Brassica olearaceae* with different doses.(i.e.400 +200 mg/kg, 200 +500 mg/kg, 400 +500 mg/kg b.w.p.o.)

Table No.1 . Grouping and treatment schedule for DTH test

Sr. No.	Groups	Test substance	Dose
1	I	Control (water)	10 ml/kg
2	II	Std (Levamisole)	50 mg/kg
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg
4	IV	*Brassica olearaceae* (Test 2)	500 mg/kg
5	V	Test 1+ Test 2	400+250 (mg/kg)
6	VI	Test 1 + Test 2	200+500 (mg/kg)
7	VII	Test 1 + Test 2	400+500 (mg/kg)

7. On 14th day, all the groups (i.e. Group I-VII) were immunized and challenged respectively, with 0.1 ml of 20 % SRBCs in normal NaCl.
8. On 21stday, all rats from all groups were challenged with 0.03ml of 1% SRBCs in subplanter region of right hind paw.
9. After the foot pad reaction was assessed after 24 hrs. i.e. On 22nd day, in terms of increase in the thickness of footpad resulting hypersensitivity reaction due to oedema.
10. The paw volume in right hind foot paw measured with the help of digital plethysmometer/Vernier calliper.

- **Antigenic material:**

- **Preparation of Sheep RBCs:**

The first step was to collect the sheep's blood in pathogen free alsever's solution in 1:1 proportion. The Alsever's solution was freshly prepared at the time of it's use. Then the blood was kept in the refrigerator and processed for the preparation of SRBCs batch, by centrifugating at 2000rpm for 10 min and additionally washing it with physiological saline for 4-5 times and then suspended it into buffered saline for longer use.

- **Composition of Alsever's Solution:**

Table No. 2. Composition of Alsever's solution for DTH Test

Chemicals	Quantity (g/L)
Sodium chloride	4.2
Sodium citrate	8.0
Citric acid anhydrous	0.55
Glucose	20.5
Distilled water q.s.	1000ml

- **Statistical analysis:**

All the results were expressed as Mean value ± SEM. Data were analysed using one-way analysis of variance (ANOVA) technique followed by Dunnets Test. P< 0.05, were considered statistically significant.

CARBON CLEARANCE TEST (10 DAYS MODEL)

- **Purpose and Rationale:**

The Phagocytic activity of reticulo-endothelial system will be calculated by carbon clearance test. The phagocytic index was calculated as the rate of carbon elimination by reticulo-endothelial system in carbon clearance test.

- **Procedure:**[14,15,16]

1. The animals were divided into seven groups consisting of six rats in each group.
2. Group-I (control group) received vehicle (water) only with a dose of 10 ml/kg b.w.p.o.
3. Group-II (standard group) received the std. drug Levamisole with a dose of 50 mg/kg b.w.p.o.
4. Group-III (Test-1) received the *Actidinia delicosa* with a dose of 400 mg/kg b.w.p.o.
5. Group-IV (Test-2) received the *Brassica olearaceae* with a dose of 500 mg/kg b.w.p.o.
6. Group-V, VI and VIII (Test 1 + Test 2) received the combination of extract of *Actidinia delicosa* and *Brassica olearaceae* with different doses.

Table No. 3. Grouping and Treatment Schedule for Carbon Clearance Test

Sr. No.	Groups	Test substances	Dose
1	I	Control (water)	10ml/kg
2	II	Std (Levamisole)	50 mg/kg
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg
4	IV	*Brassica olearaceae* (Test 2)	500 mg/kg
5	V	Test 1+ Test 2	400+250 mg/kg
6	VI	Test 1 + Test 2	200+500 mg/kg
7	VII	Test 1 + Test 2	400+500 mg/kg

7. On 10thday, 3 hours after the last dose; all the animals of each group were given colloidal carbon suspension intravenously in a volume of 10µl/gm body weight of rat.
8. Blood samples (25µl) were then collected from retro-orbital plexus at 5 and 15 min after injection of colloidal carbon ink and lysed in 0.1 % of Na_2Co_3 solution (3 ml).
9. Then the optical density measured spectrophotometrically at 650 nm.
10. The Phagocytic activity was estimated using the following formula:
    Where,

$$K = \frac{\text{Log } OD_1 - \text{Log } OD_2}{t_2 - t_1}$$

The OD_1 and OD_2 are the optical densities at time t_1 and t_2 respectively.

- **Preparation of Carbon Ink Suspension:**

The Camlin Ink was diluted 8 times with saline solution and used for intravenous injection in Carbon clearance test in dose of 10 µl/gm body weight of the rat.[17]

- **Statistical analysis:**

All the results were expressed as Mean value ± SEM. Data were analysed using one-way analysis of variance (ANOVA) technique followed by Dunnets Test. P< 0.05, were considered statistically significant.

NEUTROPHIL ADHESION TEST (16 DAYS MODEL)

- **Purpose and Rationale:**

The inducement in recruitment of neutrophil adhesion to nylon fibres was correlates with the process of margination of cell in blood vessels.

- **Procedure:**[15,16,18]

1. The animals were divided into seven group consisting 6 rats in each group.
2. Group-I (control group) received vehicle (water) only with dose of 10 ml/kg b.w.p.o.
3. Group-II (standard group) received the std. drug Levamisole with a dose of 50 mg/kg b.w.p.o.
4. Group-III (Test 1) received the extract of *Actidinia delicosa* with a dose of 400 mg/kg b.w.p.o.
5. Group-IV (Test 2) and received the extract of *Brassica olearaceae* with a dose of 500 mg/kg b.w.p.o.
6. Group- V, VI and VII (Test 1 + Test 2) received the extract of *Actidinia delicosa* and *Brassica olearaceae* with a different dose.

Table No. 4. Grouping and Treatment schedule for Neutrophil Adhesion Test

Sr. No.	Groups	Test substance	Dose
1	I	Control (water)	10 ml/kg
2	II	Std (Levamisole)	50 mg/kg
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg
4	IV	*Brassica olearaceae* (Test 2)	500 mg/kg
5	V	Test 1 + Test 2	400 + 250 mg/kg
6	VI	Test 1 + Test 2	200 + 500 mg/kg
7	VII	Test 1 + Test 2	400 + 500 mg/kg

7. On 16th day drug treatment, blood samples from each the groups were gather by puncturing the retro-orbital plexus under the mild-anaesthesia.
8. The blood was collected in the vials which are pre-treated with disodium EDTA and estimated for TLC and DLC.
9. After initial counts, blood samples were incubated with 80 mg/ml nylon fibers for 15min at 37^{0}C.
10. The incubated blood samples were again analysed for TLC and DLC.
11. The product of TLC and % Neutrophil gives Neutrophil Index (NI) of blood sample
12. The % Neutrophil Adhesion was calculated as follows:

$$\% \text{ Neutrophil Adhesion} = \frac{NI_U - NI_T}{NI_U}$$

Where, NI_U = Neutrophil index of Untreated Blood Sample.
NI_T= Neutrophil index of Nylon fibre treated Blood Sample.

- **Statistical analysis:**

All the results were expressed as Mean value ± SEM. Data were analysed using one-way analysis of variance (ANOVA) technique followed by Dunnets Test. P< 0.05, were considered statistically significant.

IN-VITRO METHODS:

T-CELL POPULATION TEST

- **Purpose and Rationale:**[19,20]

The increase in number of lymphocyte formation and rosette formation in T –cell population test indicates it's effect of extract on cell mediated immunity and T-cell activity. It shows dose dependent activity profile of the drug. Drug may the CD4 and CD8 cells which influence T-cell mechanism results increase in T-cell immune response significantly.

- **Procedure:**

1. The animals were divided into seven groups consisting 6 animals in each group.
2. Group-I (control group) received vehicle (water) only with a dose of 10 ml/kg b.w.p.o.
3. Group-II (standard group) received the standard drug Levamisole with a dose of 50 mg/kg b.w.p.o.
4. Group-III (Test 1) received the extract of *Actidinia delicosa* with a dose of 400 mg/kg b.w.p.o.
5. Group-IV (Test 2) received the extract of *Brassica olearaceae* with a dose of 500 mg/kg b.w.p.o.
6. Group V, VI and VII (Test 1+ Test 2) and received the combination of *Actidinia delicosa* and *Brassica olearaceae* extract with different doses.

Table No. 5. Grouping and Treatment schedule for T-cell population test

Sr. No.	Groups	Test substance	Dose
1	I	Control (Water)	10 ml/kg
2	II	Std (Levamisole)	50 mg/kg
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg
4	IV	*Brassica olearaceae* (Test 2)	500 mg/kg
5	V	Test 1 + Test 2	400 + 250 mg/kg
6	VI	Test 1 + Test 2	200 + 500 mg/kg
7	VII	Test 1 + Test 2	400 + 500 mg/kg

7. Antigen Challenge: On 0^{th} day of treatment, all groups were challenged with 0.1 ml of SRBCs containing 1 X 10^8 cells, i.p.
8. On 11^{th} day of the dosing, blood was withdrawn from retro-orbital plexus and anticoagulated with Alsever's solution in different test tubes.
9. Then, the test tubes containing blood were kept in sloping position (45^0C) at 37^0C for 1 hour. The RBCs are make to settle at bottom and supernant was collected from each test tube by using micropipette which contains lymphocytes.
10. Then 50µl of blood lymphocyte suspension and 50µl of SRBCs were mix in test tube and incubated.
11. The resultant suspension was centrifuge at 200 rpm for 5 minutes and kept in refrigerator at 4^0C for 2 hours.
12. The upper layered fluid was taken and 1 drop of cell suspension was placed on a glass slide. Total lymphocytes were estimated and a lymphocyte binding with three or more erythrocytes i.e. RBCs was considered as rosette and number of rosettes was calculated.

- **Antigenic material:**

Preparation of Sheep's RBCs:

Sheep blood was collected in sterile alsever's solution in 1:1 proportion, Alsever's solution(Table No.2.) was freshly prepared at the time for it's use. Then the blood was kept in the refrigerator and processed for the preparation of SRBCs batch, by centrifugating at 2000 rpm for 10 min and further washing it with physiological saline for 4-5 times and then suspending into buffered saline for further use.

- **Statistical analysis:**

All the results were expressed as Mean value ± SEM. Data were analysed using one-way analysis of variance (ANOVA) technique followed by Dunnets Test. P< 0.05, were considered statistically significant.[21]

RESULTS AND DISCUSSION

IN VIVO METHODS

DELAYED TYPE HYPERSENSITIVITY REACTION:

Effect of ethanolic extract of *Actidinia delicosa* cv.Hayward and methanolic extract of *Brassica olearaceae* var. Capitata on SRBCs induced delayed type of hypersensitivity reaction is shown in [Table No.6]. The cell mediated immune response assessed by DTH reaction using Plethysmometer showed significant increase for alone *Brassica olearaceae* (Group IV) and Combination of *Actidinia delicosa* cv. Hayward fruit and *Brassica olearaceae* var. Capitata (Group VII) showed significant (****p<0.0001) DTH response in terms of increase in the mean difference in paw volume when compared with normal control. Therefore, increase in DTH reaction in rat in response to T cell dependent antigen revealed that the stimulatory effect of Methanolic leaf extract of *Brassica olearaceae* var. Capitata on T cells.

Table No: 6. Result of DTH

Sr. No.	Groups	Treatments	Dose and Route of administration	Mean difference in paw edema in (mm) X (Mean ± SEM)
1	I	Control	10 ml/kg (p.o.)	2.677 ± 0.01926 (100 %)
2	II	Standard (Levamisole)	50 mg/kg (p.o.)	4.443 ± 0.02290**** (165.96 %)
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg (p.o.)	3.087 ± 0.01820**** (115.31 %)
4	IV	*Brassica olearaceae* (Test 2)	500 mg/kg (p.o.)	3.205 ± 0.1708**** (119.72 %)
5	V	Test 1+ Test 2	400 +250 mg/kg (p.o.)	3.580 ± 0.01732*** (133.73 %)
6	VI	Test 1 + Test 2	200 +500 mg/kg (p.o.)	3.913 ± 0.01585**** (146.17 %)
7	VII	Test 1+ Test 2	400 + 500mg/kg (p.o.)	4.233 ± 0.3565**** (158.12 %)

Values are expressed as (Mean ±S.E.M.) n=6. **** indicate p<0.0001, statistically significant when comparing with control group by ANOVA followed by Dunnett test.

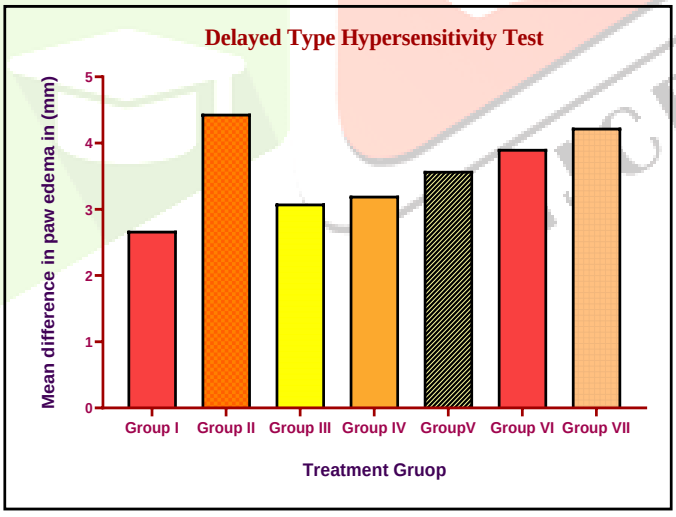

Fig No.1. Graphical representation of DTH

CARBON CLEARANCE TEST

Effect of *Actidinia delicosa* and *Brassica olearaceae* extract on the Phagocytic activity by carbon clearance test is shown in above table [Table No: 7]. The Phagocytic activity of reticulo-endothelial system is generally measured by the rate of removal of carbon particle from the blood stream. In carbon clearance test, the Phagocytic index of alone *Brassica olearaceae* treated group i.e. (Group IV) showed significant increased in Phagocytic index i.e. 140.50 % and also the combination group of *Actidinia delicosa* and *Brassica olearaceae* treated group i.e. (Group VIII) showed significant increased in Phagocytic index i.e.174.90 % when compared with normal control. This indicates stimulation of reticuloendothelial system.

Table No: 7. Results of Carbon clearance test

Sr. No	Groups	Treatments	Dose and Route of administration	Absorbance		Phagocytic Index (Mean ±SEM)
				5 min	15 min	
1	I	Control	10 ml/kg (p.o)	0.173	0.083	0.03160±0.0017 (100%)
2	II	Standard (Levamisole)	50 mg/kg (p.o)	0.0161	0.037	0.06337±0.0016**** (↑ 200.53%)
3	III	*Actidinia delicosa* (Test 1)	400 mg /kg (p.o)	0.135	0.07	0.04283±0.00081**** (↑ 135.53 %)
4	IV	*Brassica olearaceae*(Test 2)	500 mg/kg (p.o)	0.0167	0.072	0.04440 ±0.00030**** (↑ 140.50 %)
5	V	Test 1+ Test 2	400+250 mg/kg (p.o)	0.0164	0.068	0.04627±0.00033**** (↑ 146.42 %)
6	VI	Test 1+ Test 2	200+500 mg/kg (p.o)	0.170	0.063	0.04932±0.00038**** (↑ 156.07 %)
7	VII	Test 1+Test 2	400+500 mg/kg (p.o)	0.167	0.047	0.05527±0.0017**** (↑ 174.90 %)

Values are expressed as (Mean ± SEM).n=6. **** indicate $p<0.0001$, statistically significant when comparing with control group by ANOVA followed by Dunnett test.

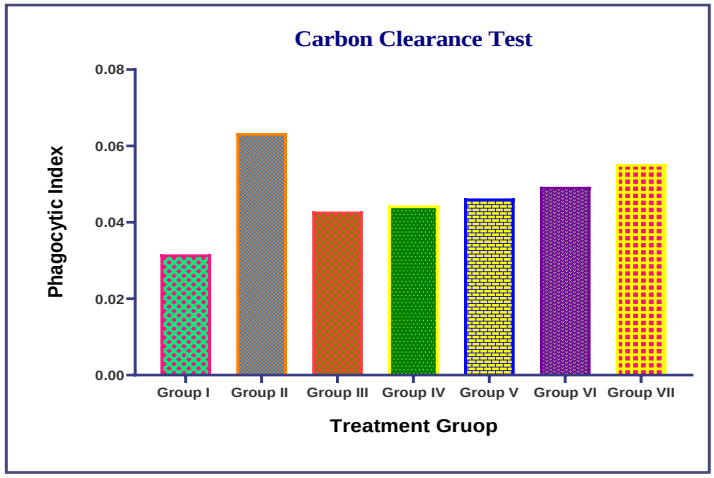

Fig No: 2. Graphical Representation of Carbon Clearance Test

NEUTROPHIL ADHESION TEST

Effect of ethanolic extract of *Actidinia delicosa* and methanolic extract of *Brassica olearaceae* on Neutrophil activation by the Neutrophil adhesion test is shown in table [Table No.8]. In present study, when blood sample were incubated with nylon fibres, a reduction in Neutrophil percentage due to adhesion of Neutrophil to the nylon fibres was observed. The percentage reduction in the Neutrophil count in nylon fibre treated blood samples from the tested group *Brassica olearaceae* (500mg/kg) and combination of *Actidinia delicosa* and *Brassica olearaceae* (400+500mg/kg) was significantly ($p<0.0001$) more compared to control group, showed possible Immunostimulant effect.

Table No: 8. Result of Neutrophil adhesion test

Sr. No	Groups	Treatments	Dose and Route of administration	% Neutrophil adhesion
1	I	Control (Water)	10 ml/kg (P.O.)	28.42 ± 2.270 (100 %)
2	II	Standard (Levamisole)	50 mg/kg (P.O.)	66.89 ± 1.974**** (235.36 %)
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg (P.O.)	48.95 ± 1.628**** (172.23 %)
4	IV	*Brassica olearaceae* (Test 2)	500 mg /kg (P.O.)	50.64 ± 2.426**** (178.18%)
5	V	Test 1+ Test2	400 + 250 mg/kg (P.O.)	53.81 ± 0.9345**** (189.33 %)
6	VI	Test 1+ Test 2	200 + 500 mg/kg (P.O.)	57.49 ± 1.363**** (202.28 %)
7	VII	Test1 + Test2	400 + 500mg/kg (P.O.)	61.58 ± 1.141**** (216.67 %)

Values are expressed as (Mean ± S.E.M) n=6. $p<0.0001$, statistically significant when compared with control group by ANOVA followed by Dunnett test.

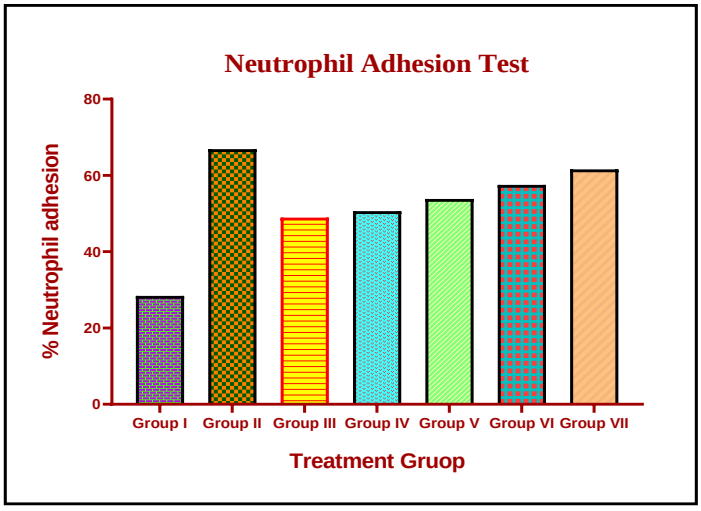

Fig No: 3. Graphical Representation of Neutrophil adhesion test

IN-VITRO METHOD

- **T-CELL POPULATION TEST**

Result of Total Lymphocyte Count for T-Cell Population Test:

Effect of *Actidinia delicosa* and *Brassica olearaceae* on T-Cell population test is shown in above table [Table No: 6.33]. Formation of lymphocyte were compared with control group, significant increase in lymphocyte formation was found to be 222.30 % in alone Group IV i.e. *Brassica olearaceae* treated group with dose 500mg/kg and Group VII i.e. combination of *Actidinia delicosa* and *Brassica olearaceae* treated group with dose 400+500 mg/kg, also shown significant increase in lymphocyte formation as 263.16%.

Table No: 9.Result Of T-Cell Population Test

Sr. No	Groups	Treatments	Dose and Route of administration	Total Lymphocyte Count Mean (per mm^3)
1	I	Control (Water)	10 ml/kg (P.O.)	695 ±93.52 (100 %)
2	II	Standard (Levamisole)	50mg/kg (P.O.)	2022 ±126.4**** (290.93 %)
3	III	*Actidinia delicosa* (Test 1)	400 mg/kg (P.O.)	1545 ±38.69**** (222.30%)
4	IV	*Brassica olearaceae* (Test2)	500 mg/kg (P.O.)	1546 ±53.14**** (222.42 %)
5	V	Test1 + Test 2	400+250 mg/kg (P.O.)	1659± 82.64**** (238.70 %)
6	VI	Test 1 + Test 2	200+500mg/kg (P.O.)	1751± 94.57**** (251.94 %)
7	VII	Test 1 + Test 2	400+500mg/kg (P.O.)	1829± 94.57**** (263.16 %)

Values are expressed as (Mean ± SEM). $p < 0.0001$ statistically when compared with control group by ANOVA followed by Dunnett test.

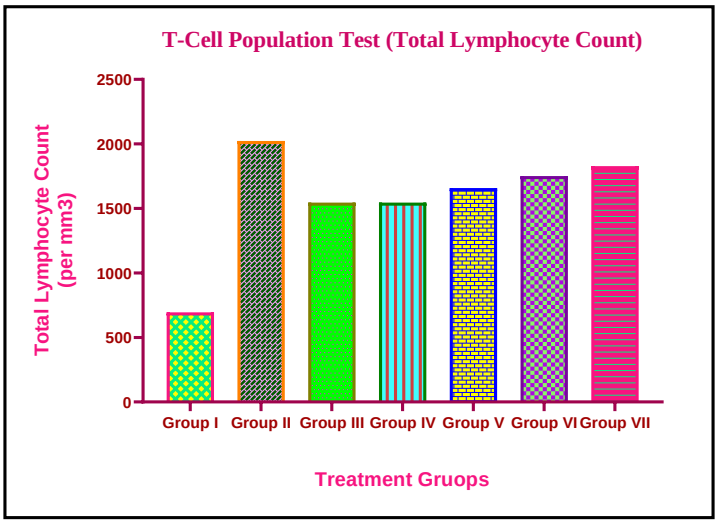

Fig No: 4. Graphical representation of T-Cell Population Test

Results of Rosettes Count For T-Cell Population Test

In this test, % increase in formation of rosette was found to be 19 ± 1.229 [i.e. 175.72%] and 24 ± 1.138 [i.e.218.18%] when animal were administered with alone *Brassica olearaceae* (500 mg/kg) treated group and combination of *Actidinia delicosa* and *Brassica olearacea*e treated group (400+500 mg/kg) respectively. Both groups shows significant activity when compared with normal control group. [Table No. 10]

Table No: 10. Result of Rosette count

Sr. No.	Groups	Treatments	Dose and Route of Administration	Number of rosette
1	I	Control	10 ml/kg (P.O.)	11± 1.145 (100 %)
2	II	Standard (Levamisole)	50 mg/kg (P.O.)	27± 1.453**** (245.45 %)
3	III	*Actidinia Delicosa* (Test 1)	400 mg/kg (P.O.)	19± 1.000**** (172.72 %)
4	IV	*Brassica olearaceae* (Test 2)	500 mg/kg (P.O.)	19±1.229**** (175.72 %)
5	V	Test 1 + Test 2	400 + 250 mg/kg (P.O.)	20 ±1.276**** (181.81 %)
6	VI	Test 1 + Test 2	200 + 500 mg/kg (P.O.)	21 ±0.5774**** (190.90 %)
7	VII	Test 1 + Test 2	400 + 500 mg/kg (P.O.)	24±1.138**** (218.18 %)

Values are expressed as (Mean ± SEM) n=6. **** indicate $p < 0.0001$, statistically significant when compared with control group by ANOVA followed by Dunnett test.

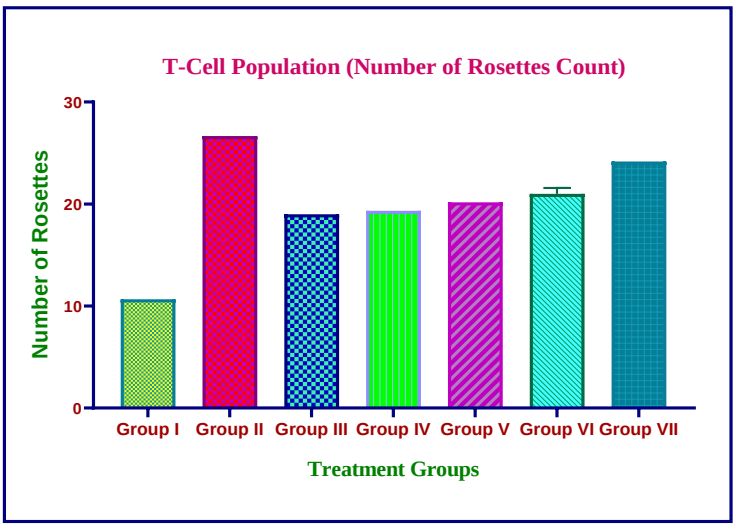

Fig No: 5. Graphical Representation of Rosette Count For T-Cell Population Test

DISCUSSION

Human survival relies upon their immune protection mechanisms in opposition to external damage, pathogenic microorganisms and cancer. It is widely recognized that the immune system facilitates the host to govern bacteria, allergen or poisonous molecules, and prevent the growth of cancer. Once the body's resistance system became defective, leads to an impaired response against infectious pathogens and cancer. The reason of impairment (Immunosuppression) may be both extrinsic or intrinsic (inherited) and called as secondary or primary immunodeficiency, respectively.[22]

In chemotherapy, Immunomodulators are used in a similar manner to adjuvants to controland prevent infections. The relation between immune states and the occurrence, growth and decline of tumor is one of the essential problems in tumor immunology. Various biological response modifiers (BRMs) such as natural products having biological activity to enhance host defense system have been considered as a useful tool to inhibit tumor growth in cancer immunotherapy. In many cases, BRMs activate immune-related cells, including NK cells, lymphokine cells and macrophages, to control cancer growth. Their clinical applications are to boost the body's general vitality or to treat a debilitating condition.[3]

The mechanisms of immunomodulatory interest arise via activation of macrophages, stimulation of phagocytosis, the stimulation of lymphocytes the development of cell immune function and action of the nonspecific cellular immune system, immunostimulation of peritoneal macrophages, increased production of antigen-specific immunoglobulin, elevated non -specific immune mediators and the number of natural killer cell, which reduces chemotherapy-induced leucopenia, and increase total circulating white blood cells counts and interleukin-2 levels.[23]

As immunostimulants, various synthetic drugs are used, consisting of levamisole, thalidomide, but these drug have a variety of side effects, such as nephrotoxicity, liver toxicity, bone marrow suppression, gastrointestinal diseases and so on. Because of the side effects associated with synthetic agents, plants are safer. Indian medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of especially granulocytes, macro-pages, natural killer cells and competent functions.[24]

DTH is antigen-specific and causes antigen to induces erythema and induction at the site of antigen infection in immunized animals. DTH histology can vary from species to species, but the common feature is that the flow of immune cells at the injection site, macrophages and basophils in mice and induction becomes apparent within 24 - 72 h. T-cells are required to initiate the reaction. Increase in the DTH response indicates that drug has a stimulatory effect on lymphocytes and necessary cell types required for the expression reaction. DTH response, which is direct co-related to cell-mediated immunity, was significantly increased with chloroform, methanol and petroleum ether fraction as compared to untreated control.[25] In present research work, it was found that the alone *Brassica olearaceae* group [3.205 ± 0.1708] i.e. 119.72 %, causes the increase in footpad edema after 24 hrs of the exposure to antigenic material i.e. SRBCs, when compared with control [2.677 ± 0.019] i.e. 100 %. and combined *Actidinia delicosa* and *Brassica olearaceae* group [4.233 ± 0.3565] i.e. 158.12 % etc. than the other doses and also Levamisole [4.443 ± 0.022] i.e. 165.96%, causes the increase in footpad edema after 24 hrs of the exposure to antigenic material i.e. SRBCs, when compared with control [2.677 ± 0.019] i.e. 100 %. This indicated stimulation of cell mediated immunity and the increase in response occurs as dose increases.

CCT was carried out to evaluate the effect of drug on the reticulo-endothelial system (RES) which is a diffuse system consisting Phagocytic cells comprising offixed tissue macrophages and mobile macrophages. The Phagocytic cells in this system comprisethe mononuclear phagocyte system (MPS), and the macrophage is the major differentiated cell inthe MPS. Cells of the RES and MPS are known to be important in the clearance of particles fromthe bloodstream. When colloidal ink containing carbon particles are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation.[26] In present research work, Phagocytic index of alone *Brassica olearaceae* group [0.0444 ± 0.0003] i.e.140.50 %, combination of *Actidinia delicosa* and *Brassica olearaceae* group [0.05527 ± 0.0017] i.e. 174.90 % etc. and Levamisole [0.06337 ± 0.0016] i.e. 200.53 %, causes increase in Phagocytic index than other alone and combination doses groups, when compared with control [0.03160 ± 0.0017] i.e.100 %. This increase in Phagocytic index indicates that there was stimulation of reticulo-endothelial system and the response increase as dose of formulation increases.

Neutrophils are a part of the cell mediated immune response responsible for the innate immunity that help to eliminate foreign bodies through recogination and migration towards the foreign body, phagocytosis, and foreign body destruction.[27] These study showed that number of neutrophils in blood samples treated with nylon fibers from the test group was reduced by a percentage. So, the alone *Brassica olearaceae* group [50.64 ± 2.426] i.e. 179.18% showed increase in % Neutrophil adhesion than *Actidinia delicosa* group [48.95 ± 1.628] i.e. 172.23%, when compared with control group [28.42 ± 2.270] i.e.100% and also combination of *Actidinia delicosa* and *Brassica olearaceae* with dose 400 + 500 mg/kg [61.58 ± 1.141] i.e. 216.67 % showed increase in % Neutrophil adhesion than other combination doses. The results showed a dose-dependent increase in the Neutrophil adhesion to the nylon fibers, which was an indication of increased neutrophil migration towards foreign bodies.

The increase in formation of rosettes and lymphocyte in the T-cell population test indicates impact of extract on cell mediated immunity. It shows a dose-dependent activity profile of the drug. Further observed that, the *Brassica olearaceae* at dose 500mg/kg [1546 ± 53.14] i.e. 222.42% shows maximum mean lymphocyte count than *Actidinia delicosa* at dose 400 mg/kg [1545± 38.69] i.e. 222.30%, when compared with control group [695 ± 93.52] i.e. 100 % and also combination of *Actidinia delicosa* and *Brassica olearaceae* group at dose 400 +500 mg/kg [1829 ± 9457] i.e. 263.16 % shows high lymphocyte count than other combination groups, when compared with control group. Both extracts can activate CD4 and CD8 cells, which influence the T-cell mechanism and leads to a significant increase in T –cell immune response.

In the presented study, the sequence of immunomodulatory activity was rooted as Levamisole > *Brassica olearaceae* var. Capitata > *Actidinia delicosa* cv. Hayward.

In the present study, it was revealed that *Brassica olearaceae* gives potent immunomodulatory activity than the *Actidinia delicosa* when doses are given alone and In combination of *Actidinia delicosa* and *Brassica olearaceae*, it showed synergistic effect.

CONCLUSION

Since ancient times, Plants have been used to cure various types of diseases. So, the present study revealed that the phytochemicals were found which is beneficial for the health, such as alkaloids, glycosides, steroids, flavonoids, saponins, tannins, terpenoids and phytosterols, which will increases the medicinal potential of kiwi and red cabbage.Therefore it can be used to treat various diseases.

In conclusion, *Actidinia delicosa* cv. Hayward and *Brassica olearaceae* var. Capitata stimulates immune system by acting through cellular and humoral immunity in experimental models of immunity in animals. The effect of this extract can be compared to the standards drug i.e Levamisole, when doses are given in combination. Based on the result of this research, the conclusion is the methanolic extract of *Brassica olearaceae* var. Capitata shows potent immunomodulatory activity than the Ethanolic extract of *Actidinia delicosa* cv. Hayward in alone doses, when compared with control and Combination of *Actidinia delicosa* and *Brassica olearaceae* at a various doses showed synergistic Immunomodulatory effect.

However, *Brassica olearaceae* var. Capitata was to be most effective than the *Actidinia delicosa* cv. Hayward due to presence of phytoconstituents such as flavonoids, alkaloids, anthocynins, tannins, steroids which is responsible for immunomodulatory activity. So, the overall order of Immunostimulatory activity is,

Levamisole > *Brassica olearaceae* > *Actidinia delicosa*.

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