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Plasma Electrophoresis and Phagocytic Index Screening of Some Indigenous Vegetables Subjected to Preclinical Models

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Abstract

50 % of methanolic extract *Colocasia esculenta, Moringa oliefera, Luffa cylindrica,* and *Hibiscus esculentus* were subjected to immunomodulatory activity in Swiss albino mice either sex. Mice were treated with five days of dosing of *Colocasia esculenta 50 mg/kg bw, Colocasia esculenta* 100 mg/kg bw, *Moringa oliefera* 200mg/kg bw, *Moringa oleifera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus* 100 mg/kg bw, and *Hibiscus esculentus* 200 mg/kg bw. Cyclosporine (2.5 mg/kg) used as a standard reference drug for 5 days. Investigation of immunomodulator activity of these 50 % of methanolic extract of drugs to parentage of yeast digestion form 24 hours the peritoneal fluid culture and electrophoretic plasma protein band albumin, alpha 1, alpha 2, beta and gamma respectively from blood plasma were observed using parameters phagocytosis and plasma electrophoresis. Also investigated the ulcerogenic effect or any toxic effect of plant extract by histopathology study of crypt, villi and goblet cells with reference to standard drug cyclosporine. As regards these parameters, *Hibiscus esculentus* 100 and 200 mg/ kg bw dose, *Moringa oleifera* 200 and 400 mg/ kg bw dose and *Luffa cylindrica* 200mg/kg bw elicited a moderately significant increase in the % of yeast digestion ($P < 0.001$) respectively and *Luffa cylindrica* 100mg/kg bw significant increase in the % of yeast digestion ($P < 0.01$). *Hibiscus esculentus* showed significant dose dependent increase and *Moringa oleifera* decrease phagocytic activity of macrophages. *Hibiscus esculentus* 200 mg/ kg bw dose and Moringa *oleifera* 200 significantly increased ($P < 0.01$) the Gamma globulin.

However, our present study revealed and signatured for their immunomodulator enhancing property. As in Asian subcontinent daily there vegetables are cooked and served with know and unknown of its potential function against different diseases. If there vegetables properly ruled out for their pharmacological aspect then it may add diamond in the crown of dietician which has been bother every day today life but over looked exponetentially

Keywords:- *Colocasia esculenta, Moringa oliefera, Luffa cylindrica,* and *Hibiscus esculentus*

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Introduction

Herbal medicine has become an integral part of standard healthcare, based on a combination of time honoured traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections [1]. The modulation of immune response with the aid of various bioactives in order to alleviate certain diseases is an active area of interest. Apart from being specifically stimulatory or suppressive, certain agents normalize or modulate pathophysiological processes and are hence called 'immunomodulatory agents'[2]. The property of any substance to enhance non-specific resistance of body against pathogens is termed 'adaptogenic.[3] Most important area in which herbal medicine has not to witness any breakthrough is the development of adjutants to be used in vaccination programs or immunosuppressants that can be safely exploited in organ transplantation and autoimmune diseases. These fundamental fields of immunomodulators are currently receiving inadequate attention.[4]

Plant derived polysaccharides have attracted widespread attention as ideal immunomodulators due to their therapeutic properties and relatively low toxicity and side effects as compared to others Many of them have been found to influence macrophage functions which play a key role in innate as well as adaptive immune responses. Thus modulation of the macrophage function by these polysaccharides can help increase phagocytosis activity, and chemotaxis and antigen presentation to T cells thus helping in preventive and therapeutic strategies against diseases.[5]

Modulation of immune system by cytotoxic agents is emerging as a major area in pharmacology, especially in Cases where undesired immunosuppression is the result of therapy [6]. A major drawback of Current cancer therapeutic practices such as chemotherapy and radiation therapy is bone marrow suppression resulting in cytopoenia and subsequent suppression of humoral and cellular as well as non-specific and specific cellular responses..[7] Other have severe side effects such as nausea, vomiting, alopecia, mucosal ulceration, pulmonary fibrosis, cardiac and hepatic toxicity etc Drugs that could alleviate these side effects will be highly useful in cancer therapy.

Immunomodulators are substances, which modify the activity of the immune system. Immunomodulators have biphasic effects; some tend to stimulate immune system which are low while others inhibit host parameters which are normal or already activated [8] Use of plant products as immunomodulators is getting more and more importance in the field of cancer research. Some of the plants with known immunomodulatory activities are Tinospora cordifolia,[9] Withania somnifera,[10] Piper longum,[11] *Viscum album*, *Panax ginseng*, *Tinospora* cordifolia, Asparagus racemosus etc. Components such as polysaccharides, lectins [12] proteins and peptides [13] present in plants have been shown to stimulate the immune system.

Green and yellow vegetables including cabbage, Brussel's sprouts and other cruciferous vegetables contain several organosulfur compounds including isothiocyanates (ITCs) and dithiolethiones. Isothiocyanates occur in cruciferous vegetables as thioglucoside conjugates called glucosinolates [14]

Materials and Methods

Materials

Animal

Adult *Swiss albino* mice (25-30 gm, body weight) have either sex.

Plant

50% methanolic extract *Luffa cylindrica* (fruits), *Moringa oliefera* (pods), *Hibiscus esculentus* (fruits), *Colocasia esculenta* (rhizome).

Instrument

Co_2 incubator, Centrifuge, Cyclomixer, Light Microscopy, Micropipette, Densitometry, Glass

slide warmer, Incubator, Moist heat autoclave, Laminar flow and image analyzer. Grinder, Weighing machine, Water bath.**,** Electrophoresis supply 606 systronic, densitometers 20s systronic Cellulose acetate strip and X ray for the loading the sample.

Chemicals and others

Methanol, Acetic Acid, Normal saline, Phosphate buffer saline, RPMI 1640 (1000 gibco BRP) media, Yeast, Agrose, Double distillation water, Geimsa Stock, NaH_2Po_4, Na_2HPO_4, EDTA, Tris HCL, Boric acid, Indicator Bromo Phenol Blue, Mercuric Chloride, Cornoy's Fixative (methanol and acetic acid), Destainer(7% acetic acid), Clearing solution, 0.25 ml cHCl, 100 ml 70% ethanol, Scott's solution, 1 liter tap water, 10 g magnesium sulfate (anhydrous), 2 g sodium bicarbonate,

Whatsman, Filter Glass slide, Syringe, Measuring cylinder, Beaker Centrifuge tube, and Separating funnel, Cellulose acetate strip and X ray for the loading the sample.

Methods

Collection of plants

Plant fruits *Colocasia esculenta, Hibiscus esculentus, Luffa cylindrica* and *Moringa oleifera* were collected during July and August from the garden of Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal.

Preparation of extracts

The fruits of *Colocasia esculenta, Hibiscus esculentus, Luffa cylindrica* and *Moringa oleifera* (1 kg all fruits) gently washed with distil water and made the small slice. The slices were allowed to shade dry for two to three weeks. The shade dried fruits were pulverized with help of grinder and used for extraction. Powder of all the vegetables was weighed (200 gm) and macerated in a separating funnel with 50% ethanol. The mixture was vigorously shaken at regular interval for 72 hrs. The extract was collected in beaker & concentrates it in a water bath at 45°C & the similar process was repeated up to three times, then a colorless marc was obtained. The concentrated extract was transferred in a Petri plate, then dried at 45°C in hot air oven and powder of crude extract was colleted and weighed, then it was kept in an airtight container. The final yield values of different plants were *Colocasia esculenta* 1.5 gm, *Hibiscus esculentus* 1.7 gm, *Luffa cylindrica* 1.6 gm and *Moringa oleifera* 1.39gm.[15]

Experimental animals and research protocol approval

Swiss albino mice (25-30 g) of either sex were provided from Jawaharlal Nehru cancer Hospital & Research Centre, Bhopal (M.P.). They were maintained at a temperature of 25±1°C and relative humidity of 45 to 55% under 12-h light: 12-h dark cycle. The animals had free access to standard food pellets (Golden Feeds 894/8 Mehrauli, New Delhi-30,) and water was available *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (CPCSEA Registration Number: 500/01/a/cpcsea/2001).

Group – 1 Represent the normal control group.
Group – 2 50% methanolic extract of *Luffa aegyptiaca* 100 mg/kg. bw. Orally[16]
Group – 3 50% methanolic extract of *Luffa aegypt*iaca 200 mg/kg. bw. orally
Group - 4 50% methanolic extract of *Moringa oleifera 200* mg/kg bw orally.[17]
Group - 5 50% methanolic extract of *Moringa oleifera* 400 mg/kg bw orally.
Group - 6 50% methanolic extract of *Hibiscus esculentus* 100 mg/kg bw orally.[18]
Group - 7 50% methanolic extract of *Hibiscus esculentus* 200 mg/kg bw orally.
Group - 8 50% methanolic extract of *Colocasia esculenta* 50 mg/kg bw orally[19].
Group - 9 50% methanolic extract of *Colocasia esculenta* 100 mg/kg bw orally.

Group - 10 Standard will be subjected to Cyclosporine 10 mg/kg bw. orally.[20]

Histopathological Studies

After regular days of dosing according to protocol, last day of dosing mice were took fasting over night fasting. Next day mice were scarified by cervical disc location. Peritoneal fluid and intestine was taken out. Intestine was cleaned with normal saline repeated it 2-3 time and removed the debris fat and omentum with help of forceps. Found out the exact location of jejunum. Cut the jejunum fixed it in 10% formalin & the sections stained with haematoxylin & eosins were studied under light microscope. Observed under the microscope to find out the effect of plant extract on jejunum, or have any toxic or side effect found out by counting and observed the crypt, villi and goblet cells.

Method of Phagocytosis

Culture

Extract will be administered to the experimental animal for 5 days regularly. Chilled Phosphate buffer saline (PBS) injected to mice Tran abdominally. Abdominal was gently messaged after 5 min. Peritoneal fluid be will aspirate and centrifuged at 1000 rpm for 15 min. The supernatant will be discarded and pellet was suspended in RPMI 1640 (gibco BRL) media. This culture will be co-incubated for 24 hrs at 37^0C in 5% CO_2 incubator.

Harvesting

After 24 hrs cultures will incubated with heat-inactivated yeast (57°C for 1 hr) in 10:1 ratio in CO_2.incubator. After 1hr., centrifuge it at 1000 rpm for 20 min. Smear will prepared on clean glass slide and fixed in cornoy's fixative for 10 min. Air dried and stain with giemsa for 20 min. Observe the phagocytic activity of macrophage against yeast under 40x magnification using light microscopy. Percentage of inhibition of yeast digestion of macrophage index was calculated by using the formulae.[21]

$$\% \text{ of inhibition of yeast} = \frac{\text{No. of active macrophage} \times 100}{\text{Total No. of macrophage}}$$

Method for Cellulose Acetate Electrophoresis

First prepare the desired buffer, equally fill the buffer tanks with prepared buffer. Cut cellulose acetate strip in upright position, holds the strip carefully with help of forceps and mark on the cellulose acetate strip with help pencils, according to need. Carefully dip the strip in buffer for 5-10 minutes, in such way that its upper surface dose not be dip in buffer this is called impregnation. After 10 minutes, strip is taking out from buffer tank and put very care fully on clean glass slide. This glass slide put on center of buffer tank.

Cut the filter paper strip and one edge of filter paper should be dip in buffer and other one edge touch cellulose acetate membrane. Before loading of sample, tank is saturated for 15 minutes. The sample runs at constant voltage, sample is loaded exactly on the mark point. After band has traveled to the maximum, height without any deviation current is stopped. Then strip is dipped in Ponceau S stain (Protein stain). After 30 minutes, the strip is distained with help of 7 % of acetic acid. Destain the strip by occasional rinsing the distaining solution until the background become weight. Dehydrated the strip in pure methanol for 1 minute. Place the cellulose strip on a glass plate. Immerse this glass plate in cleaning solution for 1 minute at room temperature or on hot plate at 20°C. Use densitometer for quantitative determination of protein fraction.[22]

Method for Agrose Gel Electrophoresis

Weight 1gm of agrose and place it in conical flask. Add 100 ml. of serum electrophoresis buffer and boil until a transparent gel is formed. Layer meted agrose on glass plate. After the formation of gel, cut slot on gel across the middle. Apply the serum by means micropipette. Place the gel slide on buffer tank. Add the appropriate amount of buffer in anodic and cathodic tank respectively Connect the gel to buffer by means of whatsman filter paper. Put on

Tables

S./N Animal groups	Albumin Mean± S.d (%)	Alpha 1 Mean ± S.d (%)	Alpha 2 Mean ± S.d (%)	Beta % Mean ± S.d (%)	Gamma Mean± S.d (%)
1. Control	44.55±1.31	14.23± 4.96	12.89± 4.05	5.23±1.28	23.37±2.83
2. Standard	75.23±3.98**	5.27 ±1.55	4.57± 1.93	3.70±0.81	11.38±3.11
3. Colocasia esculenta 50 mg/kg bw	14.68±3.01**	14.69 ± 4.62	42.02± 17.3*	23.83±11.88	4.768±4.643
4. Colocasia esculenta 100 mg/kg bw	23.39±12.5	14.48 ± 6.97	15.16± 7.24	35.56±22.41*	11.40±15.35
5. Moringa oliefera 200mg/kg bw	19.05±9.34*	17.68 ± 5.94	9.448± 8.07	13.80±8.266	40.02±26.89
6. Moringa oliefera 400 mg/kg bw	34.26±5.28	9.100 ± 3.02	8.188± 4.80	11.27±9.404	37.17±7.404
7. Luffa cylindrica 100 mg/kg bw	26.47±15.0	14.59 ± 23.0	15.77± 12.4	16.84±9.887	26.30±11.64
8. Luffa cylindrica 200 mg/kg bw	31.58±16.1	13.26 ± 6.23	12.12± 5.16	11.63±1.739	31.38±11.77
9. Hibiscus esculentus 100 mg/kg bw	12.99±10.0**	31.99 ± 12.7	20.99± 9.15	11.86±9.140	22.16±17.8
10. Hibiscus esculentus 200 mg/kg bw	16.25±8.12**	21.47 ± 14.0	14.52± 7.53	4.800±3.065	42.92 ±17.9

Normal range percentage fraction of plasma protein (%) Albumin: 42-60, Alpha 1: 2-11, Alpha 2: 9-21, Beta: 4.5-21, Gamma 9-25.

Table no:- 2 Showing the Mean and Standard deviation of % fraction of electrophoresis plasma protein from *Colocasia esculenta 50 mg/kg bw, Colocasia esculenta*100 mg/kg bw, *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus*100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw treated Swiss albino mice Data are expressed as means ± SD of four animals in each group. *P<0.05, **P<0.01 as determined by One way analysis of variance (ANOVA).

power supplier and voltage to 300. Put slide in staining solution for 1 hr. and distain. Dry the slide at room temperature use, Densitometry for quantitative determination of protein fraction.[23]

Densitometry analysis (Banloregenii)

A densitometer is an instrument for measuring the darkness or light stopping power of exposed and processed photographic material i.e. its density.

Observation and results

We observed from table 1and 2 that control group revealed phagocytic index 5.603 ± 0.3597 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 44.55 ± 1.31,14.23 ± 4.96, 12.89± 4.05, 5.23±1.28, 23.37±2..83 respectively. Control group revealed that all the plasma protein falls under normal range.

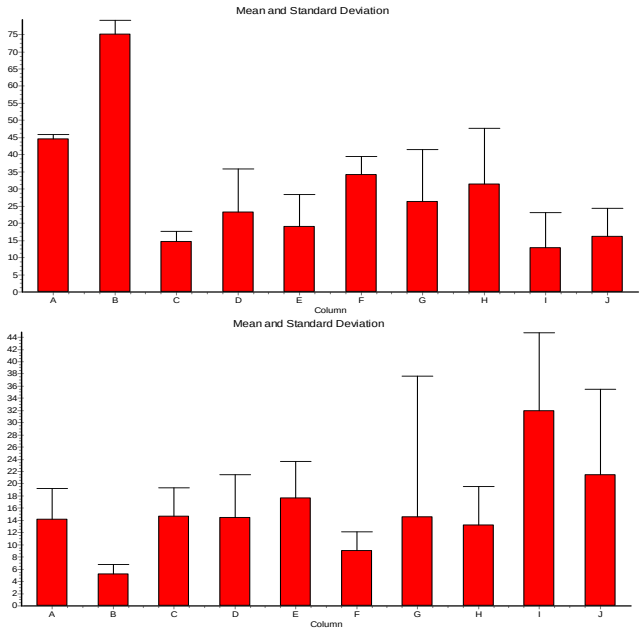
Standard group revealed that albumin plasma protein moderately increase, Alpha 2 protein and beta protein slightly lower than the normal range. However, Alpha 1 and Gamma proteins fall under the normal range.

Tables:-2

Test groups	Mean ± S.D
1. Control	5.603± 0.3597
2. Standard	4.210± 0.3966
3. Colocasia esculenta 50 mg/kg bw	8.063±1.103
4. Colocasia esculenta 100 mg/kg bw	7.955±0.9180
5. Moringa oliefera 200mg/kg bw	15.40±1.662***
6. Moringa oliefera 400 mg/kg bw	14.68±1.774***
7. Luffa cylindrica 100 mg/kg bw	9.808±0.2948**
8. Luffa cylindrica 200 mg/kg bw	13.30±0.413***
9. Hibiscus esculentus 100 mg/kg bw	11.30±2.222***

10. Hibiscus esculentus 200 mg/kg bw	13.66±2.600***

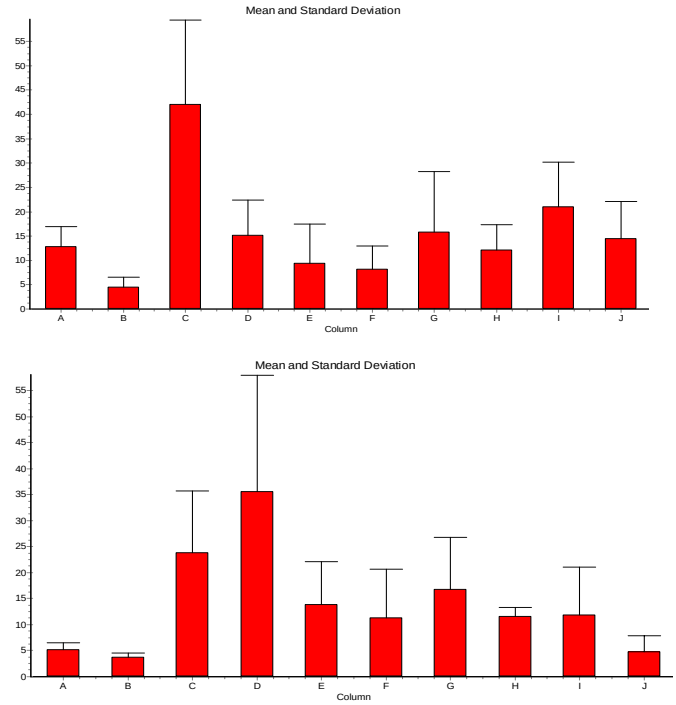
Table :- 2 Showing effect of *Colocasia esculenta50 mg/kg bw*, *Colocasia esculenta*100 mg/kg bw, *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus*100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw on macrophage induce % of yeast digestion response in mice . Data are expressed as means ± SD of four animals in each group. **P<0.01, ***P<0.001 as compared to control determined by One way analysis of variance (ANOVA)



Graph-1 and 2: Showing effect of A-J group on percentage fraction of electrophoresis plasma protein Albumin, Alpha 1 and comparison with standard and control group. On x axis A, B, C, D, E, F, G, H, I and J mark expressed as group of *Colocasia esculenta 50 mg/kg bw*, *Colocasia esculenta*100 mg/kg bw, *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus*100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw. On y-axis % fraction of plasma protein Albumin and Alpha 1. Graphs are expressed as means ± SD of four animals in each group. Data determined by One-Way analysis of variance (ANOVA).

Colocasia esculenta 50mg/kg bw revealed phagocytic index 8.063 ± 1.103 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 14.68 ± 3.010, 14.69 ± 4.620, 42.02± 17.37, 23.83±11.88, 4.768 ±4.643 respectively. *Colocasia esculenta* (100mg/kg bw) revealed phagocytic index 7.955 ± 0.9180 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 23.39 ± 12.52 14.48 ± 6.977, 15.16 ± 7.245, 35.56±22.4, 11.40 ±15.31 respectively.

Colocasia esculenta 50 mg/kg bw revealed that albumin and alpha 2 protein decreased and increased respectively 2-3 times higher However, alpha 1 and beta protein slightly increased than the normal range.

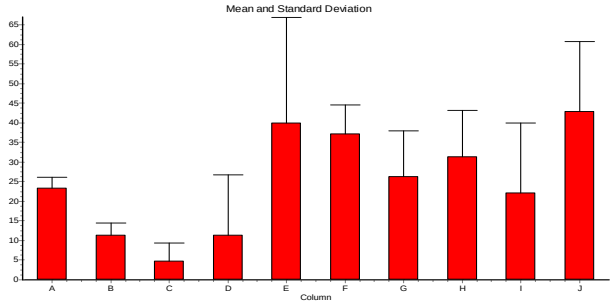


Graph-3 and 4: Showing effect of A-J group on percentage fraction of electrophoresis plasma protein Alpha 1, alpha and comparison with standard and control group. On x axis A, B, C, D, E, F, G, H, I and J mark expressed as group of *Colocasia esculenta 50 mg/kg bw*, *Colocasia esculenta*100 mg/kg bw, *Moringa oleifera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus*100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw. On y-axis % fraction of plasma protein Alpha 1 and Beta. Graphs are expressed as means ± SD of four animals in each group. Data determined by One-Way analysis of variance (ANOVA).

Moringa oliefera 200 mg/kg bw revealed phagocytic index 15.40 ± 1.662 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 19.05 ± 9.34, 17.68 ± 5.948, 9.448 ± 8.072, 13.80±8.26,6 40.02±26.89 respectively.

Moringa oliefera 200 mg/kg bw revealed that albumin 2-3 times lower, alpha 1 slightly increased However, Gamma plasma protein 2-3 times higher than the normal range.

Moringa oliefera 400mg/kg bw revealed phagocytic index 14.68 ± 1.774 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 34.26± 5.285, 9.100 ± 3.028, 8.188 ± 4.802, 11.27±9.404, 37.17 ±7.404 respectively.



Graph-5: Showing effect of A-J group on % fraction of electrophoresis plasma protein Gamma and comparison with standard and control group. On x axis A, B, C, D, E, F, G, H, I and J mark expressed as group of *Colocasia esculenta 50 mg/kg bw*, *Colocasia esculenta*100 mg/kg bw, *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus*100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw. On y-axis % fraction of plasma protein Gamma. Graphs are expressed as means ± SD of four animals in each group. Data determined by One-Way analysis of variance (ANOVA

Moringa oliefera 400 mg/kg bw revealed that albumin slightly decreased and gamma plasma protein 2-3 times higher than the normal range However, remaining plasma protein falls under the normal range.

Luffa cylindrica 100mg/kg bw revealed phagocytic index 9.808 ± 0.294 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 26.47 ± 15.08, 14.59 ± 23.01, 15.77 ± 12.44, 16.84±9.887,26.30±11.64 respectively.

Luffa cylindrica 100 mg/kg bw revealed that albumin slightly decreased However, alpha 1 and gamma plasma protein slightly increased respectively than the normal range and remaining plasma protein falls under the normal range.

Luffa cylindrica 200mg/kg bw revealed phagocytic index 13.30 ± 0.413 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 31.58 ± 16.13, 13.26 ± 6.232, 12.12 ± 5.162,11.63 ± 1.739, 31.38 ± 11.77 respectively.

Luffa cylindrica 200 mg/kg bw revealed that albumin slightly decreased and alpha 1 protein slightly increased However, Gamma plasma protein 2-3 times higher than the normal range.

Hibiscus esculentus 100mg/kg bw revealed phagocytic index11.30 ± 2.222 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 12.99± 10.09 31.99 ± 12.47, 20.99 ± 9.158, 11.86±9.140, 22.16 ±17.85 respectively.

Hibiscus esculentus 200mg/kg bw revealed phagocytic index 13.66 ± 2.600 and percentage fraction of plasma protein revealed albumin, alpha 1, alpha 2, beta, gamma 16.25± 8.127, 21.47 1± 4.03, 14.52 ± 7.537, 4.800±3.065, 42.92 ±17.91 respectively *Hibiscus esculentus* 100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw revealed that albumin and alpha 1 plasma protein in both doses of the drugs moderately increased while the gamma protein moderately increased with *Hibiscus esculentus* 200 mg/kg bw not in *Hibiscus esculentus* 100 mg/kg bw.

Statistical analysis

The data were expressed as mean ± SD .the statistical significance between means was analyzed using one way analysis of variance (ANOVA) followed by Tukey - Kramer multiple comparison test. A $P<0.05$ was considered as statistical significant.

Discussion and Conclusion

Discussion

The world is facing an explosive increase in the incidence of immunodeficiency disease and autoimmuno disease. In cancer treatment (chemotherapy), AIDS patient and organ transplantation immunity power of patient is decrease at this moment immunomodulator and immunosuppressant drugs are beneficial for patient, so that effective complementary therapies are needed.

In this present study, 50 % methanolic extract of *Colocasia esculenta* 50 mg/kg bw, *Colocasia esculenta* 100 mg/kg bw, *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, *Hibiscus esculentus* 100 mg/kg bw and *Hibiscus esculentus* 200 mg/kg bw was investigated for immunomodulator activity by plasma electrophoresis and phagocytosis method.

In our study, orally administered of Effect 50 % methanolic extract of *Colocasia esculenta* (50 mg/kg bw and 100 mg/kg bw) for regular five days as reflected increase % the of yeast digestion as compared to all the group, both drug were partially significant (*P< 0.05) the % of yeast digestion as compared to standard group, therefore *Colocasia esculenta* 50 mg/kg bw had lowest % of yeast digestion effect among the all the drugs but increased the dose (100 mg/kg bw) of this just slightly increase the % of yeast digestion. Therefore it has lowest immunomodulator effect among the all the plants. *Moringa oliefera* 200 mg/kg bw and *Moringa oliefera* 400 mg/kg bw dose moderately significant (**P< 0.001). with *control, standard, Colocasia esculenta* 50 mg/kg *bw, Colocasia esculenta* 100 mg/kg bw, However, *Luffa cylindrica* 100 mg/kg bw *and Luffa cylindrica* 200 mg/kg bw. *Moringa oliefera* 200 mg/kg bw were partially significant (*P< 0.05) the % of yeast digestion as compared to all the group. Therefore *Moringa oliefera* 200 mg/kg bw had highly phagocytosis activity among the all the test group but *Moringa oliefera* 400 mg/kg bw had a slightly lower effect than *oliefera* 200 mg/kg bw dose.

Luffa cylindrica 200 mg/kg bw and *Luffa cylindrica* 100 mg/kg bw dose moderately significant (**P< 0.001) with standard and control group as compared with all the group. While *Luffa cylindrica* 200 mg/kg bw and *Hibiscus esculentus* 100 mg/kg had lower phagocytic activity than the *Luffa cylindrica* 100 mg/kg bw.

Hibiscus esculentus 100 mg/kg bw dose moderately significant (**P< 0.001) with standard and control group as compared with all the group however *Hibiscus esculentus* 200 mg/kg bw moderately significant (**P< 0.001) with the *Colocasia esculenta* (50 mg/kg bw and 100 mg/kg bw) *Hibiscus esculentus* 200 mg/kg bw doses showed slightly higher effect than the *Hibiscus esculentus* 100 mg/kg bw.

In case of Albumin globulin, standard group showed the moderately increase % fraction of Albumin globulin at ***P< 0.001 as compared with reaming entire group.(*Colocasia esculenta* 50 and 100 mg/kg bw. dose Control, *Luffa cylindrica* 100mg/kg bw, *Luffa cylindrica* 200 mg/kg bw *Moringa oliefera* 200 and 400mg and *Hibiscus esculentus(* (200 and 400mg) While Control group partially significant (*P< 0.05) with *Colocasia esculenta* 50 mg/kg bw, *Moringa oliefera* 200 and *Hibiscus esculentus (*200 and 400mg).

Colocasia esculenta 50mg/kg bw dose was reflected increase the % fraction of Alpha 2 plasma protein treatment with regular five days. *Colocasia esculenta* 50mg/kg bw dose moderately significant (**P< 0.001) alpha 2 plasma as compression with *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw dose and *Hibiscus esculentus* 200 mg/kg bw dose while partially significant (*P< 0.05) with *Luffa cylindrica* 100 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw, standard and control as compared with all the group *Hibiscus esculentus* 100 mg/kg bw. *Colocasia esculenta* 100mg/kg bw had partially significant (*P< 0.05) with standard, control and *Hibiscus esculentus* 200 mg/kg bw.

Colocasia esculenta had a lower immunomodulator effect as compared with the entire test group because lower % fraction gamma and beta globulin as compared to other test group. For immunomodulator activity % of alpha 2, beta and gamma globulin is considered. (Considering order of globulin gamma > beta > alpha)

% fraction of gamma globulin in *Moringa oliefera* 200 mg/kg bw showed partially significant *P< 0.05 than *Colocasia esculenta* 50 mg/kg bw group as compared with all the test

group. *Moringa oliefera* 200 mg/kg bw had moderately immunomodulator activity but lower than *Hibiscus esculentus* 200 mg/kg bw and higher than the *Moringa oliefera* 200 mg/kg bw .
Among the *Luffa cylindrica* 100mg/kg bw and *Luffa cylindrica* 200 mg/kg bw, *Luffa cylindrica* 200 mg/kg bw had higher immunomodulator activity than the the *Luffa cylindrica* 100mg/kg bw but lower than the *Moringa oliefera* 200mg/kg bw, *Moringa oliefera* 400 mg/kg bw, and *Hibiscus esculentus* 200 mg/kg bw.
% fraction of gamma globulin in *Hibiscus esculentus* 200 mg/kg bw. had partially significant *P< 0.05 than *Colocasia esculenta* 50 mg/kg bw group as compared with all the test group. It had extremely good immunomodulatory activity than all the test group because of increment of gamma globulin had higher than the all the test group.
Dietary contribution for different aliments is big question marks for its affinity and it is potentially toward the different disease hypothetically. We claim that all the dietary supplement and dietary aliment is beneficial to human health. However, no proper validation engraved in such a situation where the purity of vegetables consistent could not able to define. Therefore our present study we conclude that *Hibiscus esculentus* and *Moringa oleifera* independently proven as best immunomodulator enhancing property strong immense immunomodulator modle parameter to be taken to lot of their potentiality. However, our present study revealed and signatured for their immunomodulator enhancing property. As in Asian subcontinent daily there vegetables are cooked and served with know and unknown of its potential function against different diseases. If there vegetables properly ruled out for their pharmacological aspect then it may add diamond in the crown of dietician which has been bother every day today life but over looked exponetentially

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