IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

STUDY OF IMMUNOMODULATORY ACTIVITY OF *GRACINIA GUMMI GUTTA FRUIT* EXTRACT IN EXPERIMENTAL ANIMALS

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ABSTRACT

The aim of the present study was to study the immunomodulatory activity of *Gracinia Gummi Gutta Fruit* extract (GGGFE) in experimental animals at two doses of 200 mg/kg and 400 mg/kg orally. The effect was studied in Cyclophosphamide induced neutropenia, Carbon clearance test, Serum immunoglobulin levels and Indirect haemagglutination. Cellular immunity was carried out by Cyclophosphamide induced neutropenia and Carbon clearance test whereas humoral immunity was analyzed by serum immunoglobulin levels and indirect haemagglutination test. The *Ocimum sanctum* extract (OSE, 100 mg/kg, p.o) was used as standard. The study of immunomodulatory potential of *Gracinia Gummi Gutta Fruit* extract (GGGFE) evoked a significant ($p<0.05$) protection against cyclophosphamide induced neutropenia indicating its effect on cell mediated immunity and significant ($p<0.05$) increased carbon clearance, indicating the stimulation of Reticulo-endothelial system. On the other hand, GGGFE produced a significant increase in serum immunoglobulin levels and a dose dependent increase in haemagglutination titer values suggesting its effect on the humoral arm of the immune system. Thus, it was concluded that the GGGFE explored a significant effect on both cell mediated and humoral immunity.

Keywords: *Gracinia Gummi Gutta Fruit* extract, Neutropenia, Carbon clearance, Serum immunoglobulin, Haemagglutination titre.

INTRODUCTION

The term immunity defines body's natural defence system against a vast array of diseases.[1]Immune system dysfunction is responsible for various diseases like allergy, arthritis, ulcerative colitis, asthma, parasitic diseases, cancer and infectious diseases.[2]With the number of hungry people rising from 785 million in 2015 to 822 million in 2018 is a very alarming situation which can cause various implications such as undernourishment and immune deficiency syndromes.[3]

Malnutrition contributes a major role in immunodeficiency of elderly patients in developing world. The leading infectious causes of death are respiratory tract infections, tuberculosis, diarrheal diseases, malaria and AIDS, which together represent > 90% of deaths in developing world and the remaining 10%, are due to tropical diseases and various other infections.[4] Malnutrition increases the risk of infection due to the impaired cell-mediated immunity and cytokine, complement, and phagocytic function.[5]

Immunology is one of the most rapidly developing areas of biomedical research. It has great promises with regard to the prevention and treatment of wide range of disorders.[6] Immunomodulation means the alteration of immune response which may increase or decrease the immune responsiveness, where immunostimulation is the enhancement in the immune responsiveness and reduction in the immune responsiveness is called immunosuppression. An immunomodulatory agent can be either biological or synthetic substance which can stimulate, suppress or modulate any of the components of the immune system including the innate and adaptive arms of immune response.[7]

Various allopathic drugs are used to modulate the immune system but these drugs are very expensive for poor people and are not easily accessible. However, in most cases they are associated with adverse drug reactions.[8] Immunomodulators such as Levamisole, IFN and IL- 2 are used in combination with cisplatin, adriamycin,5-flurouracil etc. in the treatment of various types of carcinomas, and the challenges faced for these synthetic immunomodulators is their side effects such as fatigue, neutropenia, myalgias, anorexia, elevated transaminases etc.[9]

Cyclophosphamide is clinically used for tumor therapy and it exhibits strong immunosuppressive effects in patients, by causing damage to the immune system and hematopoietic function. Moreover, it causes senescence

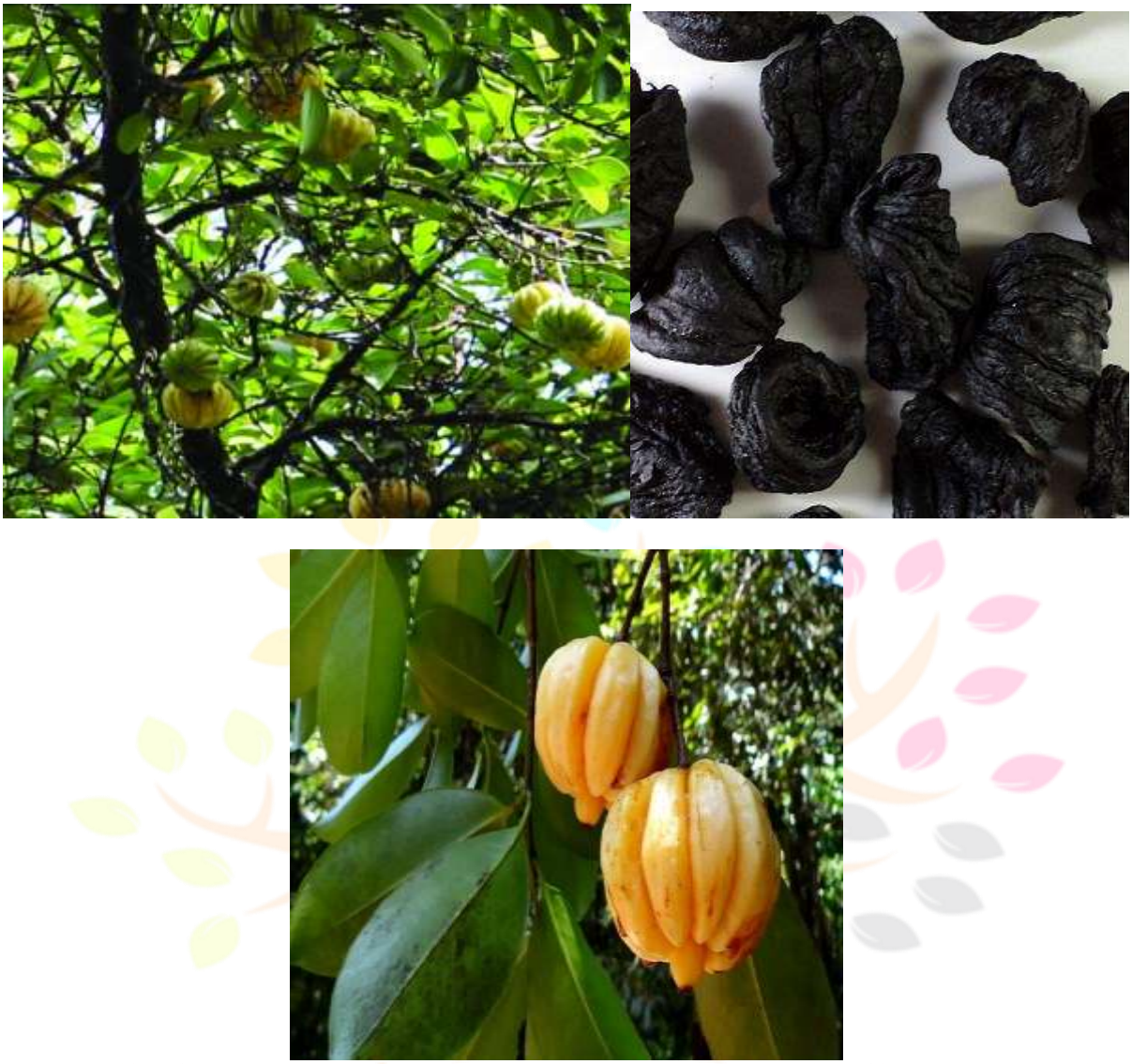
by disturbing the balance of the oxidative system.[10] other immunosuppressive agents such as azathioprine and 6-mercaptopurine can causes medullar suppression, and cyclosporine causes nephropathy, hypertension, convulsions, hepatitis etc. Tacrolimus and Mycophenolate are used as alternatives to cyclosporine but these drugs may increase the risk of diabetes.[11]

Hence, now days the use of medicinal plants being increased in the rural area of developing world and several distinct studies established the capacity of medicinal plants to enhance or reduce the immune response. For example, Ginseng enhances β and T cells, immunomodulatory effect of garlic on cell mediated immunity has been reported.[12] Some of the other plants which used as alternative herbal medicines including immunomodulatory effects are *Ocimum sanctum, Tinospora cordifolia, Eclipta alba* etc. Secondary metabolites such as polysaccharides, lectins, proteins, peptides, alkaloids, flavonoids, saponins, coumarins, and triterpenoids present in plants have been shown to immunomodulatory activity.[13, 14]

Gracinia gummi gutta belonging to the Clusiaceae family. Commonly Known as Malabar tamarin. Secondary metabolites like xanthones, bioflavonoids, coumarin, benzophenones are present. The preliminary qualitative chemical tests showed that leaves have high content of alkaloids, tannins, phenolic flavonoids, carbohydrates and proteins. Steroids, terpenoids, phlobatannin and cardiac glycosides were found in small quantity. It was found that the polyisoprenylated benzophenone and xanthone derivatives have antioxidant, apoptotic, anticancer, antiviral, antiulcer and antiprotozoal properties.[15] The potential medicinal value *of Gracinia Gummi Gutta indicates* the possibility of modulation of immune system. But still now no scientific evidence is available against it, so the present study is designed to explore the immunomodulatory effect of *Gracinia Gummi Gutta Fruit* extract GGGFE) by using different experimental models.

Garcinia gummi-gutta. Roxb Syn. Garcinia cambogia, popularly known as Malabar tamarind/kudampuli, belonging to the family Cluisaceae. Garcinia gummi-gutta is a tropical species of Garcinia native to Indonesia. In India, species of Garcinia grow extensively in semi wild state, in the Konkan region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, and evergreen forests of Assam, Khasi, Jantia hills, Nagaland, West Bengal, and Gujarat. In Malabar and Konkan regions of Southern India. In Karnataka it is found in Kodagu and Uttar Kannada. Fruits are ovoid, 2 inches in diameter, yellow when ripe, with 6-8 grooves, seeds 6-8 surrounded by succulent aril. The fruits have been reported to be used in traditionalAyurvedic medicine for the treatment of aliments like delayed menstruation, diarrhoea, haemorrhoids, dysentery, ulcer, rheumatism and heart

complaints.



***Gracinia gummi gutta* plant, fruit**

The fruit is well known for the acidic nature and the chemistry and analytical techniques of hydroxy citric acid, the major organic acid in *Gracinia gummi-gutta*. Benzophenones are the major secondary metabolites in *Gracinia gummi-gutta*, followed by xanthones and bioflavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garcinol, xanthochymol and guttiferone.

G. gummi-gutta is traditionally used as a condiment for flavouring curries. The seeds yield an oil, which is used in medicine. The dried rind was used for polishing gold and silver and also used as a substitute for acetic and formic acids in the coagulation of rubber latex. and has been used traditionally for the treatment of edema, delayed menstruation, ulcers, open sores, hemorrhoids, fever, rheumatism, and also against intestinal parasites. The astringent properties of the rind make it an indispensible ingredient in gargles for weak gums, bowel complaints, constipation, diarrhoea and dysentery.

Methodology

Experiment models include:

Cyclophosphamide induced neutropenia and Carbon clearance test

Serum immunoglobulin levels and Indirect Haemagglutination test

Chemicals used apparatus used

SI. No	Chemicals	Manufacturer
	Indian ink	Loba chemicals Pvt Ltd. Mumbai
2.	Sodium carbonate	Finarchemicals, Ahmedabad
3.	Cyclophosphamide	Cadila healthcare Ltd, Goa
4.	Leishman's stain	Nice chemicals Pvt Ltd, Cochin
5.	WBC dilution fluid	B.N Laboratories
6.	Sodium citrate	Thomas Baker chemicals Pvt Ltd, Mumbai
7.	Sodium chloride	RankemLtd, New Delhi
8.	Sodium dihydrogen phosphate	Merck specialities Pvt Ltd, Mumbai
9.	Sodium azide	Merck specialities Pvt Ltd, Mumbai
10.	Sheep blood	Procured from local slotter house

11.	Zinc sulfate	RankemLtd, New Delhi
12.	Barium chloride	Finarchemicals, Ahmedabad
13.	Sulphuric acid	Finarchemicals, Ahmedabad

Apparatus used

SI. No	Instruments used	Company name
1.	Analytical balance	Schimadzu, Japan
2.	Centrifuge	Remi, India
3.	UV Spectrophotometer	Schimadzu, Japan
4.	Haemocytometer	Superior Marienfeld, Germany
5.	Nephaloturbidity meter	Systronics
6.	Microscope	Biocraft
7.	Microtitre plates	Laxbro, Pune

Experimental animals:

Laboratory breed Wistar albino rats (180-200), 12-15 weeks and Swiss albino mice (20-30g), 4 weeks of either sex respectively was used for the study. Animals were housed under Standard standard conditions of

temperature (25˚C), 12h/12h light/ dark cycles and fed with standard pellet diet containing (%w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.15, sand (silica) 1.12, and water adlibitum. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep animals clean and dry. The animals were maintained under standard conditions in an animal house approved by Committee for the purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were subjected for quarantine (10days) prior to experimentation. The Institutional Animal ethical committee approved the experimental protocol on animals.

Preparation of fruit extract[76]:

The fruit of *Gracinia gummi gutta* was collected from in and around Kodagu district, Karnataka, India during the winter season in the month of August. The fruit rinds weighing 5kg were separated along the grooves and deseeded. They are cleaned, shade dried, pulverized, and stored in airtight containers until the commencement of the extraction process. Hydroalcoholic extract was prepared. About 40-45g of dried powder was packed in the thimble of Soxhlet apparatus, and extraction was carried out with 95% ethanol refluxing at 50-70^0 C which yielded a dark brown sticky mass. The extract was concentrated and dried using a ventilated oven at 45^{0}C for 24h. The stock powder is stored in a glass desiccator at 4^0 C. The chemical constituent of extract was identified by qualitative analysis.

Extraction of *Gracinia gummi gutta fruit.*

Phytochemical estimation of the extract[76]:

Gracinia gummi gutta fruit extract were subjected to qualitative analysis to investigate the presence of various phytochemical constituents such as flavonoids, phenolic compounds, alkaloids, glycosides, tannins and saponins etc.

Tests for detection of steroids

Salkowski test

3ml of chloroform was mixed with 5 mg of the extract followed by shaking with 3 ml concentrated sulphuric acid. The presence of steroids was confirmed by the development of red colour.

Lieberman Burchardt test

The extract (5mg) was mixed with 3 ml of chloroform in a test tube, followed by addition of 1 ml of concentrated sulphuric acid and five drops of acetic anhydride through the sides of the test tubes. The presence of steroids was confirmed by the development of a reddish ring at the junction of two layers.

Tests for Detection of Alkaloids

The extract (0.5 g) was mixed with 5 ml of ammonia, 5ml of chloroform and 5 ml dilute hydrochloric acid. The acid layer obtained was used for the following chemical tests for alkaloids.

Mayer's test

A few drops of Mayer's reagent were added to 1ml of acid layer. The presence of alkaloids was confirmed by the development of a creamy white precipitate. Mayer's reagents were made by mixing 1.358 g of mercuric chloride dissolved in 60 ml of water and 5 g of potassium iodide dissolved in 10 ml of water and make up the final volume to 100 ml with distilled water)

Wagner's test

1 ml of the acid extract was mixed with a few drops of Wagner's reagent. The presence of alkaloids was indicated by the development of reddish-brown precipitate. (Wagner's reagent was prepared by mixing 2 g of iodine and 6 g of potassium iodide followed by dissolving in 100 ml of water)

Hager's test

1ml of the acid extract was mixed with a few drops of Hager's reagent were mixed. Development of yellow precipitate indicated the presence of alkaloids. (Hager's reagent was made by mixing 1 g of picric acid in 100 ml of water)

Dragendroff's Test:

1ml of acid extract was mixed with a few drops of Dragendroff's reagent. Development of a reddish-brown precipitate indicated the presence of alkaloids. (Dragendroff's reagent was prepared by mixing Stock solution (1) and Stock solution (2) which was then mixed with 7ml of concentrated hydrochloric acid and 15 ml of water. The final volume was made up to 400 ml with distilled water. Stock solution (1) was made by dissolving 0.6 g of bismuth sub nitrate in 2 ml of concentrated hydrochloric acid and 10 ml of water. Stock solution (2) was made by dissolving 6 g of potassium iodide in 10 ml of water)

Test for detection of phenolic compounds

Five drops of 10 per cent ferric chloride was added to the plant extract (5gm), dissolved in 1ml of water. The presence of phenolic compounds was confirmed by the development of dark blue colour.

Test for detection of tannins

Gelatin test the extract (0.5 g) was mixed with a few drops of 1% solution of gelatin dissolved in 10% sodium chloride. The presence of tannins was confirmed by the development of a white precipitate.

Ferric chloride test

3ml of 1% ferric chloride solution was mixed with 2mg of the plant extract. The presence of tannins was confirmed by the development of a blue, green or brown colour indicated.

Tests for detection of flavonoids

Lead acetate test

The alcoholic solution (2ml) of the extract was mixed with a few drops Journal of Ayurvedic and Herbal Medicine|April-June|2021 83 of neutral 10 % lead acetate. Development of yellow precipitate confirmed the presence of flavonoids. The alcoholic solution of the extract was prepared by dissolving 0.5 g extract in 10 ml methanol.

Ferric chloride test

2ml of alcoholic solution of the extract was mixed with a few drops of neutral ferric chloride solution. The presence of flavonoids was confirmed by the development of green colour.

Tests for detection of glycosides

Sodium hydroxide test

5-6 drops of sodium hydroxide solution (10%) were added to 5mg of the plant extract dissolved in 1 ml water.

Development of yellow colour indicated the presence of glycosides.

Benedict's test

5ml of Benedict's reagent was added to the extract (0.5 g) was dissolved in 1ml of water. The presence of glycosides was confirmed by the development of brown or red colour on boiling for two minutes.

Tests for detection of saponins

Foam tests the extract (5 mg) was shaken with equal volume of water. The presence of saponins was confirmed by development of the foam that persisted for 10 minutes.

Tests for detection of diterpene

The extract (5mg) was mixed with 3 ml of 5 per cent copper acetate solution. the presence of diterpenes was indicated by the development of green colour.

Tests for detection of triterpenes

Salkowski test

The extract (3 mg) was added to 3 ml of chloroform followed by shaking with 3ml conc. sulphuric acid. The presence of triterpenes was indicated by the development of yellow colour in lower layer on standing.

Lieberman Burchardt test

The extract (3 mg) was mixed with 3 ml of chloroform in a test tube. 1 ml of concentrated sulphuric acid and 5 drops of acetic acid were added slowly along the sides of the test tube. The presence of triterpenes was indicated by the development of a deep red ring at the junction of two layers.

Dose selection:

Experimental doses of ethanolic extract of Gracinia gummi gutta fruit were selected based on previous literature. For this research work, the selected doses were 200 and 400mg/kg, body weight of animal by oral route.[77]

EXPERIMENTAL MODEL

Cyclophosphamide induced neutropenia and carbon clearance test[78-82.]

Group-I: Normal (0.9% Normal saline) (1ml/kg, p.o).

Group- II: Control group (Cyclophosphamide 200mg/kg).

Group-III: Standard (extract of *Ocimum sanctum* (OSE) 100mg/kg, p.o) +

Cyclophosphamide (200mg/kg i.p)/Indian ink(0.1ml).

Group-IV: Low dose of GGGFE (LDGGG) (200mg/kg, p.o) +

Cyclophosphamide (200mg/kg i.p)/Indian ink (0.1ml).

Group- V: High dose of GGGFE (HDGGG) (400mg/kg, p.o) +

Cyclophosphamide (200mg/kg i.p)/Indian ink(0.1ml).

In Cyclophosphamide induced neutropenia, Swiss albino mice of either sex were pretreated with *Ocimum sanctum* extract (OSE) and low and high dose of GGGFE (200mg/kg and 400mg/kg) or normal saline (0.9% 1ml/kg) orally for 10 days. On the 10th day, a neutropenic dose of cyclophosphamide (200mg/kg, i.p) was administered. The total leucocyte count (TLC) and % reduction in neutrophil count were performed prior to and on the day after injection of cyclophosphamide. The TLC and % reduction in neutrophil count in treated groups were compared with the values of the control group.

➢ **Blood sampling and determination of parameters in cyclophosphamide induced neutropenia**

Blood samples for the determination of Total leucocyte count (TLC) and % reduction in neutrophil were collected from the animals through retro-orbital plexus in to heparinised tubes on day 0 before injection and on day 3 after injection of cyclophosphamide[78].

➢ **The parameters estimated were:**

Total leukocyte count (TLC) test

This test is also called as total white blood cell (WBC) count test. For this test, 0.38ml of 1% glacial acetic acid (WBC diluting fluid) mixed with 0.02ml of blood in a test tube and resultant mixture was counted with the improved Neubauer counting chamber using x 40 magnification lens. The four corner squares of the central square were counted and the number of cells (cells/l) counted was recorded[78].

% Neutrophil count test (DLC test)

The test was carried out by fixing the blood on a slide and staining with Leishman's stain, then the slides were kept for 8minute and the excess stain was washed off with water were air dried. oil immersion was added on the slides which were subsequently examined under a light microscope using x 100 magnifications. Thus, the percentage neutrophils (cellµl/l) were determined.

In the Carbon clearance test, swiss albino mice of either sex was administered with OSE and low and high dose of GGGFE (200mg/kg and 400mg/kg) or Normal saline for 10 days orally. After 48h of the last dose of the drug, mice were injected with 0.1ml of Indian ink via the tail vein. Blood samples was withdrawn at 0min and 15min.The effectiveness of pretreatment will be evaluated by the estimation of phagocytic index.[78]

➢ **Blood sampling and determination of phagocytic index in carbon clearance test**

Blood samples were withdrawn from the retro-orbital plexus of the animals at 0 min before and 15 min after injection of the Indian ink. A 50μl blood sample was mixed with 4ml of sodium carbonate solution (0.1%) to lyses the erythrocytes. The optical density was recorded using UV visible spectrophotometer at 660nm.[78]

➢ **The parameters estimated were:**

The phagocytic index, K, which is the rate of carbon elimination from the reticuloendothelial system, was calculated using the following equation:

Phagocytic index, K =log OD1- log OD2/15,

Where, OD1 and OD2 are the optical densities at 0 and 15 min respectively.[78]

Serum immunoglobulin level and Indirect Haemagglutination test[83-85]:

Group- I: Normal (0.9% Normal saline) (1ml/kg p.o).

Group-II: Standard (extract of *Ocimum sanctum* (OSE) 100mg/kg, p.o).

Group-III: Low dose of GGGFE (LDGGG) (200mg/kg) +SRBC.

Group-IV: High dose of GGGFE (HDGGG) (400mg/kg) + SRBC.

In indirect haemagglutination test, rats of various groups were pre-treated with OSE and high and low doses of GGGFE (200mg/kg and 400mg/kg) orally for 14 days and all rats of entire groups immunized intraperitoneally with sheep red blood cells (SRBCs). The drug treatment was continued for 14 more days. The influence of the prophylactic treatment was evaluated by the determination of heamagglutination (HA) titre value.

➢ **Antigen and immunization**

The sheep red blood cells (SRBCs) were used as an antigenic material. Fresh sheep blood was collected in a mixture of 0.49% w/v EDTA and 0.9%w/v sodium chloride solution from sheep sacrificed in the local slaughter house. (SRBCs) were washed 3times in large volumes of pyrogen free normal saline by centrifugation at 3000rpm for 10 min on each cycle and adjusted to concentration of 0.5×109 cells/mL for immunization. Each rat was immunized by injecting 0.1mL of SRBC (sheep red blood cells) suspension containing of 0.5×109 cells intraperitoneally, including the control rats. The day of immunization was referred to as day zero. The drug treatment was continued for another 14 days[86,87].

➢ Blood sampling and determination of Haemagglutination titer

Blood samples were collected in micro centrifuge tubes from each rat by retro orbital puncture on day 15. The blood samples were centrifuged and serum was obtained, which was used for hemagglutinating antibody (HA) titer. The titer value was determined by titrating serum dilutions with SRBC (0.025×109 cells) in microtiter plates.[87]

➢ Method of serum Dilutions (Double dilutions)

Antibody level was evaluated by hemagglutination technique using 96 wells bottomed titre plate (12 × 8). The wells of titre plate were marked 1 to 12. In the first and last well, 25 µL of the serum collected from treated animals was added and left for inactivation at 56 °C for 30– 40 min. Thereafter, 25 µL of PBS was added to all the wells except well number 12, and mixed properly. Then 25 µL of blood sample was taken from the first well, added to second well and serially diluted up to well number 10. Thereafter, 25 µL sample was taken out from well number 10 and discarded. Finally, 25 µL of 1% suspension of SRBCs was added to all the wells and incubated at 37 °C for 1 h. Each well of the titre plate was examined visually for hemagglutination. The reciprocal of the highest dilution of the test serum giving agglutination was examined as the antibody titre.[8]

In serum immunoglobulin levels, albino rats of either sex were pretreated with the OSE and low and high doses of GGGFE (200mg/kg and 400mg/kg) or normal saline orally for 21 days. The immunomodulatory effect of prophylactic treatment was analysed by determining the serum immunoglobulin levels of treated and control group[83-85].

➢ Preparation of standard Barium sulfate solution

A solution of barium chloride (1.15g/100mL) was prepared. From this solution, 3mL was made up to 100 mL with 0.2 N sulphuric acid. The turbidity obtained with this solution was expressed as 20 zinc sulfate turbidity (ZST) units.[78]

➢ Blood sampling and determination of serum immunoglobulin levels

After six hours of last dose of drug, blood was collected and serum was used for estimation of immunoglobulin levels. For each sample to be analyzed, a control tube containing 6ml of distilled water and a test tube containing 6ml of zinc sulfate solution were prepared. To each, 0.1ml of serum was added from a pipette and they were inverted to enable complete mixing of the reagents and left to stand for 1 hour at room temperature. The first tube served as a blank and the second tube was taken as sample. The turbidity obtained was measured using digital

nepheloturbidity meter. The turbidity obtained i.e.; sample-blank was compared with that obtained with standard Barium sulfate (BaSo4) solution.[78]

Statistical analysis:

The statistical significance was assessed in Graph Pad Prism 9 software using one-way analysis of variance (ANOVA) followed by Tukey-Kramer comparison test. The values are expressed as mean ±SEM and$p<0.05$ was considered significant.

RESULTS

Preparation of fruit extract of *Gracinia gummi gutta:*

The fruit extract of GGGFE was prepared by the fruit rind was separated, cut into small pieces; shade dried and were pulverized. The dried powder obtained was extracted with 95% ethanol using Soxhlet apparatus, followed by concentration in a vacuum rotary evaporator and kept under refrigeration for further use. The yield of the extract was 10% on dry matter basis.

Table 5.1 Phytochemical investigation

SL NO	TEST	INFERENCE	RESULT
1.Test for Alkaloids			
1.a	Mayer's test	Creamy white precipitate	+
1.b	Wanger's test	Reddish brown precipitate	+
1.c	Hanger's test	Yellow precipitate	+
1.d	Dragendroff's test	Reddish brown precipitate	+

2.Test for Steroids			
2.a	Salkowski test	Red colour	+
2.b	Liberman Burchardt test	Reddish ring	+
3.Test for Phenols			
3.a	Ferric chloride test	Dark blue colour	+
4.Test for Tannins			
4.a	Gelatin test	White precipitate	+
4.b	Ferric chloride test	Blue colour	+
5.Test for Flavonoids			
5.a	Lead acetate test	Yellow precipitate	+
5.b	Ferric chloride test	Green colour	+

6.Test for Glycosides			
6.a	Sodium hydroxide test	Yellow colour	+
6.b	Benedict's test	Red colour	+
7.Test for Saponins			
7.a	Foam test	No foam	-
8.Test for Triterpens			
8.a	Salkowski test	Yellow colour	+
8.b	Liberman Burchardt test	Deep red ring	+

' + ' indicates positive result, ' - ' indicates the negative result.

Cyclophosphamide induced neutropenia and Carbon clearance test

Cyclophosphamide induced neutropenia

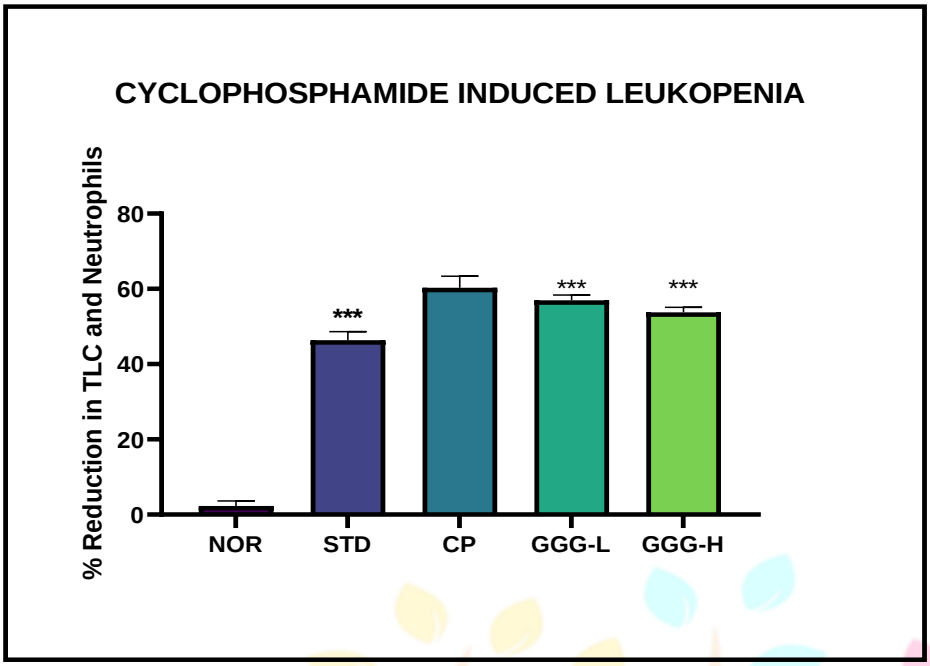
The pretreatment of animals was done with (GGGFE) 10 days before cyclophosphamide administration showed 56.52% (*p<0.05) and 55.64% (*p<0.05) reduction in TLC with low and high doses respectively, While the cyclophosphamide treated group (control) showed a 62.43% reduction. The pretreatment of animals with *Ocimum sanctum* Extract (OSE) showed only 43% fall in TLC when compared to initial values (Table 5.3). The low and high doses of GGGFE demonstrated 44.27% (*p<0.05) and 43.61% (p*<0.05) reduction in neutrophil count compared to initial values. While the cyclophosphamide treated group showed a 58.07% reduction (Table 5.3).

And however, the statistical analysis revealed that there was a significant difference in the TLC and % reduction in neutrophil before and after the cyclophosphamide administration in different groups compared to control.

Effect of GGGFE on cyclophosphamide induced leucopenia in mice.

Treatment	Total leukocyte count(cells/mm^3)		%Reduction
	Before	After	
Normal	5355±84.14	5225.17±73.62	2.1256±1.30
Standard (OSE) (100mg/kg, po)	5967.17±47.79	3196.83±50.73	46.905±2.30***
LDGGG (200mg/kg, p o)	5598±72.133	2409.5±61.74	56.685±1.43***
HDGGG (400mg/kg, p o)	5658±57.67	2615.67±55.43	53.585±1.35***

All values are mean ± SEM, n=6, ***p<0.001, **p<0.01, *p<0.05when compared to control.



All values are mean ± SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compared to control.

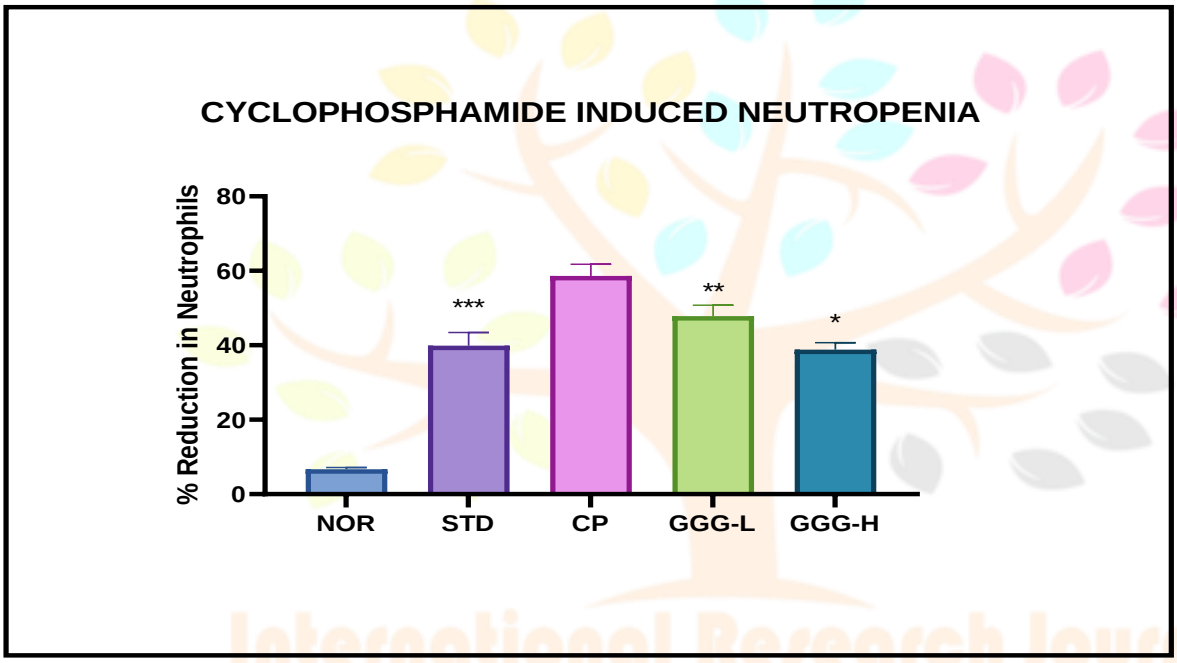
Figure5.1 Effect of GGGFE on Cyclophosphamide induced leukopenia in mice.

Effect of GGGFE on cyclophosphamide induced neutropenia in mice.

Treatment	**Neutrophil count (cells/mm)**		**%Reduction**
	Before	**After**	
Normal	15.71±0.47	14.6±0.45	6.67±0.47
Standard (OSE) (100mg/kg, p.o)	25.85±0.88	15.50±0.48	39.91±3.52***
Diseased group (cyclophosphamide,200mg/kg i.p)	15.85±0.43	6.55±0.31	58.64±3.13

LDGGG (200mg/kg, po)	21.22±0.42	11.06±0.49	47.81±2.94**
HDGGG (400mg/kg, po)	21.6±0.37	13.28±0.20	38.81±1.89*

All values are mean ± SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compared to control.



All values are mean ± SEM, n=6, ***p<0.001, **p<0.01, *p<0.05 when compared to control.

Effect of GGGFE on Cyclophosphamide induced neutropenia in mice.

Carbon clearance test:

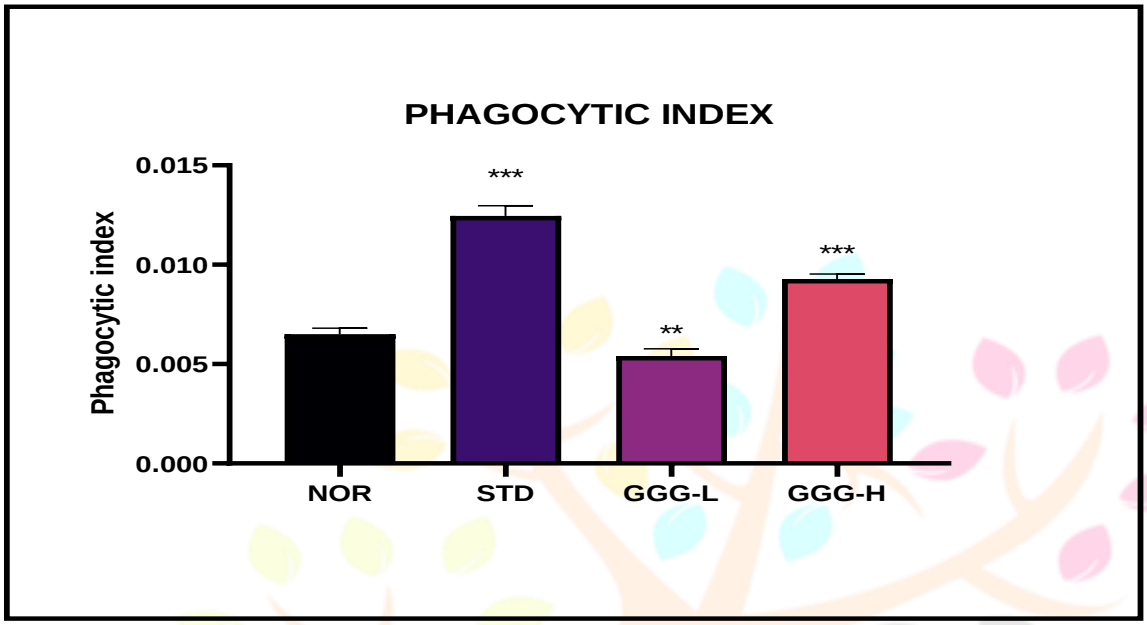
Administration of HDGGG (400mg/kg, p.o) and OSE (100mg/kg, p.o) produced an increase inclearance of carbon particles from blood as indicated by a moderately significant increase inphagocytic index (p*<0.05), while LDGGG (200mg/kg) also showed significant increase (p*<0.05) when compared to the Normal.

Figure 5.4 Effect of GGGFE on Phagocytic index in carbon clearance test in mice.

Treatment	Phagocytic index
Normal	0.0065±0.0003
Standard (OSE) (100mg/kg, p.o)	0.0124±0.0005***

LDGGG (200mg/kg)	0.0054±0.0003**
HDGGG (400mg/kg)	0.0093±0.0002***

All values are mean ± SEM, n=6, *** p<0.001, **p<0.01, *p<0.05when compared to normal



All values are mean ± SEM, n=6, *** p<0.001, **p<0.01, *p<0.05when compared to normal

Effect of GGGFE on Phagocytic index in carbon clearance test in mice.

Serum immunoglobulin levels and Indirect haemagglutination test

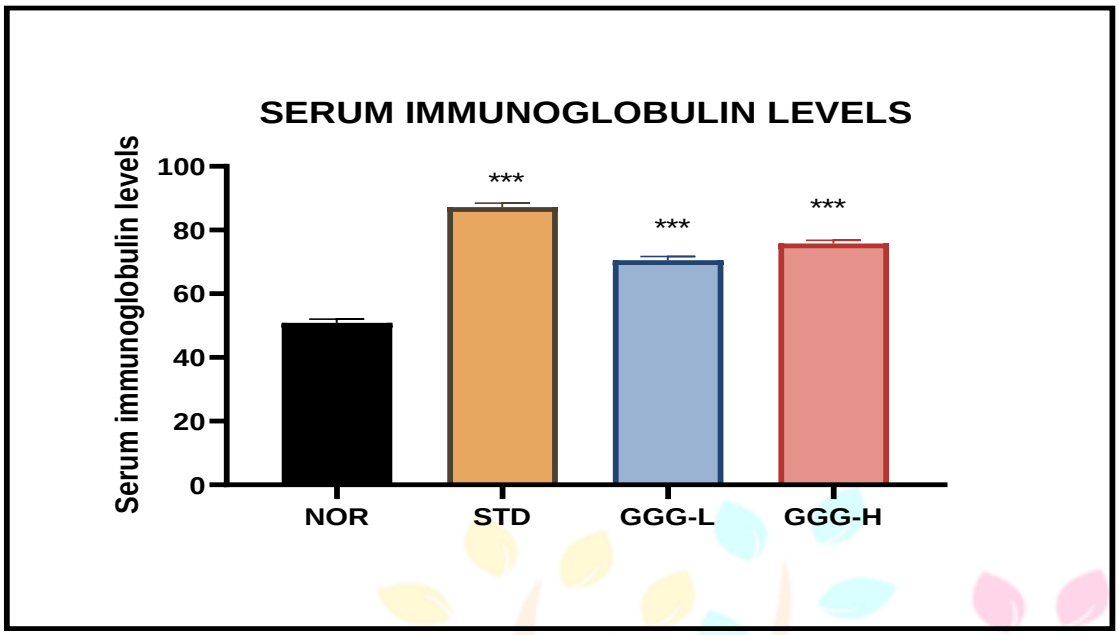
Serum immunoglobulin levels

Prophylactic treatment in the different experimental group such as OSE (100mg/kg, p.o)LDGGG (200mg/kg, p.o) and the HDGGG (400mg/kg, p.o) demonstrate a significant and moderately significant (*p<0.05 and **p<0.01 respectively) increase in serum immunoglobulin level respectively when compared to normal.

Effect of GGGFE on Serum immunoglobulin levels in rats.

Treatment	Serum immunoglobulinlevel (ZST units)
Normal	50.855±1.186
Standard (OSE) (100mg/kg, p.o)	87.166±1.310
LDGGG (200mg/kg)	70.7±1.213
HDGGG (400mg/kg)	75.95±0.996

All values are mean ± SEM, n=6, *** p<0.001, **p<0.01, *p<0.05 when compared to normal.



All values are mean ± SEM, n=6, *** p<0.001, **p<0.01, *p<0.05 when compared to normal.

Effect of GGGFE on Serum immunoglobulin levels in rats.

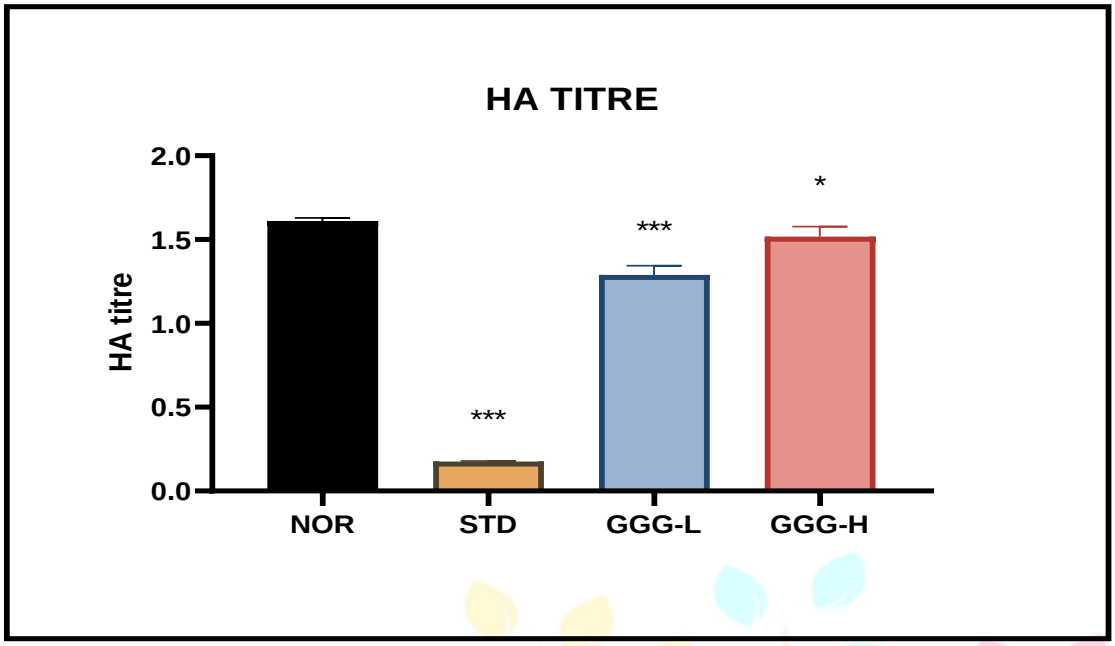
Indirect Haemagglutination test

The hemagglutinating antibody (HA) titre value was significantly reduced (p*<0.05) and (p**< 0.01) in animals that received vaccination along with low and high dose of GGGFE when compared to animal that received vaccination alone.

Table 5.6 Effect of GGGFE on Indirect haemagglutination in rats

Treatment	HA titer
Normal	1.617±0.017
Standard (OSE) (100mg/kg, p.o)	0.175±0.004
LDGGG (200mg/kg)	1.285±0.055
HDGGG (400mg/kg)	1.525±0.059

All values are mean ± SEM, n=6, *** p<0.001, **p<0.01, *p<0.05 when compared to normal.



All values are mean ± SEM, n=6, *** p<0.001, **p<0.01, *p<0.05 when compared to normal.

Figure 5.5 Effect of GGGFE on Indirect haemagglutination in rats.

DISCUSSION

The aim of the present study was to assess the immunomodulatory activity of *Gracinia gummi gutta* fruit extracts on laboratory animals. The study reported that *Gracinia gummi gutta* activates immune system through cell mediated and humoral immunity in several experimental animals at both low and high doses.

The objective of the current study was to assess the immunomodulatory activity of *Gracinia gummi gutta* fruit extracts (GGGFE) on various immune system components using a variety of immunomodulatory models, including carbon clearance test, indirect haemagglutination test, neutropenia induced by cyclophosphamide, and serum immunoglobulin levels.

Gracinia gummi gutta belonging to the Clusiaceae family. The fruit is well known for the acidic nature. Hydroxy citric acid, the major organic acid in *Gracinia gummi-gutta.* Secondary metabolites like xanthones, bioflavonoids, coumarin, benzophenones are present. Benzophenones are the major secondary metabolites in *Gracinia gummi-gutta*, followed by xanthones and bioflavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garcinol, xanthochymol and guttiferone. Hydroxy citric acid (HCA) is the important one, being an anti-obesity agent. It was found that the polyisoprenylated benzophenone and xanthone derivatives have antioxidant, apoptotic, anticancer, antiviral, antiulcer and antiprotozoal properties. And also, has been used traditionally for the treatment of edema, delayed menstruation, ulcers, open sores, hemorrhoids, fever,

rheumatism, and also against intestinal parasites. The astringent properties of the rind make it an indispensible ingredient in gargles for weak gums, bowel complaints, constipation, diarrhoea and dysentery.[75]

In addition to having anticancer characteristics, Cyclophosphamide induces myelo suppression in the experimental animals. It belongs to is a nitrogen mustard subclass of alkylating agents that suppresses the immune system and is known to produce neutropenia by suppressing the myelocytes.[83]To examine the impact of GGGFE on hemopoiesis, neutrophil counts and total leukocyte counts were measured both before and after the administration of cyclophosphamide. Both low and high doses of GGGFE caused 56.4% ($p<0.05$) and 44% ($p<0.05$) reduction in cyclophosphamide induced neutropenia when compared to cyclophosphamide treated group with 59.85% ($p<0.05$) reduction. Suggesting that it attenuates the effect of cyclophosphamide on the haemopoietic system. The activation of macrophages, which release a number of mediators including colony-stimulating factor and interleukin, could be the way cyclophosphamide-induced neutropenia may be prevented.[84] Phagocytosis is the process through which phagocytes eat and eliminate bacteria, malignant cells, inorganic particles, and tissue debris as part of the immune system's non-specific defence reaction against germs.[87] Phagocytic index was calculated in the current study using the carbon clearance method. It was used to assess the impact on reticuloendothelial cell-mediated phagocytosis. The RE cells are composed of fixed and sessile macrophages, Kupffer's cells, as well as various free histiocytes including monocytes and neutrophils. These RE cells serve as the body's initial line of defence by phagocytizing debris, germs, and other foreign substances. Through phagocytosis, RE cells can swallow carbon particles and eliminate them from the body.[89] The macrophages consume the colloidal carbon ink's carbon particles when it is given intravenously. Phagocytic index refers to the rate at which (carbon particle) ink is cleared from blood. An exponential equation controls how quickly macrophages remove carbon from blood when colloidal ink containing carbon particles is introduced directly into the systemic circulation.[90] Ethanolic extract of *Gracinia gummi gutta fruit* extracts at both the doses in mice stimulate the reticuloendothelial system by significant increase in phagocytic index ($p<0.05$) when compared to normal. Hence, these agents may stimulate the reticuloendothelial system.

The estimation of serum immunoglobulin level is a direct indicator of humoral immunity. Serum immunoglobulin refers to a group of serum molecules produced by B lymphocytes, they are soluble and secreted form of B-cell receptors and are produced to a maximum level to counter the invasion by an antigen and hence they are also called as antibodies. Based on the rate of electrophoretic migration, blood contains three different types of

globulins: alpha, beta, and gamma. A zinc sulphate turbidity test was used in the current investigation to estimate the serum immunoglobulin levels (ZST).

This examination establishes the quantity of immunoglobulins in the serum. A little amount of serum was added to a zinc sulphate solution, which was then given a one-hour incubation period at room temperature. The immunoglobulins precipitate as a result of zinc sulphate, which causes the solution to become cloudy. Although this test performs a fine job of detecting immunoglobulins, it does not quantify them very well, making it difficult to determine whether a condition is borderline. However, this examination is reasonably rapid and inexpensive. One of its shortcomings is that results depend on a variety of variables, including pH of the reaction mixture, time, temperature, and others.[91]

The indirect haemagglutination test was carried out to examine GGGFE's impact on the humoral immune system. B cells are key players in humoral immune responses and generate antibodies.[86] B cells engage with the antigen during the humoral immune response, and then they multiply and differentiate into plasma cells that secrete antibodies. The titer values of the circulating antibodies increased when the test animals were given OSE and GGGFE. The results showed that *Gracinia gummi gutta* fruit extract significantly increased circulating antibody titre at high dose ($p<0.01$) and low dose ($p<0.05$) when compared to normal group. This shows that the T and B cell subsets responsible for creating antibodies are more sensitive to stimuli. The test involves the preparation of double dilutions of serum samples and addition of constant amount of SRBC. As a result of the construction of antibody bridges with the surrounding erythrocytes, which occur when the serum includes antibodies to the SRBC, there will be agglutination and these bridges will eventually settle at the bottom as latex. The result showed that high values of hemagglutinating antibody titer obtained in the case of ethanolic extract of *Gracinia gummi gutta* indicated that immune stimulation was achieved through humoral immunity.[84]

CONCLUSION

The current study showed that GGGFE (200 mg/kg and 400 mg/kg, p.o) explored immunomodulatory activity in experimental animals. From the results of the present study, it can be concluded that the immune system is stimulated by low and high doses of GGGFE in different experimental animal models of immunity through cellular and humoral immunity. The efficiency of *Gracinia gummi gutta* fruit extract may result from the individual or combined effects of the phytochemical constituents present in it. The immunomodulatory effect of

fruit extract in various experimental animals may be due to the presence of flavonoids, coumarins, and steroids, which are responsible for antioxidant, anti-microbial, anti-inflammatory, and hepatoprotective activity. Further research is required to establish the fact clinically.

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