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TO EVALUATE IMMUNOMODULATORY ACTIVITY OF ELEUSINE INDICA IN CYCLOPHOSPHAMIDE INDUCED IMMUNOSUPPRESSIVE WISTAR ALBINO RATS

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ABSTRACT

The objective of this study was to assess the immunomodulatory effects of Eleusine indica in Wistar albino rats with cyclophosphamide-induced immunosuppression. Wistar albino rats were divided into five groups, with each group comprising six rats. Group I served as the normal control, Group II received cyclophosphamide (20 mg/kg, i.p.) for 10 consecutive days, Group III received cyclophosphamide along with the standard drug levamisole (50 mg/kg, p.o.) for 10 consecutive days, Group IV received cyclophosphamide along with Eleusine indica extract (200 mg/kg, p.o.) for 10 consecutive days, and Group V received only Eleusine indica extract (200 mg/kg, p.o.) for 10 consecutive days. Following the treatment period, various immunological parameters such as hematological parameters, humoral immune response, and cell-mediated immune response were assessed. Cyclophosphamide administration led to a significant reduction in hematological parameters, humoral immune response, and cell-mediated immune response. However, treatment with Eleusine indica extract significantly mitigated these cyclophosphamide-induced alterations. Additionally, Eleusine indica extract exhibited immunostimulatory effects in normal rats. The findings of this study suggest that Eleusine indica possesses notable immunomodulatory properties and could serve as an adjunctive therapy in immunocompromised conditions.

Key words - Eleusine Indica, Immunomodulatory activity, Cyclophosphamide, Immunosuppression.

INTRODUCTION

For the vast majority of people worldwide, medicinal plants remain the primary source of healthcare, and they have been integral to traditional medical systems since ancient times.

In Africa, the practice of healing with plants has endured for generations, with knowledge passed down orally among specific groups like herbalists and traditional healers. Researchers have identified over 814 species with therapeutic properties from 130 different plant groups. Among these, Eleusine indica holds significance as a medicinal plant in Benin. Eleusine indica, a member of the Poaceae family, is indigenous to tropical and subtropical regions and is commonly considered a weed species [1-4].

E. indica is believed to possess various pharmacological properties including anti-inflammatory, antioxidant, antibacterial, hepatoprotective, antiplasmodial, and antidiabetic effects. In Malaysia, E. indica, locally known as "rumput sambau," is the most accessible species of Eleusine and is commonly found growing along sidewalks and highways. Throughout history, traditional medicine has employed E. indica to address diverse conditions such as symptoms of microbial infections, muscle sprains, bloody coughs, and poisoning from scorpions or centipedes. Literature reviews indicate that traditional healers worldwide extensively use E. indica to manage conditions like inflammation and autoimmune disorders. Additionally, in India, young plant seedlings of E. indica are consumed either raw or cooked as a side dish with rice during periods of famine [5-8].

According to nutritional analysis, E. indica contains a variety of essential nutrients, including fibre, vitamins, minerals, proteins, lipids, and carbohydrates. Eleusine indica L., commonly known as goose grass, is a C4 grass that typically behaves as a summer annual in temperate climates. In turf grass systems, it can be particularly problematic in high-traffic areas where turf grass is less competitive. The immune system operates with a multi-layereddefence mechanism. The initial line of defence against infections is provided by the skin, which serves as the most basic barrier.

Physiological barriers also come into play, altering environmental factors such as pH and temperature to create unsuitable conditions for non-native species. Upon entry of pathogens into the body, both the innate and adaptive (or acquired) immune systems are activated to combat them. These systems consist of various chemicals and cells that intricately interact to detect and eliminate infections [9-13].

1. The innate immune system
    - The complement system
    - Macrophages
    - Cytokines and natural killer cells
2. The adaptive immune system
3. Cytokines/ interleukins
4. C-reactive protein
5. Immunomodulators

Factors associated with the modification of immune system [14, 15]

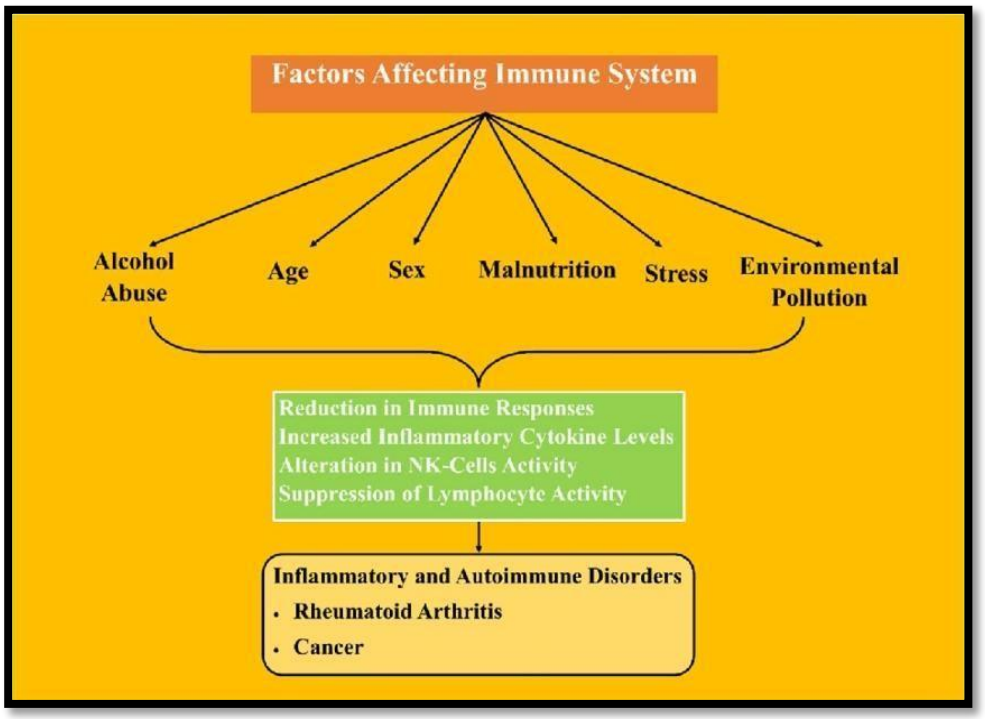

Fig. 1 Factors affecting immune system

Cyclophosphamide (CP), a potent alkylating agent, is extensively utilized in chemotherapy to treat diverse cancers and autoimmune disorders. Although CP effectively targets rapidly dividing cells, such as cancer cells, its administration often results in immunosuppression. This immunosuppressive effect arises from CP's capability to disrupt the proliferation and functionality of immune cells, ultimately compromising the immune response and heightening susceptibility to infections [16-18].

In recent years, there has been a surge in interest regarding the exploration of natural products as potential immunomodulatory agents. These natural compounds present several advantages over conventional drugs, such as their favourable safety profile, minimal side effects, and ability to modulate the immune system without causing undue harm. Eleusine indica, commonly known as "goose grass" or "wiregrass," is a plant that has been traditionally utilized in various regions across the globe for its medicinal properties [19-22].

Eleusine indica

Eleusine indica (L.) Gaertn, a perennial plant belonging to the Poaceae family, has been valued for its therapeutic properties for an extended period. It is predominantly found in the Pacific Islands and tropical regions. The most accessible species of Eleusine is E. indica, known locally as "rumput sambau" in Malaysia, and it tends to proliferate like a weed along sidewalks and roadways. Traditional medical practices across the globe have utilized E. indica to address various conditions, including symptoms of microbial infections, muscle injuries, bloody coughs, and incidents of scorpion or centipede poisoning [23, 24].

Fig 2. *Eleusine indica*

Taxonomy of *Eleusine indica*[25, 26]

Table 1. Taxonomy of *Eleusine indica*

Common names	Waghnakhi, goose grass
Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Cyperales
Family	Poaceae
Genus	*Eleusine*
Species	*Eleusine indica*

Eleusine indica has been reported to possess a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties. However, its potential immunomodulatory effects, particularly in the context of CP-induced immunosuppression, remain relatively unexplored.

Pharmacological properties of Eleusine indica

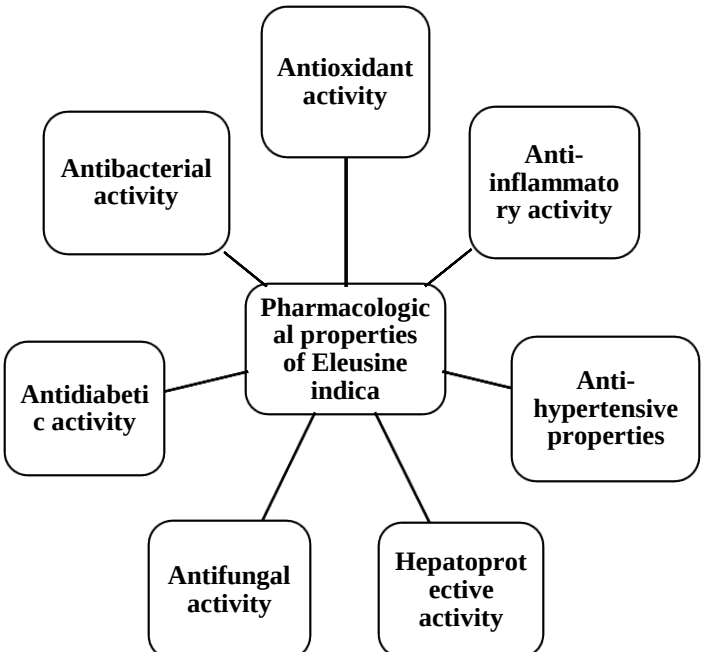

Fig 3. Pharmacological properties of *Eleusine indica*

In this study, our objective was to examine the immunomodulatory effects of Eleusine indica in Wistar albino rats with cyclophosphamide-induced immunosuppression. We hypothesized that the extract of Eleusine indica would alleviate the immunosuppressive effects caused by cyclophosphamide. To investigate this hypothesis, we evaluated different aspects of the immune system, such as hematological parameters, immune organ indices, and specific immune responses, in rats treated with Eleusine indica extract subsequent to cyclophosphamide-induced immunosuppression [27-30].

MATERIALS AND EQUIPMENTS[31-36]

Material for Animal Trial

Table 2. Requirement of Animals

Sr. No	**Species**	**Rat**
1	Strain	Wistar Albino Rats
2	Sex	Male
3	Body weight range	200-250 gm
4	No. of animals	72

Study Design

Six animals in each group were used for this study, Extract was dosed as per standard protocol and animals were observed for signs and symptoms along with weekly Body weight. Collection of Sheep RBC - Sheep RBC (SRBC) were collected in saline, washed three times in phosphate buffered solution (PBS) and adjusted to a concentration of 0.5X109 cells/ml.

Haemagglutination Antibody (HA) Titre:-

Table 3. Haemagglutination Antibody (HA) Titre

Group	**Name of Group**	**Dose**	**No. of Animals (Wistar rats)**
1.	Control	Only vehicle for 21 days	6

2.	Change	Vehicle for 21 days and Cyclophosphamide 30mg/kg i.p. on 18th,19th & 20th day	6
3.	Standard	Levamisole 50 mg/kg p.o for 21 days and Cyclophosphamide 30mg/kg i.p on 18th, 19th & 20^{th} day	6
4.	Experimental -1 (Extract 1)	Extract-1 200 mg/kg p.o. for 21 days and Cyclophosphamide 30mg/kg i.p. on 18th, 19th & 20th day	6
5.	Experimental -2 (Extract 2	Extract-2 400 mg/kg p.o. for 21 days and Cyclophosphamide 30mg/kg i.p. on 18th, 19th & 20th day.	6
6.	Experimental -2 (Extract 3)	Extract-2 600 mg/kg p.o. for 21 days and Cyclophosphamide 30mg/kg i.p. on 18th, 19th & 20th day.	6

Carbon Clearance Assay (Phagocytic Response):-

For a duration of 21 days, the rats received treatment with extracts and a standard drug. On the 21st day, the rats were administered an intravenous injection of 0.2 ml/animal Indian ink dispersion (pre-warmed at 37 °C) through the tail vein. Blood samples were collected at 0 and 15 minutes following the injection by retro-orbital puncture and placed in micro-centrifuge tubes.

Subsequently, 25µl of blood samples were added to 2 ml of 0.1% Na2CO3 solution. The absorbance was measured at 660 nm using a UV-visible spectrophotometer. The rate of carbon clearance, also known as the phagocytic index (K), was calculated using the formula k = Loge (OD1).

OBSERVATION AND RESULTS

1) Haemagglutination Antibody (HA) Titre

- **Body Weight HA**

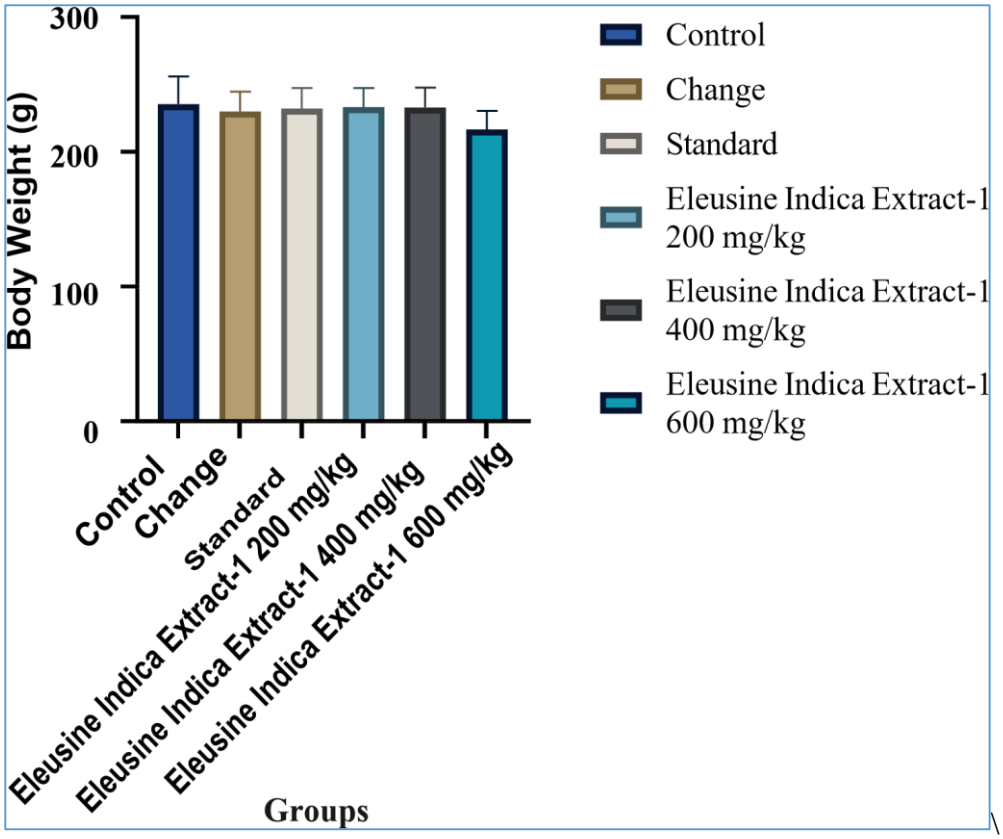

Fig 4. Body Weight HA

- **HA Titer**

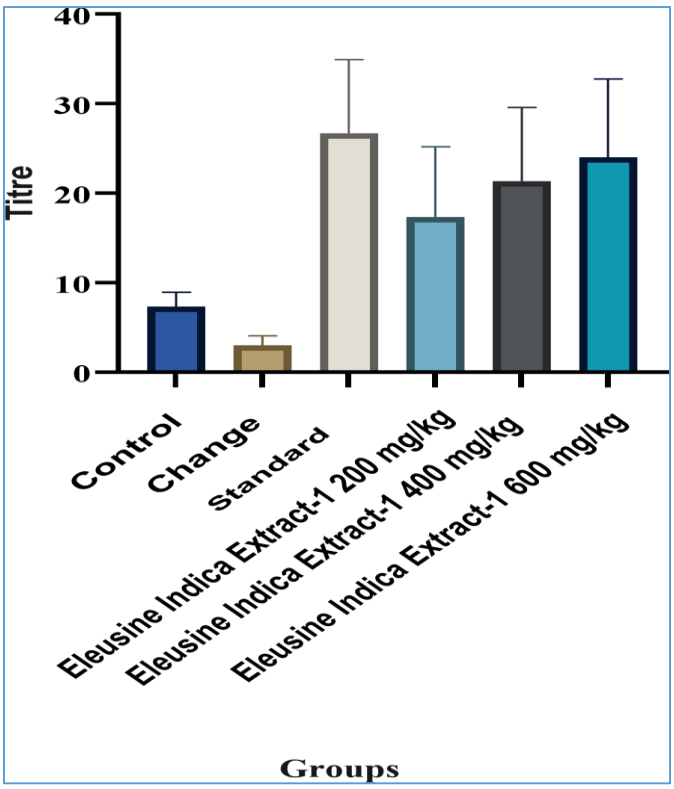

Fig 5. HA Titre

- **IL 6 HA**

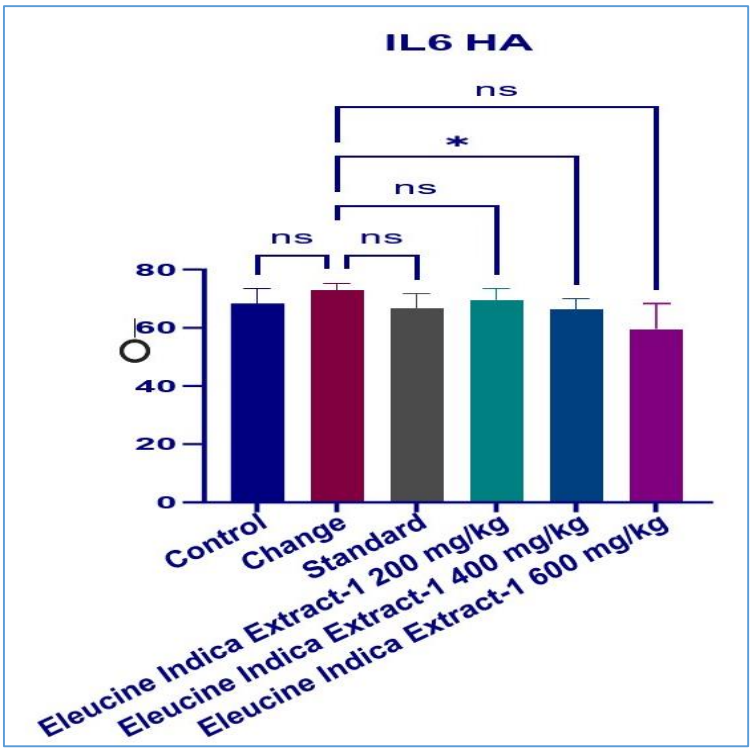

Fig 6. IL 6HA

- **TNF HA**

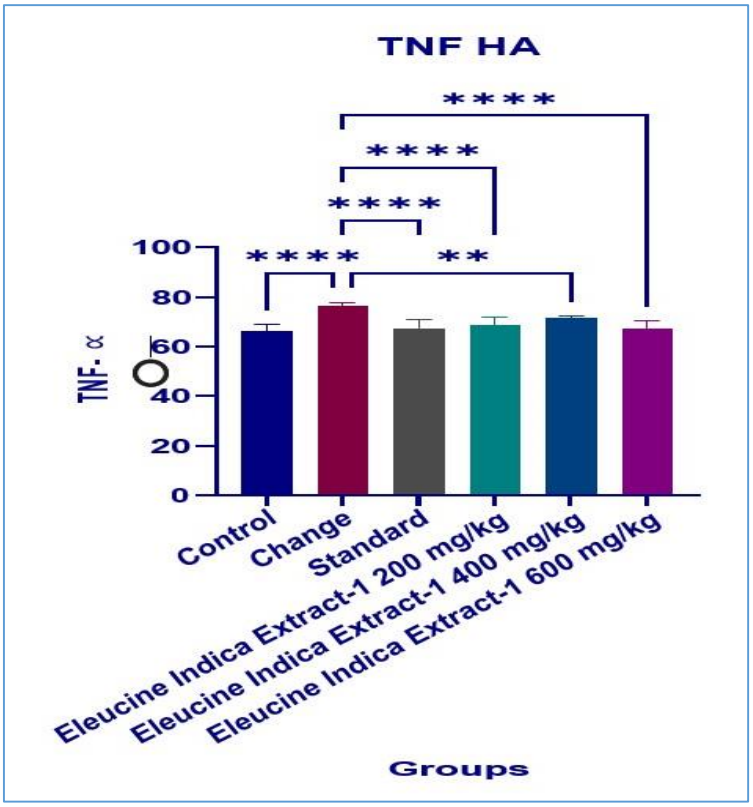

Fig 7. TNF HA

- **CRP HA**

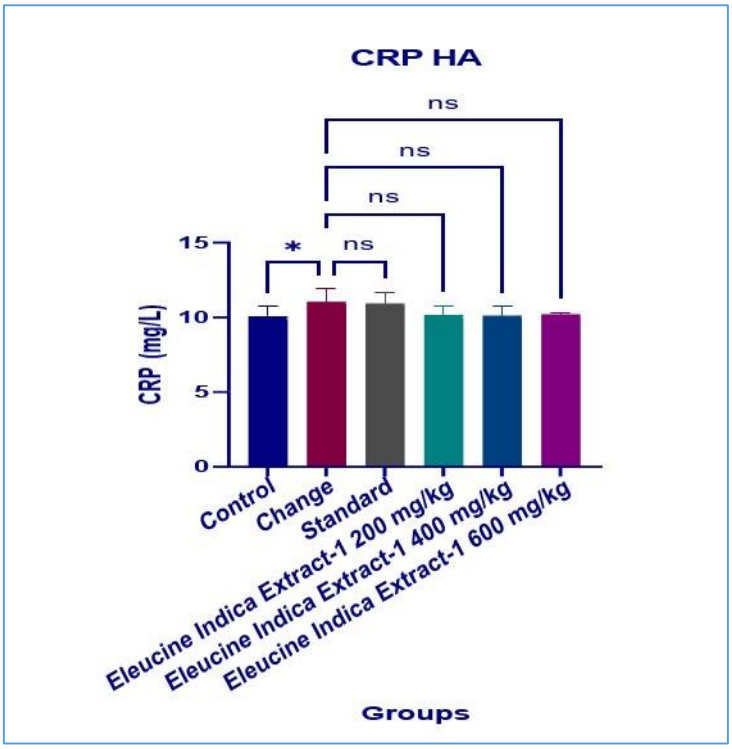

Fig 8. CRP HA

2) Carbon Clearance Test

- **Body Weight**

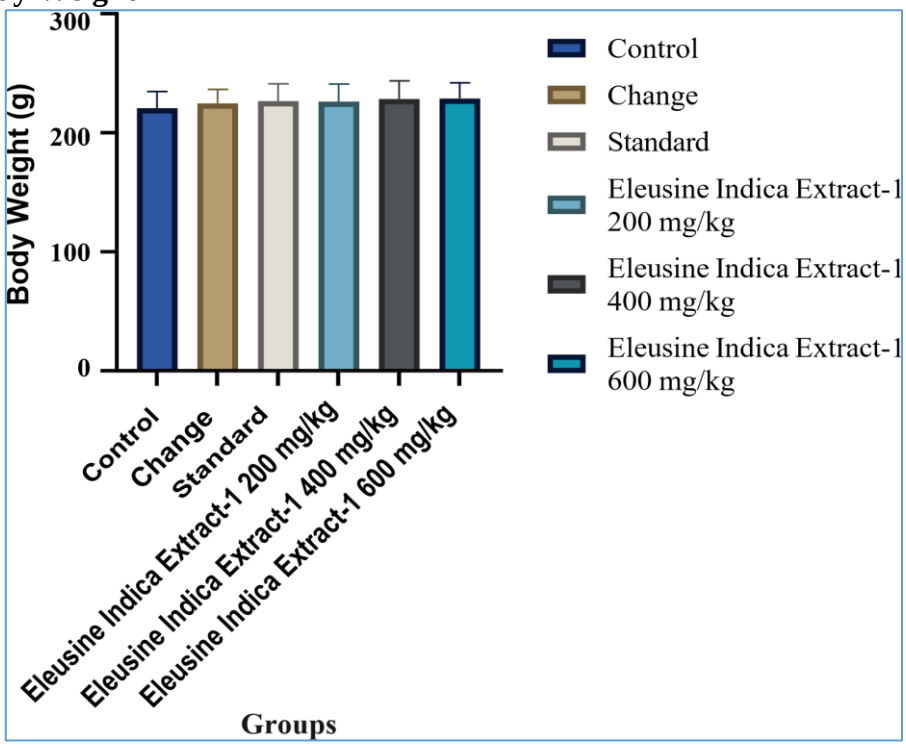

Fig 9. Body weight CCT

- **Phagocytic Index**

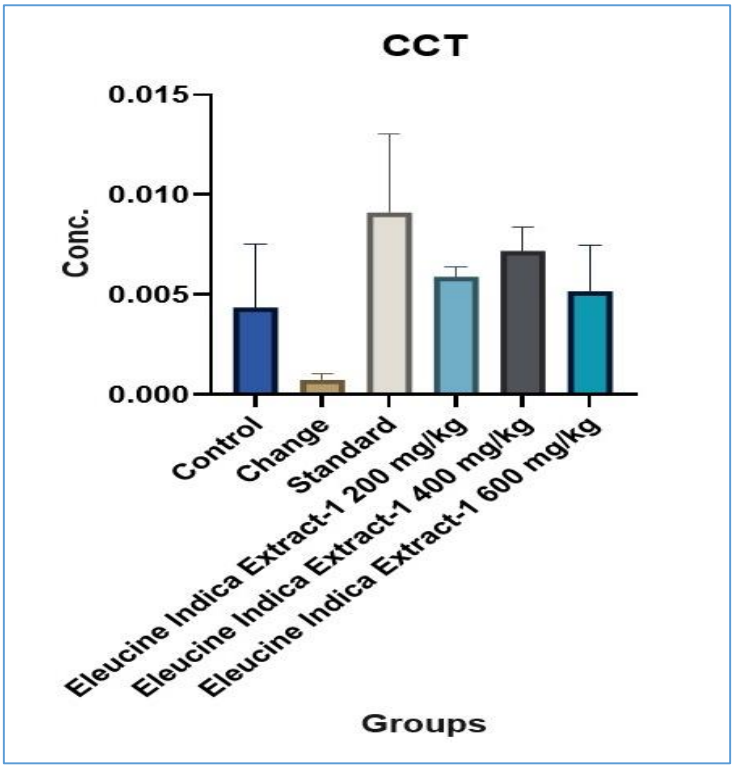

Fig 10. Phagocytic Index CCT

- **IL-6**

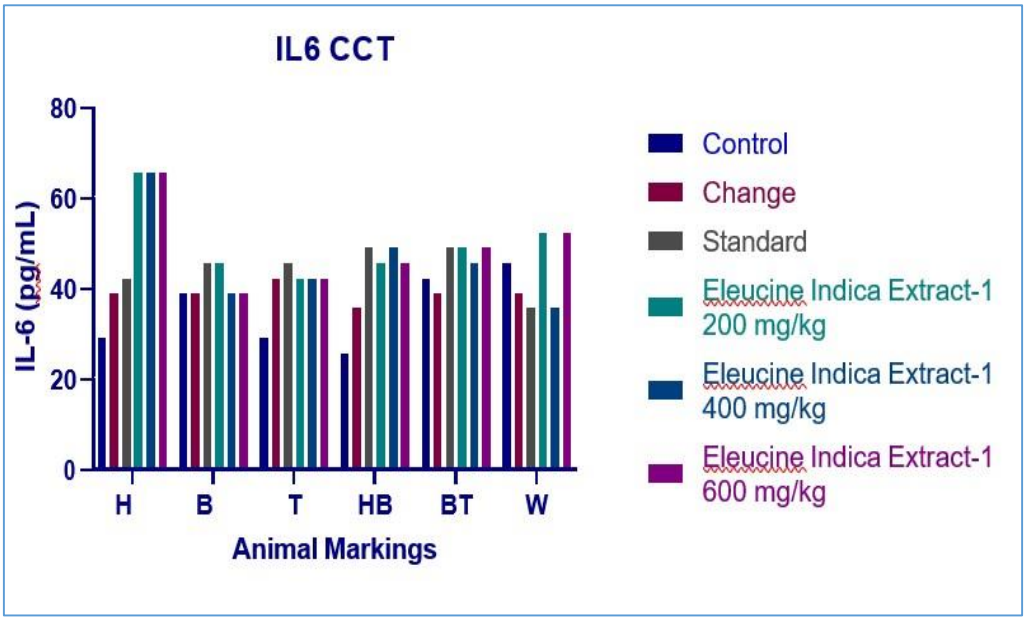

Fig 11. IL-6 CCT

- **TNF-α**

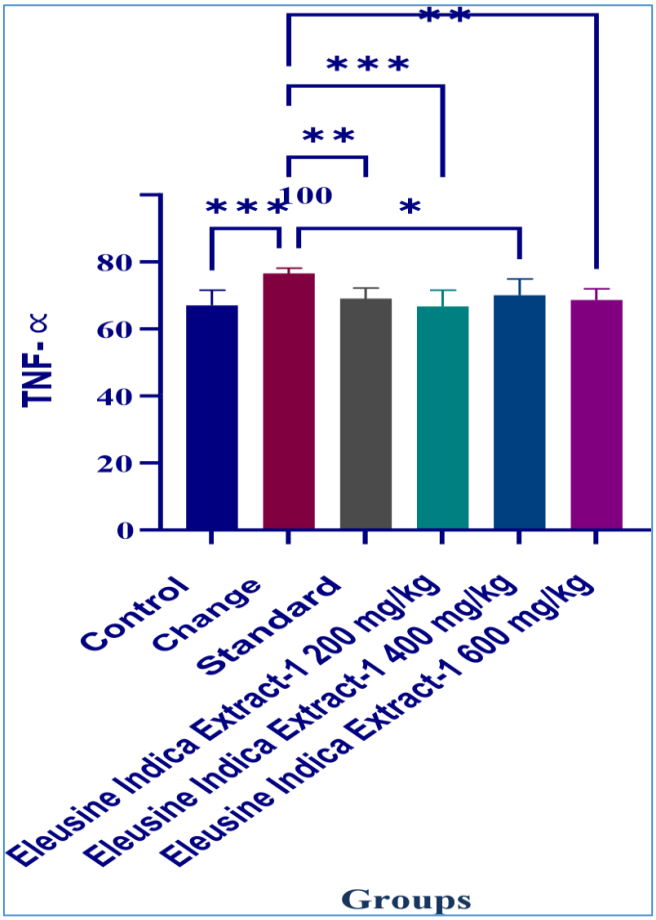

Fig 12. TNF-α CCT

- **CRP**

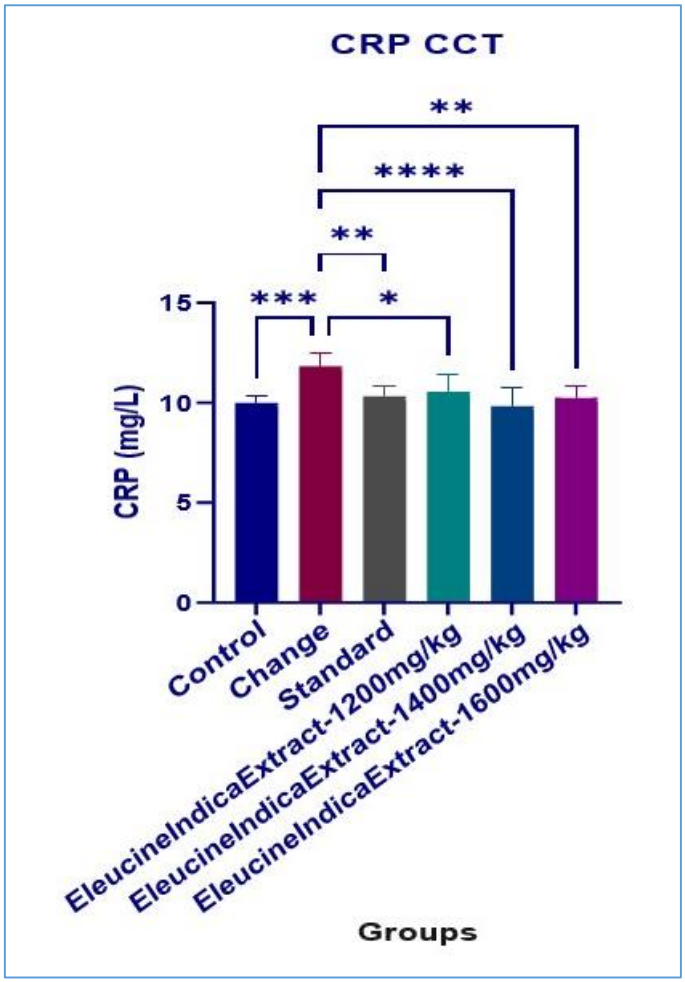

Fig 13. CRP CCT

DISCUSSION

The objective of our study was to explore the immunomodulatory potential of Eleusine indica plant extract and its potential therapeutic applications. Our results indicate that Eleusine indica extract exhibits substantial immunomodulatory effects, underscoring its significance as a valuable resource in herbal medicine.

Summary of Findings: Our investigation revealed that Eleusine indica extract exerted notable effects on several immune parameters. These effects included increased cytokine production, enhanced lymphocyte proliferation, and heightened phagocytic activity. These positive outcomes suggest that Eleusine indica has the ability to modulate immune responses without specifically inducing either stimulation or suppression [37, 38].

The selection of Eleusine indica was based on several compelling reasons. Firstly, Eleusine indica, commonly known as goose grass, has been recognized for its significant medicinal value and has a long history of traditional use in diverse folk medicine practices across different cultures. Secondly, numerous studies have highlighted its potential medicinal properties, which include anti-inflammatory effects, antioxidant activity, wound healing properties, diuretic effects, and antimicrobial activity. Lastly, based on both scientific research and traditional medicinal uses, Eleusine indica was identified as a promising candidate for its anticipated immunomodulatory activity [39].

Eleusine indica is rich in various chemical constituents, including alkaloids, flavonoids, phenolic compounds, triterpenoids, saponins, and coumarins. These compounds contribute to

its diverse pharmacological activities, such as antioxidant, anti-inflammatory, and immunomodulatory properties.

Our study provides compelling evidence supporting the positive immunomodulatory activity of Eleusine indica plant extract. These findings underscore the potential of Eleusine indica as a valuable natural resource for maintaining immune balance and promoting overall health. However, further research is necessary to fully elucidate the mechanisms of action and therapeutic applications of Eleusine indica in immune modulation [40, 41].

Hemagglutination Antibody (HA) Titre: The hemagglutination test performed on Wistar albino rats provided valuable insights into the immunomodulatory potential of Eleusine indica. The observed increase in hemagglutination titres suggests enhanced antibody production, indicating an activated humoral immune response [42].

Carbon Clearance Test: The carbon clearance assay demonstrated the phagocytic response of the reticuloendothelial system to Eleusine indica extract. The results suggest that the extract may enhance the body's ability to clear foreign particles, indicating its potential in modulating immune function [43].

C - reactive protein (CRP) Test: Changes in CRP levels in rats treated with Eleusine indica extract suggest that the extract may influence the immune system by suppressing inflammation or stimulating immune responses [44].

Interleukin-6 (IL-6) Test: The IL-6 test showed alterations in IL-6 levels in rats treated with Eleusine indica extract, indicating its potential to modulate inflammatory responses or immune cell activity [45].

The findings of our study lend support to the potential immunomodulatory properties of Eleusine indica and emphasize its therapeutic promise in regulating immune function. Further research is warranted to delve into the mechanisms underlying these effects and to explore the potential applications of Eleusine indica in treating inflammatory and immune-related conditions. This ongoing investigation holds promise for uncovering new avenues for the development of novel therapies targeting immune dysregulation.

CONCLUSION

From present Study it was concluded that Eleusine indica extract is safe in doses up to 400mg/kg and 600mg/kg and has good immunostimulant action when compared to the control group.

The immunostimulant activity is due to,

1. HA Titre
2. Phagocytic index
3. Proliferation of B cell

In the future, Eleusine indica holds potential as a candidate for cancer treatment due to its demonstrated immunostimulant activity. Additionally, its immunomodulatory properties suggest potential applications in combating infectious diseases. Continued research into the mechanisms of action and efficacy of Eleusine indica in these contexts may pave the way for its development as a therapeutic agent for cancer treatment and infectious diseases.

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