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# COMPARISON OF IMMUNOMODULATORY ACTIVITY OF *ANDROGRAPHIS PANICULATA* AND *TINOSPORA CORDIFOLIA*

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## ABSTRACT

The work mainly focus on the immunomodulatory potential of methanol extracts of two Indian medicinal plants- *Andrographis paniculata* (AP) and *Tinospora cordifolia*(TC). The coarse powder of aerial parts of AP and mature stems of TC (500g each) was subjected separately to successive extraction with 1000ml each of petroleum ether (60-80$^{o}$C) followed by methanol. The dose of methanol extracts were selected and were administered orally at doses of 200 mg/kg body weight to healthy rats and compared with the control group and standard drug i.e. cyclophosphamide (100mg/kg) respectively by using different animal models like modulation of macrophage function, humoral response in normal and cyclophosphamide induced immunodeficient rats, neutrophil adhesion (NA) test, haemagglutinating antibody (HA) titre, delayed type hypersensitivity (DTH) response in rats. The methanol extract of the aerial parts of *A. paniculata* exhibited appreciable activity as compared to the methanol extract of mature stems of *Tinospora cordifolia*. After arriving to a conclusion that *Andrographis paniculata* exhibited best immunostimulant potential, indicating that it has promising immunomodulatory activity, as proposed from commercial point of view.

**KEYWORDS:** Methanol extract, *Andrographis paniculata, Tinospora cordifolia* Haemagglutination, Humoral and cellular responses, Immunostimulant.

## 1. INTRODUCTION

Indian traditional systems of medicines like Ayurveda and Siddha have the potential to the body's natural resistance to disease.[1] Recent studies with plants have revealed many compounds with potent antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential.[2] It is believed that the immunomodulatory drugs promote positive health and maintain organic resistance against infections by establishing body's equilibrium and conditioning the body tissues.[3] The restorative and rejuvenating power of herbal remedies might be due to their action on the immune system and thereby responsible for the protection of the organism from extraneous substances and maintaining the homeostasis. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people.[1] The plants use as an immunomodulator namely *Andrographis paniculata* and *Tinospora cordifolia*. They have good impact in the treatment and management of HIV-AIDS because these plant not only treats disease but also enhance the body vitality and immunity. The humoral and cell-mediated immune response was observed through delayed type hypersensitivity (DTH) model.

*Andrographis paniculata*, is an herbaceous plant of family Acanthaceae, also called King of Natural Bitters and is a traditional India, Southeast Asian and Chinese herb, used for centuries in Ayurvedic medicine. Since ancient times, *A. paniculata* is being used in Ayurvedic and traditional Siddha systems as well as in tribal medicine in India and some other countries for multiple clinical applications. It has three major spheres of influence, first by offering extensive immune system support, second to protect the liver and third to strengthen the cardiovascular system[4]. The herb has been revered for treating infectious diseases and is highly regarded for preventating many diseases, due to its powerful immune strengthening benefits. It's most active and major constituent-Andrographolide is a Lactone ring in basic diterpene glycoside molecules.[5] *Andrographis paniculata* is also reported to possess anti-inflammatory[6], Anti-Oxidation[7], Anti-hepatotoxicity[8], Anti-hyperglycemic effect[9], Anti-infection[10], Anti-cancer[11], Anti-atherosclerosis activity.[12]

*Tinospora cordifolia* is a large extensively spreading, perennial climber belonging to the family Menispermaceae. It is widely distributed throughout tropical and subtropical India. In Hindi, the plant is commonly known as Giloya, Giloe or Amrita. Giloya is a

Hindu mythological term that refers to the heavenly elixir which has saved celestial beings from old age and kept them eternally young.[13] The active adaptogenic constituents are diterpene compounds including tinosporone, tinosporic acid, cordifolisides A to E, syringen, the yellow alkaloid, berberine, giloin, crude giloininand, a glucosidal bitter principle as well as polysaccharides, including arabinogalactan polysaccharide (TSP).[14] It shows significant bactericidal activities. It improves bacterial clearance as well as improves phagocytic and intracellular bactericidal capacities of neutrophils. It also stimulates macrophage action. As a result it stimulates immune system of body.[15] In Ayurveda also called as Amrita, it is used as "*rasayana*" which has powerful immunostimulant activity.[16] Charaka described rasayana as antiaging, which increased the life span, promoted intelligence, improved memory and ensured freedom from diseases, indicating immunostimulant effect.[17] *T. cordifolia* is used to strengthen the immune system of the body by keeping the function of its various organs in harmony. It has great potency to alleviate impurity of body organs. *T. cordifolia* in Vedic age was considered as one of the most rejuvenating herbs working well on the entire seven Dhatus (the constituent elements of the body) keeping the bodies free from all types of illness.[18] Therefore, in the today's world of modern medicine *T. cordifolia* is rightly called as "The Magical Rejuvenating Herb". Categorized as "rasayana" in traditional Indian System of Medicine it is used as general tonic because of its anti-inflammatory, anti-arthritic, anti-allergic, anti-malarial and immunomodulatory properties.[19-21]

*Andrographis paniculata* and *Tinospora cordifolia* both have *some* common medicinal properties like immunomodulatory, anti-oxidant, anti-inflammatory, anti-malarial, hepatoprotective, anti-diabetic etc.The objective of the present study was to compare the immunomodulatory activity of *Andrographis paniculata* aerial parts with *Tinospora cordifolia* mature stems in methanol extracts using standard methods.

## 2. MATERIALS AND METHODS

**2.1 Plant material:** The Aerial parts of *Andrographis paniculata* and selected mature stems of the *Tinospora cordifolia* were collected from medicinal plant garden of Department of Pharmaceutical Sciences, University Campus, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.).The plant material was identified and authenticated taxonomically at the Botany Department of Dr. H.S Gour University, Sagar M.P. (Ref no-1417 and 1418, respectively, dated- 28.01.2011). A voucher specimen of the collected sample was deposited in the Departmental herbarium for future reference.

**2.2 Preparation of extracts:** The collected plant materials were washed with water to make them free from any dust or foreign matter. *A. paniculata* (aerial parts) was dried as such in open shade whereas for convenience of drying *T. cordifolia* (mature stems) were cut into small pieces, crushed and dried in open shade. After air drying the plant materials were powdered (40 mesh size). 500g of each powder was separately extracted by soxhlet successively with 1000ml each of petroleum ether (60-80$^{o}$C) followed by methanol. The appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator and dried over a desiccator at room temperature to obtain total extracts i.e. petroleum ether and methanol extracts of *A. paniculata* and *Tinospora cordifolia*. After completion of extraction, extracts were weighted (w/w); percentage yield was calculated and abbreviated separately.

**2.3 Animal selection:** Swiss Albino mice of either sex (20-25gms.) and Wistar albino rats of either sex (200-250gms.) were selected for carrying out Pharmacological activity. Animals were housed at room temperature (23±2$^{0}$C) with 12h light and 12h dark cycle and relative humidity (55±10%) and were given water (*ad libitum*) and were fed with rat pellet feed. Experiments on animals were conducted after getting approval from Institutional Animal Ethics Committee Dr. H.S. Gour Central University, Sagar, M.P. (Registration No. 379/01/ab/CPCSEA) which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

**2.4 Acute toxicity studies:** The acute oral toxicity studies and selection of dose was done as per guidelines of Organization for Economic Co-operation and Development (OECD), draft guidelines 423 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy albino mice of either sex were used for acute toxicity study to determine $LD_{50}$ of investigating the methanol extracts of AP and TC. The animals were randomly selected, marked to permit individual identification and kept in the cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substances of AP and TC were administered in a single dose by gavage. Three mice (n=3) were used in each category and starting dose lied in the range of 5-5000mg/kg body weight (OECD guideline 423). Dose ranging between 5, 300, 2000 and 5000mg/kg of body wt. of extract combinations were administered stepwise to the mice according to their weights. After the treatment, mice were observed individually once during the first 30 minutes, and then periodically during the first 24 hrs. There was no mortality till the dose of 2000mg/kg body weight in extract combinations. Considering this dose of 2000mg/kg body weight, 1/10$^{th}$ of this dose i.e. 200mg/kg body weight was taken as effective dose for methanol extracts of *A. paniculata* (MEAP) and *T. cordifolia* (METC) for carrying out their immunomodulatory screening.

**2.5 Dose selection and Preparation for study**

After carrying out acute toxicity studies, it was observed that lethal dose for methanol extracts was 2000mg/kg body weight and hence 1/10$^{th}$ of the dose i.e. 200mg/kg body weight was taken as effective dose for methanol extracts for performing the Immunomodulatory Screening.

**2.6 Standard Drugs, Chemicals and reagents**

The drugs, chemicals, and reagents procured from S.D. Fine Chemicals, (Mumbai, India) were of analytical grade. **Cyclophosphamide** (German Remedies Limited, Kundaim Industrial Estate, Ponda, Goa) was used to produce immunosuppression in rats (Dose- 100mg/kg body weight). **Sheep Red Blood Cells (SRBCs)** at dose of 0.5ml were injected intraperitoneally for immunization and challenge to the rats. **Alsever's solution-** for collection of blood from sheep and **Phosphate Buffer saline (PBS -7.2pH)-** for collection of peritoneal fluid form mice were used.

**2.7 Sheep Red Blood Cells (SRBCs)**

Blood from healthy Sheep was collected from local butcher house in **Alsever's solution**. It was washed three times with pyrogen free 0.9% normal saline and centrifuged at 3000 rpm for 5min. Supernatant was discarded. The settled SRBC was then suspended in normal saline and total SRBC counted using Neubauer chamber and RBC of this suspension was adjusted to a concentration of $5\times10^9$ SRBC (0.5ml) and injected intraperitoneally for immunization and challenge.[22]

**2.8 Preparation of Alsever's Solution Formula.**

| | |
|---|---|
| Citric acid | 0.055gm |
| Sodium citrate | 0.8gm |
| Glucose | 2.05gm |
| Sodium chloride | 0.42gm |
| Distilled water to make volume up to | 100 ml |

All the above ingredients were weighed and dissolved in distilled water and the volume was made up to 100ml. The solution was stored in refrigerator.[23]

**2.9 Preparation of Phosphate Buffer saline, (PBS) (7.2 pH)**

Take 50ml of 0.2M of Pot. di hydrogen phosphate ($KH_2PO_4$), [27.218gms. $KH_2PO_4$ in 1000ml. water] was taken in 200ml. volumetric flask and 34.7ml. of 0.2M Sod. hydroxide was added and volume was made up to 200ml.[24]

**2.10 Statistical Analysis of Experimental Data**

All the experimental data for statistical analysis were presented as mean± SEM (n=4 in each group). Results obtained were statistically analyzed by using One-way ANOVA followed by Dunnett's comparision test. Statistical significance on comparison with control group was indicated by *mark, where *P<0.05 was considered as significant value.

**2.11 Determination of Immunomodulatory activity**

**2.12 Modulation of Macrophage Function:** Albino male mice (20-25g) of either sex were housed under standard laboratory conditions prior to experimentation. They were fasted for a period of 24hrs. allowing free access to drinking water, prior to p.o. drug administration.

Group-1: (control, n=4) received only clean tap water.
Group-2 and 3: (received methanol extract of AP and TC, n=4) @ 200mg/kg body weight.

Peritoneal macrophage was isolated from the treated mice (n=4) on day 5$^{th}$, 10$^{th}$ and 15$^{th}$ consecutively and also from control mice. Peritoneal fluid was collected in Phosphate buffer saline (PBS, ph 7.2) and the macrophage count was done.[25]

**2.13 Humoral response in normal and cyclophosphamide induced immunodeficient rats**

**2.13.1 Treatment schedule:** In this procedure male albino rats (200-250g) were divided into three groups. Group- A: (–ve control, n=4) was administered tap water from day (-9) to day (+5) and on day (+2) 100mg/kg body weight of cyclophosphamide was administered orally in addition to water. Group-B: (+ve control, n=4) was administered tap water from day (-9) to day (+5). Group-C1 and C2: (methanol extracts, n=4) was administered 200mg/kg body weight of MEAP and METC once a day orally from day (-9) to day (+5) and on day (+2) 100mg/kg body weight of cyclophosphamide was administered orally in addition to AP and TC treatment.

- On day 0, rats in all groups were immunized (ip) with 0.5ml/100g body weight with SRBCs.
- On day (+6), blood was collected from each rat and serum separated.
- The value of highest serum dilution carrying visible hemagglutination was taken as the antibody titre.[26]

**2.14 Immunostimulant Activity of Drugs treated (MEAP and METC) was performed on the following:**

**(a)** Neutrophil Adhesion (NA) Test
**(b)** Hemagglutinating Antibody (HA) Titre
**(c)** Delayed Type Hypersensitivity (DTH) response in rats.

**Treatment:** Albino Wistar male rats (200-250g) were used for the study. Animals were housed properly under standard conditions of temperature (23±2°C), 12:12h light/dark cycles and fed with standard pellet diet and water ad libitum. Fresh SRBC in Alsever's solution were obtained from authentic source. The animals were divided into three groups consisting of four animals per group. A group of four untreated rats was taken as control (Group 1). The Drugs treated (MEAP and METC) were fed orally for 14 days at a dose of 200mg/kg body weight (Group 2 and 3) for assessment of immunomodulatory effect. On 14th day, all groups of

rats were challenged with SRBCs (0.5ml/100g body weight I.P.).

**(a) Neutrophil Adhesion (NA) test**

On 14th day of Drugs treatment, blood samples were collected (before challenge) by puncturing the tail-vein into heparanized vials and analyzed for total leukocyte counts (TLC) and differential leukocyte counts (DLC). After initial counts, blood samples were incubated with 80mg/ml of nylon fibers for 15min. at 37$^0$C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percentage neutrophil gives Neutrophil Index (NI) of the treated and untreated blood samples and the difference was taken as index of neutrophil adhesion (NA).[27]

Percent neutrophil adhesion was calculated as below

$$\textbf{Neutrophil adhesion (NA) (\%)} = \frac{\textbf{NIu} - \textbf{NIt}}{\textbf{NIu}} \textbf{ X 100.}$$

Where
NIu = Neutrophil index of untreated blood sample.
NIt = Neutrophil index of treated blood sample.

**(b) Hemagglutinating Antibody (HA) titre**

Rats of groups MEAP and METC were pretreated for 14 days and each rat was immunized with SRBC (0.5ml/100g body weight I.P.), including control rats. The animals were treated with drug combinations for 14 more days and blood samples were collected from each rat on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBCs. The micro titre plates were incubated at room temperature for 2 hours and examined visually for agglutination. The highest number of dilution of serum showing hemagglutination was expressed as HA titre.[28]

**(c) Delayed Type Hypersensitivity (DTH) response**

DTH response was determined by the significant decrease or increase in paw volume. All the groups of SRBCs immunized rats were challenged by subcutaneous administration of SRBCs 0.5ml in right hind foot pad on 28th day and 0.2ml of 0.9% normal saline was similarly injected into left hind foot pad as control. The cell mediated immune response was measured at 24h after SRBCs challenged on 28th day in terms of increase in paw volume (plethysmometrically). The DTH response was expressed as the mean percent increase in paw volume between the right foot pad injected with SRBCs and left foot pad injected with normal saline.[29]

## 3. RESULTS

The drugs namely *A. paniculata* and *T. cordifolia* were collected and authenticated. The drugs were cut into pieces and powdered to a coarse consistency, which were subjected to extraction (soxhlet) by using pet. ether (60-80) and methanol in succession as solvents. The extracts obtained were concentrated and dried in desiccators **(Table-1).** All the individual dried extracts were checked for their active ingredients by proximate chemical analysis and it was found that the bio-actives were present in methanol extracts of AP and TC. For acute oral toxicity studies, it was observed that administration of single dose of methanol extracts of AP and TC at dose of 200mg/kg, orally did not have any deleterious effects **(Table 2)**.

**Table 1: Yield of extraction derived extracts of *A. paniculata* and *T. cordifolia.***

| Plant material | Extracted material | | | |
|---|---|---|---|---|
| | **Extract** | **Nature** | **Color** | **%Yield (w/w)** |
| ***A. paniculata* (Aerial parts)** | Petroleum ether (60-80°C) Extract (PEAP) | Solid | Light - brown | 1.06 |
| | Methanol Extract (MEAP) | Solid | Dark brown | 12.36 |
| ***T. cordifolia* (stems)** | Petroleum ether (60-80°C) Extract (PETC) | Solid | Light brown | 2.31 |
| | Methanol Extract (METC) | Semi solid | Light brown | 11.58 |

**Table 2: Acute toxicity study for $LD_{50}$ determination of methanol extracts from AP and TC.**

| S.No. | Drugs | $LD_{50}$ Cut-off | Vehicle |
|---|---|---|---|
| 1 | MEAP | 2000 mg/kg | Tween-80 |
| 2 | METC | 2000 mg/kg | Tween-80 |

***Abbreviation:*** *MEAP = Methanol extract of Andrographis paniculata; METC= Methanol extract of Tinospora cordifolia.*

The time dependent effect of drugs treated (MEAP and METC) on morphometric functional changes in mice (peritoneal macrophages) were evaluated where MEAP treated (200mg/kg b. wt. p.o.) animals showed a very significant increase in the macrophage count and the maximum number of macrophage cells (8,233±176.38, 9,243±208.17 and 9,891±218.58) were found to be on the 5th, 10th and 15th days respectively of extract administration as compared to control (3,233±497.77, 3,600±461.88 and 3,233±523.87) **(Table 3 and Fig. 1)**.

**Table 3: Effect of drugs treated (MEAP and METC) on morphometric and functional changes of macrophage in male albino mice**

| Macrophage count(Cell/mm3) | Days after Drugs extracts (AP and TC) (200mg/kg. P.O.) | | |
|---|---|---|---|
| | Day 5th | Day 10th | Day 15th |
| **Control Group** | 3,233±497.77 | 3,600±461.88 | 3,233±523.87 |
| **MEAP Group** | 8,233±176.38** | 9,243±208.17** | 9,891±218.58** |
| **METC Group** | 5,966±120.19 | 6,500±360.56* | 7,333±240.37* |

*Values are expressed as mean±SEM, (n = 4), Comparison of Group I (Control) was made with all groups. **p<0.01 Very Significant compared to control group (ANOVA followed by Dunnett's test). *p<0.05 Significant compared to control group (ANOVA followed by Dunnett's test).*

***Abbreviation:*** *MEAP = Methanol extract of Andrographis paniculata; METC= Methanol extract of Tinospora cordifolia.*

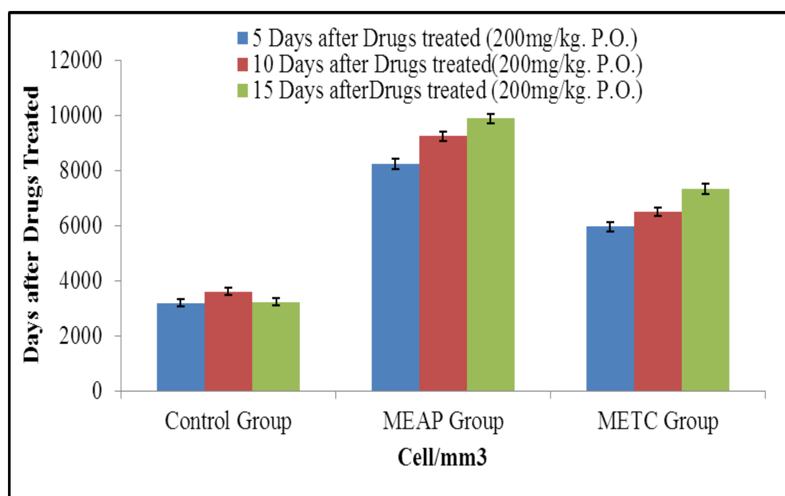


**Figure 1: Effect of drugs treated (MEAP and METC) on morphometric and functional changes of macrophage in male albino mice.**

The findings of cyclophosphamide induced immunosuppression model reveals that methanol extract on Anti-SRBC-hemagglutination antibody titre in the MEAP treated cyclophosphamide induced rats was found to be (9.49±3.74) as compared to cyclophosphamide induced control rats (1.96±0.18). The Anti-SRBC-hemagglutination antibody titre in the control rats was (4.32±2.64). The suppressive effect of cyclophosphamide was protected by animals treated with Drugs and result shown in **Table 4 and Fig. 2** have revealed that administration of Drugs of MEAP and METC could stimulate the haemopoetic system.

**Table 4: Effect of drugs treated (MEAP and METC) on hemagglutination antibody titre in normal and cyclophosphamide (CYMP, 100mg/kg) induced immunodeficient rats**

| Group | Treatment | Value |
|---|---|---|
| A | –ve Control (SRBC + CYMP) | 1.96±0.18 |
| B | +ve Control (SRBC) | 4.32±2.64 |
| C1 | Methanol extract (AP+SRBC+CYMP) | 9.49±3.74** |
| C2 | Methanol extract (TC-V+SRBC+CYMP) | 6.19±2.03* |

*Values are expressed as mean±SEM, (n = 4), Comparison of Group I (Control) was made with all groups. **p<0.01Very Significant compared to control group (ANOVA followed by Dunnett's test). *p<0.05 Significant compared to control group (ANOVA followed by Dunnett's test).*

***Abbreviation:*** *MEAP = Methanol extract of Andrographis paniculata; METC= Methanol extract of Tinospora cordifolia.*

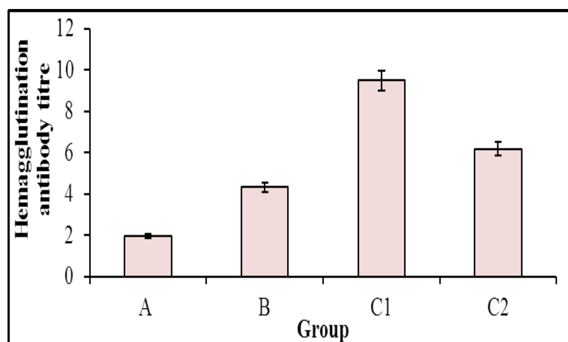


**Figure 2: Effect of drugs treated (MEAP and METC) on hemagglutination antibody titre in normal and cyclophosphamide (CYMP, 100mg/kg) induced immunodeficient rats.**

As per the present findings on neutrophil adhesion, the % neutrophil adhesion in control group animals was 32.12, whereas in Drugs treated group animals, at dose of 200mg/kg body weight, it was 54.76 and 35.91 respectively. The treatments showed very significant (P<0.01) increase at a dose of 200mg/kg body weight in MEAP treated group animals as compared to control, proving the immunostimulant action of methanol extract of AP (**Table 5 and Fig. 3**).

**Table 5: Effect of drugs treated (MEAP and METC) on neutrophil adhesion in rats**

| S. No. | Group | TLC($10^3/mm^3$) (A) | | Neutrophil % (B) | | Neutrophil Index (A X B) = (C) | | Neutrophil Adhesion (%) |
|---|---|---|---|---|---|---|---|---|
| | | UB | FTB | UB | FTB | UB | FTB | |
| 1 | Control | 3.7±0.05 | 2.7±0.05 | 38.3±0.66 | 35.6±0.66 | 141.7±2.42 | 96.2±0.87 | 32.12 |
| 3 | MEAP | 4.6±0.06 | 3.1±0.05 | 48.6±0.33 | 32.6±0.57 | 223.5±2.81 | 101.1±1.52 | 54.76** |
| 6 | METC | 5.1±0.05 | 4.2±0.05 | 52.3±0.33 | 40.6±0.33 | 268.7±1.32 | 172.2±2.31 | 35.91* |

*Values are expressed as mean±SEM, (n = 4), Comparison of Group I (Control) was made with all groups. **p<0.01Very Significant compared to control group (ANOVA followed by Dunnett's test). *p<0.05 Significant compared to control group (ANOVA followed by Dunnett's test).*

***Abbreviations:*** *MEAP = Methanol extract of Andrographis paniculata; METC= Methanol extract of Tinospora cordifolia; UB= Untreated Blood, FTB = Fiber Treated Blood*

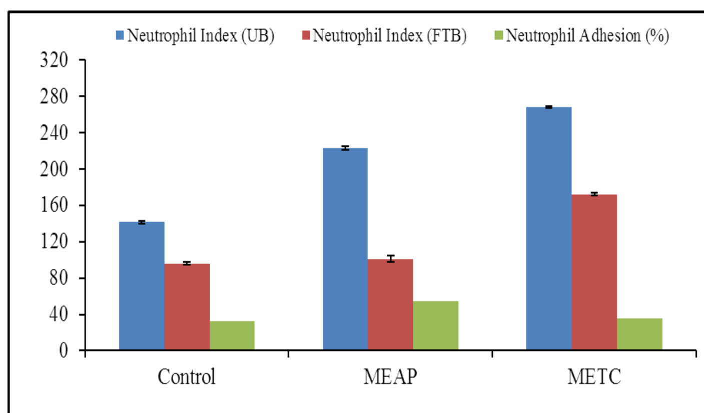


**Figure 3: Effect of drugs treated (MEAP and METC) on neutrophil adhesion in rats**

In this test, serum containing antibodies was collected from animals of each group and serial dilutions were done in microtiter plate on Hemagglutinating Antibody (HA) titer, the animlas were treated with MEAP and METC for 14 more days and blood samples were analyzed from each rat on day 15 for HA titre where values were very significantly (P<0.01) increased at the dose of 200mg/kg body weight for Drug (MEAP) (8.59±0.221) as compared to control (3.58±0.077), suggesting that 14 days pretreatment of drug of AP was capable to enhance responsiveness of macrophages and lymphocytes involved in antibody synthesis and showed possible immunostimulant action of methanol extracts of AP (**Table 6 and Fig. 4).**

**Table 6: Effect of drugs treated (MEAP and METC) on HA titre to antigenic challenge by sheep RBCs in rats**

| Group | H A Titre |
|---|---|
| Control | 3.58±0.077 |
| MEAP | 8.59±0.221** |
| METC | 5.20±0.201* |

*Values are expressed as mean± SEM, (n = 4), Comparison of Group I (Control) was made with all groups. **p<0.01Very Significant compared to control group (ANOVA followed by Dunnett's test). *p<0.05 Significant compared to control group (ANOVA followed by Dunnett's test).*

***Abbreviation:*** *MEAP = Methanol extract of Andrographis paniculata; METC= Methanol extract of Tinospora cordifolia*

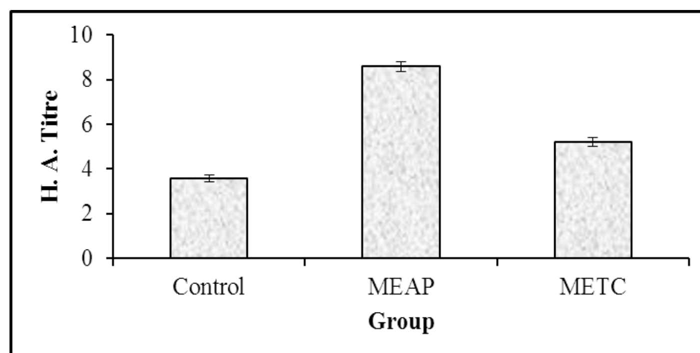


**Figure 4: Effect of drugs treated (MEAP and METC) on HA titre to antigenic challenge by sheep RBCs in rats.**

In DTH investigation, reaction was used to study the effect of drugs (MEAP and METC), using SRBC as an antigen. On 28th day after 24h of challenge in the control group animals, paw edema was 2.03±0.141while in drugs treated group animals at dose of 200mg/kg body weight, it was 8.82±0.228 and 4.76±0.217respectively **(Table 7 and Fig. 5)**. Therefore, the peak edema after 24h of challenge was the evaluating parameter. The results with Drug of AP (200mg/kg body weight) was statistically very significant (P<0.01) with regard to increase in paw volume compared to control treatment.

**Table 7: Effect of drugs treated (MEAP and METC) on DTH response to antigenic challenge by sheep RBCs in rats.**

| Group | DTH response (%increase in paw volume) |
|---|---|
| Control | 2.03±0.141 |
| MEAP | 8.82±0.228** |
| METC | 4.76±0.217* |

*Values are expressed as mean±SEM, (n = 4), Comparison of Group I (Control) was made with all groups. **p<0.01Very Significant compared to control group (ANOVA followed by Dunnett's test). *p<0.05 Significant compared to control group (ANOVA followed by Dunnett's test).*

***Abbreviation:*** *MEAP = Methanol extract of Andrographis paniculata; METC= Methanol extract of Tinospora cordifolia*

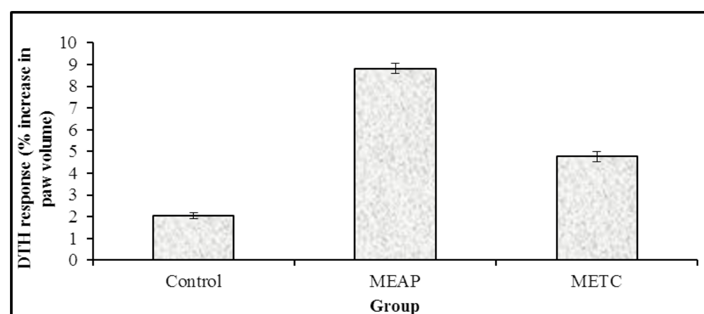


**Figure 5: Effect of drugs treated (MEAP and METC) on DTH response to antigenic challenge by sheep RBCs in rats.**

## 4. DISCUSSION

There are several herbal preparations used in the indigen ous system of medicinewhich can enhance the body's im mune status. The effects of drugs treated at dose 200mg/kg b.wt. p.o. on morphometric and functional changes of macrophages in mice showed very significant increase (p<0.01) in the number of macrophage cells on the $5^{th}$, $10^{th}$ and $15^{th}$ days of drug administration. Thus it significantly activated macrophages and enhanced their function as compared to control, suggesting that MEAP

possess potential immunostimulant effect. The depression in immune system associated with cancer, surgery, infection and certain drugs is characterized by the reduction in the number and function of neutrophils and macrophages as well as in intracellular bactericidal capacity of these cells.

Cyclophosphamide is converted in the organism from a non-reactive to a highly reactive form. This alkylating agent, which is inactive in vitro, is activated in vivo by enzymatic cleavage of the phosphamide group, which releases the active portion of the compound once the molecule is split. The alkylating agents are thought to act by combining directly with certain intracellular molecules, particularly nucleic acid and proteins. It blocks the conversion of a precursor population (possibly small lymphocytes) to lyphoblasts and the thymus dependent lymphocytes are preferentially affected by cyclophosphamide. Cyclophosphamide induced immunosuppression model shows that the MEAP very significantly ($P<0.01$) protected cyclophosphamide induced humoral immunosuppression in rats as compared to control groups. This suggested a significant potentiating action of AP on humoral immunity, as plant extracts significantly protected cyclophosphamide induced humoral immunosuppression in rats. Immunostimulants, also known as immunostimulators, are substances (plants and nutrients) that stimulate the immune system by inducing activation or increasing activity of any of its components. Immunostimulant therapy may be beneficial for patients under a variety of settings that include prevention and treatment of various infectious diseases. It is important to know the appropriate use of such treatments so that the ideal immunostimulant preparation is selected for each individual patient. An ideal situation when a host is exposed to pathogen challenge (e.g. bacteria or virus) is to have optimal immunity that protects the host from disease. In many cases specific therapy in the form of antibacterial, antiprotozoal, antiparasitic or antifungal therapy will work in combination with the immune system to aid with pathogen clearance. In some instances, the addition of an immunostimulant will aid in "boosting" the immune response.[30] Neutrophil Adhesion Test is an indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation. Hemagglutination reaction: The antigen antibody reaction results in agglutination. The relative strength of an antibody titer is defined as the reciprocal of the highest dilution which is still capable of causing visible agglutination. The augmentation of humoral response by drugs treated, as evidenced by an enhancement of antibody responsiveness to SRBC in rats, indicated the enhanced responsiveness of macrophages and B lymphocytes subsets involved in antibody synthesis. Agglutination tests can be used to measure the level of antibodies to particulate antigens. In the present investigation, DTH reaction was used to study the effect of drugs (MEAP and METC) on cell mediated immunity, using SRBC as an antigen. Thus, the results obtained with MEAP treatment concluded that the AP is able to stimulate the macrophages function to stimulate T cells for the hypersensitivity reaction in the immunized rats. Increase in DTH response indicated that AP has a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction i.e. cell mediated immunity.[32]

## 5. CONCLUSION

For catering the need of the hour for management of immune responses, the present study was designed to evaluate the immunomodulation potential of both highly valued drugs. The present study was focused on their methanol extracts as they were found to possess important bio-actives with proven immunomodulatory activities. The overall present studies revealed that methanol extract of Andrographis paniculata had good percentage of immunomodulatory activities; the above findings recommend the further investigation of Andrographis paniculata to evaluate their chemical potential. Further studies are needed to isolate and characterize the active principles to elucidate their immunomodulatory mechanism.

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