

***Immunomodulatory Efficacy of Phyllanthus
Emblica and Costus Speciosus Aqueous Extracts
for Immunosuppressive Rats.***

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Abstract

The immune system helps in eliminating toxic or allergenic substances that enter through mucosal surfaces. The immune system's ability to mobilize a response to an invading pathogen, toxin or allergen is its ability to distinguish self from non-self. This investigation aimed to study the immunomodulatory efficacy of phyllanthus emblica and costus speciosus aqueous extracts for immunosuppressive rats. Forty two mature male albino rats weighing 150-200 g were used in this work. Rats were divided into 6 equal groups (n=7 rats); one group kept as a control negative, while the rest five groups were once injected intraperitoneally with a single dose (200 mg/kg body weight) of cyclophosphamide for immunosuppression, then divided to five equal groups, one of them left as control positive group (C +ve) while the rest four groups orally ingested with two doses (250 and 500 mg/kg) of phyllanthus emblica and costus speciosus aqueous extracts for each of them. At the end of experimental period (45 days), blood samples were collected and CD4, CD8, CD16 and CD19 were analyzed by flow cytometric

analysis. IgM, IgA and IgG were determined using indirect enzyme-linked immunosorbent assay (ELISA). The levels of serum albumin and globulin were determined. The obtained results concluded that phyllanthus emblica and costus speciosus enhanced immunomodulatory efficacy by increasing blood levels of CD4, CD8, CD16, CD19, IgM and IgG, moreover, increasing serum levels of albumin and globulins. The group of costus speciosus at a dose of 500 mg/kg. b.wt., declared the best significant results to boost immunity compared to all experimental groups. The present investigation revealed that Phyllanthus emblica and costus speciosus aqueous extracts possess immunomodulatory efficacy with regard to immunosuppressive animals.

Introduction

The immune system helps in eliminating toxic or allergenic substances that enter through mucosal surfaces. The immune system's ability to mobilize a response to an invading pathogen, toxin or allergen is its ability to distinguish self from non-self (**Chaplin, 2010**).

The immune system cells must be able to discriminate between non-self-molecules which are harmful (e.g., those from pathogens) and innocuous non-self-molecules (e.g., from food), accordingly, the main issue of nutrients explores the relationship among diet, nutrients and immune function (**Childs et al., 2019**).

Herbal medicine as an imperative branch of complementary and alternative medicine has increasingly grown in the last decades (**Dai, et al., 2017**). Numerous plants constituents have unique ability in modulating immune system including glycosides, polysaccharides,

alkaloids, saponins, flavonoids, sterols and sterolins (**Chandra et al., 2015**).

Phyllanthus emblica Linn commonly known as Indian gooseberry or Amla, is the most important medicinal plant in the Indian traditional system of medicine. Furthermore, experimental studies reported that Phyllanthus emblica Linn and its phytochemicals exhibit anticarcinogenic properties. which possess radiomodulatory, chemomodulatory, chemopreventive, free radical scavenging, antioxidant, anti-inflammatory, antimutagenic and immunomodulatory activities (**Bhandari and Kamdod, 2012**).

Phyllanthus emblica Linn contains phenolic compounds, phyllembelic acid, tannins, phyllembelin, rutin, emblicol and curcuminoids as antioxidants. Moreover, it is rich in tannins including gallic acid, phyllembelic acid, ellagic acid and emblicol, and a wide range of phytochemical components including alkaloids, terpenoids, flavonoids, and tannins (**Krishnaveni and Mirunalini 2010**).

Costus speciosus is among the most effective Islamic traditional medicinal plants (Emami et al., 2016). It has been proven in the authentic Hadith present in Sunan Abi Dawud, the Book of Medicine (Kitab Al-Tibb) in which Umm Qais, the daughter of Mihsan said: I brought my son to the Messenger of Allah while I had compressed his uvula for its swelling. He said: Why do you afflict your children by squeezing for a swelling in the uvula? Apply this Indian aloeswood (costus), for it contains seven types of remedies (**Khalifa and Al-Elyani 2012**). Moreover, C. speciosus is an important medicinal plant which used widely for the treatment of various diseases which possesses multiple active constituents and has many pharmacological activities including antioxidant,

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anti-inflammatory, steroidogenic, anticancer, antidiabetic, hypolipidemic, hepatoprotective, adaptogenic, and antimicrobial characteristics (*EI-Far et al., 2018*). In vitro trials have stated the antioxidant activity of *C. speciosus* methanolic, ethanolic, and chloroform extracts of leaves, stem peel, peeled stem, and roots assessed 2' azino bis 3 ethylbenzothiazoline 6 sulfonic acid, and thiobarbituric acid (TBA) showing more hydroxyl radical scavenging activity and free-radical quenching ability (*Vijayalakshmi and Sarada 2008*). Furthermore, phytochemical composition of *costus speciosus* showed the presence of saponins, alkaloids, tannins, flavonoids, and phenols in the aqueous stem extract, moreover, it could serve as source of bioactive compounds for therapeutic purposes and nutrition (*Ukpabi et al., 2012*).

Accordingly, this investigation aimed to study the immunomodulatory efficacy of *phyllanthus emblica* and *costus speciosus* aqueous extracts for immunosuppressive rats.

Materials and Method

Materials

Experimental rats

Forty-two (42) Sprague – Dawley male albino rats weighing 150-200 g b.wt., used in this investigation, obtained from Animal House of Ophthalmology Hospital, Giza, Egypt.

Basal diet

Basal diet consisted of casein (12.5%), corn oil (10%), vitamins mixture (1%) 13, cellulose (5%), salt mixture (4%), sucrose (22%), choline chloride (0.2%) and corn starch (up to 100%) (*Hegsted et al., 1941*).

Phyllanthus emblica and costus speciosus

Phyllanthus emblica and *costus speciosus* were used as dry substances, obtained from Faculty of Agriculture, Damanhour University.

Cyclophosphamide

Cyclophosphamide was used as a dry substance purchased from Al-Gomhoria Company for Chemicals, Cairo, Egypt.

Methods

Preparation of aqueous extracts

One gram of powdered *phyllanthus emblica* or *costus speciosus* mixed with 100 ml distilled water were boiled for 10 min then cooled for 15 min., filtered then freeze-dried and the desired dose of aqueous extracts prepared as the method prescribed by **(Lemhadri et al., 2004)**. The extracts were orally given to different groups at doses of 250 and 500 mg/kg body weight.

Experimental design

Forty-two (42) male albino rats divided to six equal groups (n=7 rats) as follows: **Group (1)** was fed on the basal diet only during the whole experimental period as control negative group (C -ve). The rest five groups were once injected intraperitoneally with a single dose (200 mg/kg body weight) of cyclophosphamide as the method prescribed with **Mythili et al., (2004)**, then divided into the following groups:

Group (2): Orally administered once by cyclophosphamide at a dose of 200mg/kg b.wt., and fed on basal diet along the experimental period as (C +ve).

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Group (3): Rats ingested by cyclophosphamide at a dose of 200mg/kg b.wt., orally ingested with aqueous extract of *phyllanthus emblica* at a dose of 250 mg/kg b.wt., for 45 days.

Group (4): Rats ingested once by cyclophosphamide at a dose of 200mg/kg b.wt., ingested with aqueous extract of *phyllanthus emblica* at a dose of 500 mg/kg b.wt., for 45 days.

Group (5): Rats ingested once by cyclophosphamide at a dose of 200mg/kg b.wt., orally ingested with aqueous extract of *costus speciosus* at a dose of 250 mg/kg b.wt., for 45 days.

Group (6): Rats ingested once by cyclophosphamide at a dose of 200mg/kg b.wt., orally ingested with aqueous extract of *costus speciosus* at a dose of 500 mg/kg b.wt., for 45 days.

Biochemical Evaluation

At the end of experimental period (45 days), blood samples were collected to determine the following parameters: CD4, CD8, CD16 and CD19 were analyzed by flow cytometric analysis as the methods prescribed by **Adrienne et al., (2013)**. IgM and IgG were determined using indirect enzyme-linked immunosorbent assay (ELISA) for antibody detection as the methods described by **Toledo et al. (2003)**. The levels of serum albumin and globulin were determined using a method of **Doumas et al. (1971)**.

Statistical Analyses

Statistical analyses were performed by using SPSS (v.16, IBM SPSS Statistics, US) at $p \leq 0.05$ by using the means and one-way ANOVA followed by low significant difference post hoc multiple comparisons (**SPSS, 1998**).

Results and Discussion

Immunomodulatory efficacy for CD4 and CD8

Results listed in table (1) display the immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for CD4 and CD8 as types of leucocytes that play an important role in the immune system function by fighting infection which made in the bone marrow, and mature in the thymus gland which circulate throughout the body in the bloodstream, spleen and the lymph nodes.

It could be concluded from the results of the table that CD4 and Cd8 in control +ve intoxicated group were 225.00 ± 7.73 and 125.00 ± 8.37 Cells/ μ L but in control -ve healthy group were 850.00 ± 9.13 and 550.00 ± 7.51 Cells/ μ L,. These results denote that there were significant decrease for CD4 and CD8 in control +ve intoxicated group (Immunosuppressive rats without treatment) as compared to control -ve healthy group (normal rats). All rats orally ingected with all experimental doses of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts showed significant increases in CD4 and CD8 count when compared to control positive (C +ve) group. The group orally administered with *Costus speciosus* at a dose of 500/mg/kg showed the highest significant improvement not only in CD4 but also in CD8 when compared to all experimental groups.

These results confirmed by the results of ***Bhandari and Kamdod, (2012)*** who reported that *Phyllanthus emblica* Linn and its phytochemicals exhibit anticarcinogenic properties according their possession of radiomodulatory, antimutagenic, chemomodulatory, free radical scavenging, chemopreventive, antioxidant,

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anti-inflammatory, and immunomodulatory activities. Furthermore, **Charoenteeraboon et al., (2010)** concluded that *Phyllanthus emblica* is a rich source of vitamin C and containing a mixture of phenolic compounds and a potential source of natural antioxidants, having free radical scavenging activity, acting as cyto-, hepato-, and radio-protection.

Moreover, **Selim and Al-Jaouni (2016)** recorded that *Costus speciosus* is a widely an important medicinal plant used in many indigenous medicinal formulations suggesting that diosgenin isolated from *C. speciosus* possess anticancer, apoptotic and inhibitory effects on cell proliferation. Furthermore, **Kala et al., (2015)** showed that n-butanolic fraction as a content of *costus speciosus* koen rhizome hydroalcoholic extract possessed immunostimulatory activity.

Immunomodulatory efficacy for CD16 and CD19

Table (2) results show the immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for CD16 and CD19 as an important role in the immune system function. It is cleared from the data in the table that there were significant decrease in CD16 and CD19 in control +ve intoxicated group (Immunosuppressive rats without treatment) when compared to control -ve healthy group (normal rats) which the values of mean \pm SE were 75.00 \pm 5.71 and 345.00 \pm 9.20 Cells/ μ L for CD16, and 80.00 \pm 7.41 and 300.00 \pm 5.29 Cells/ μ L for CD19, respectively. Intoxicated rats which orally ingested with *Costus speciosus* at a dose of 500 mg/kg. b. wt., showed the highest significant improvement in CD16 and CD19 followed by *Phyllanthus emblica* at a dose of 500/mg/ kg. b. wt., group, then *Costus speciosus* at a dose

of 250 mg/kg. b. wt., group, and finally, *phyllanthus emblica* at a dose of 250/mg/ kg. b. wt., group as compared to control positive groups.

These findings agreed the findings of **Sai et al., (2002)** who demonstrated that *Phyllanthus emblica linn* relieved the immunosuppressive effects of chromium on lymphocyte proliferation and restored gamma interferon production considerably. Also, **Pandey (2011)** revealed that *Phyllanthus emblica* possess the ability to inhibit mutations in genes and repairs the chromosomal abnormalities related to the presence of ellagic acid which act as a powerful antioxidant. Furthermore, **Ngamkitidechakul et al., (2010)** suggested that *Phyllanthus emblica Linn* exhibits anticancer activity against selected cancer cells, suggesting further study as a possible chemopreventive agent.

Morover, **Chandra et al., (2015)** studied the immunostimulatory activity of *Costus speciosus* referring to the mechanism of immune stimulatory action of saponins found in it, reporting that saponins induced the production of cytokines as to interleukins and interferons that might stimulate the immune stimulant action.

Immunomodulatory efficacy for IgM and IgG

Data present in table (3) show the immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for immunoglobulin M (IgM) which found mainly in blood and lymph fluid and the first antibody the body makes when fights a new infection, and immunoglobulin M (IgM) the most common antibody in the blood and other body fluids which protects against bacterial and viral infections.

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It could be noticed from the results in the table that the mean \pm SE levels of IgM and IgG in control positive intoxicated group (C +ve) were 7.52 \pm 0.42 and 1.55 \pm 0.05 compared to 14.20 \pm 0.19 and 5.27 \pm 0.02 mg/dl in normal healthy control rats (C -ve), respectively.

These results showed that there were significant decrease in the levels of IgM and IgG in control positive intoxicated group as compared to normal healthy rats. All rats injected intraperitoneal with a single dose (200 mg/kg body weight) of cyclophosphamide for immunosuppression then orally administered with *Phyllanthus emblica* and *Costus speciosus* aqueous extracts at all tested doses showed significant improvements in the levels of IgM and IgG as compared to control positive intoxicated groups. Rats orally ingested with *Costus speciosus* at a dose of 500/mg/kg. b. wt., showed the highest significant improvements in the levels of IgM and IgG when compared to all experimental groups.

These results are in agreement with the results of **Luo et al., (2011)** who demonstrated that phenolics found in *Phyllanthus emblica* had active radical scavenging and strong inhibition ability of lipid peroxidation. Also, **Verma and Verma, (2010)** mentioned that *Phyllanthus emblica* Linn contains vitamin C which act as an effective antioxidant constituent.

Moreover, **Chandra et al., (2015)** estimated the serum immunoglobulins (Ig) level and phagocytic index as a model for evaluation of immunomodulatory activity, suggesting that both low and high doses (100 and 200 mg/kg b. wt.) of *Costus speciosus* koen rhizome extract elevated serum immunoglobulin G (IgG) level and phagocytic index, moreover, the effect at higher dose was highly significant than lower dose. Furthermore, **Ilango et al., (2020)**

demonstrated that ethanolic extract of *Costus speciosus* rhizome possesses potential to prevent noise stress induced changes in rodent immune system as soon as a strong applicable immunomodulatory prospective to clinical practices.

Immunomodulatory efficacy for albumin and globulin

The immunomodulatory efficacy of orally ingestion with *Phyllanthus emblica* and *Costus speciosus* aqueous extracts for albumin and globulin in immunosuppressive rats is recorded in table (4). It could be observed from the results in the previous table that the means \pm SE for albumin and globulin in control +ve intoxicated groups were 1.55 \pm 0.09 and 0.94 \pm 0.04 mg/dl but in control –ve healthy group were 4.11 \pm 0.05 and 3.21 \pm 0.02 mg/dl, respectively.

These results revealed that there were significant decreases in albumin and globulin in control +ve intoxicated group (immunosuppressive rats without treatment) as compared to control –ve healthy group (normal rats). All rats orally administered with all experimental doses of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts showed significant increase in albumin and globulin when compared to control positive (C +ve) intoxicated group. The group orally ingested with *Costus speciosus* at a dose of 500/mg/kg b. wt., showed the highest significant improvements in the level of albumin and globulin when compared to all experimental groups. At the same time, there were no significant differences occurred among the groups orally administered with *Costus speciosus* at a dose of 250/mg/kg b. wt., and *Phyllanthus emblica* at doses of 250 and 500 mg/kg b. wt.

Data agreed with the findings of **Suresh and Vasudevan (1994)** who reported that *Phyllanthus emblica*, an excellent source of

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vitamin C (ascorbate), improved natural killer (NK) cell activity and antibody dependent cellular cytotoxicity. Moreover, **Lue et al (2011)** and **Nuzul et al., (2020)** clearly showed the presence of fatty acids in *Phyllanthus emblica* which play a main role in traditional medicines efficacy particularly as antioxidant and antimalarial.

Meanwhile, **Pawar and Pawar (2012)** concluded that *Costus speciosus* contains an important medicinal and ornamental properties used to cure different diseases by possessing many pharmacological activities such as antibacterial, antifungal, anticholinesterase, antioxidant, antihyperglycemic, antiinflammatory, antidiuretic, larvicidal, analgesic, antipyretic, antistress and estrogenic activity. Furthermore, **Jayasri, et al., (2009)** evaluated the antioxidant activity of the plant *Costus speciosus* suggesting that this plant can be used as a potent source of natural antioxidants.

Conclusion

The present investigation revealed that *Phyllanthus emblica* and *Costus speciosus* aqueous extracts possess immunomodulatory efficacy with regard to immunosuppressive rats.

Table (1): Immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for CD4 and CD8.

Groups	Parameters	* CD4 (Cells/ μ L)	** CD8 (Cells/ μ L)
Control -ve		850.00 \pm 9.13a	550.00 \pm 7.51 a
Control +ve		225.00 \pm 7.73 f	125.00 \pm 8.37 f
<i>Phyllanthus emblica</i> 250/mg/kg. b.wt.		300.00 \pm 3.25e	200.00 \pm 5.28 e
<i>Phyllanthus emblica</i> 500/mg/kg. b.wt.		450.00 \pm 6.38c	400.00 \pm 9.04 c
<i>Costus speciosus</i> 250/mg/kg. b.wt.		350.00 \pm 5.91d	250.00 \pm 5.41 d
<i>Costus speciosus</i> 500/mg/kg. b.wt.		600.00 \pm 2.41b	450.00 \pm 3.98 b

* CD4 (T-helper/inducer cell marker), normal range equals 300–1400 cells/ μ L.

** CD8+ (Cytotoxic T/suppressor cell marker), normal range equals 200–900 cells/ μ L.

- Values denote arithmetic means \pm standard error of the mean.

- Means with different letters (a, b, c, etc.) in the same column differ significantly at $p \leq 0.05$,

- using one-way ANOVA test, while those with similar letters are non-significant.

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Table (2): Immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for CD16 and CD19.

Parameters Groups	* CD16 (Cells/ μ L)	** CD19 (Cells/ μ L)
Control -ve	345.00 \pm 9.20 a	300.00 \pm 5.29 a
Control +ve	75.00 \pm 5.71 f	80.00 \pm 7.41 f
<i>Phyllanthus emblica</i> 250 mg/kg. b.wt.	125.00 \pm 8.28 e	105.00 \pm 3.05 e
<i>Phyllanthus emblica</i> 500 mg/kg. b.wt.	205.00 \pm 3.39 c	195.00 \pm 2.28 c
<i>Costus speciosus</i> 250 mg/kg. b.wt.	165.00 \pm 7.04 d	125.00 \pm 4.33 d
<i>Costus speciosus</i> 500 mg/kg. b.wt.	295.00 \pm 2.49 b	250.00 \pm 8.32 b

* CD16 (natural killer cell marker), normal range equals 90–600 cells/ μ L.

** CD19 (mature B cell marker), normal range equals 100–500 cells/ μ L.

- Values denote arithmetic means \pm standard error of the mean.

- Means with different letters (a, b, c, etc.) in the same column differ significantly at $p \leq 0.05$,

using one-way ANOVA test, while those with similar letters are non-significant.

Table (3): Immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for IgM and IgG.

Parameters Groups	IgM (mg/dl)	IgG (mg/dl)
Control -ve	14.20±0.19 a	5.27±0.02 a
Control+ve	7.52±0.42 f	1.55±0.05 f
Phyllanthus emblica 250 mg/kg. b.wt.	8.50±0.22 e	2.09±0.07 e
Phyllanthus emblica 500 mg/kg. b.wt.	9.30±0.37 d	2.29±0.01 d
Costus speciosus 250 mg/kg. b.wt.	11.53±0.06 c	3.03±0.03 c
Costus speciosus 500 mg/kg. b.wt.	12.74±2.11 b	3.41±0.06 b

- Values denote arithmetic means±standard error of the mean.

- Means with different letters (a, b, c, etc.) in the same column differ significantly at $p \leq 0.05$, using one-way ANOVA test, while those with similar letters are non-significant.

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Table (4): Immunomodulatory efficacy of *Phyllanthus emblica* and *Costus speciosus* aqueous extracts in immunosuppressive rats for albumin and globulin.

parameters Groups	Albumin (mg/dl)	Globulin (mg/dl)
Control -ve	4.11±0.05 a	3.21±0.02 a
Control +ve	1.55±0.09 e	0.94±0.04 e
<i>Phyllanthus emblica</i> 250 mg/kg. b.wt.	2.71±0.05 d	1.19±0.03 d
<i>Phyllanthus emblica</i> 500 mg/kg. b.wt.	2.89±0.06 cd	1.25±0.05 cd
<i>Costus speciosus</i> 250 mg/kg. b.wt.	3.01±0.01 c	1.33±0.04 c
<i>Costus speciosus</i> 500 mg/kg. b.wt.	3.56±0.03 b	2.41±0.03 b

- Values denote arithmetic means±standard error of the mean.

- Means with different letters (a, b, c, etc.) in the same column differ significantly at $p \leq 0.05$, using one-way ANOVA test, while those with similar letters are non-significant.

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الفعالية المناعية للمستخلصات المائية للأملج والقسط الهندي لدى الفئران
المثبطة مناعيا

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المخلص العربي

يساعد الجهاز المناعي على التخلص من المواد السامة أو المسببة للحساسية التي تخترق الجسم من خلال الأسطح المخاطية. إن قدرة الجهاز المناعي على التأهب للاستجابة لمسببات العوامل الممرضة أو المواد السامة أو مسببات الحساسية هي قدرته على تمييز العامل الذاتي عن غير الذاتي. وقد هدف البحث الى دراسة الفعالية المناعية للمستخلصات المائية لكل من الاملج والقسط الهندي لدى الفئران المثبطة مناعيا. تم استخدام ٤٢ فأر أبيض بالغ تتراوح أوزانهم بين ١٥٠-٢٠٠ جرام، وقد قسمت الفئران الى ست مجموعات متساوية (٧ فئران في المجموعة) تركت احداهم كمجموعة ضابطة سالبة (طبيعية) بينما الخمس مجموعات المتبقية فقد حقنت في الغشاء البروتوني بجرعة ٢٠٠ملج/كجم من وزن الفئران بمادة السيكلوفوسفاميد لإحداث التثبيط المناعي، ثم قسمت الفئران المثبطة مناعيا الى خمس مجموعات تركت احداها كمجموعه ضابطة موجبة أما الاربع مجموعات المتبقية فقد تم معالجتهم بالمستخلصات المائية لكل من الأملج والقسط الهندي بجرعتين ٢٥٠ و ٥٠٠ ملليجرام /كجم لكل منهم على حده. وبنهاية التجربة (٤٥ يوم) تم تجميع عينات الدم وقياس CD4, CD8, CD16, CD19, IgM and IgA علاوة على ذلك قياس مستوى الألبومين والجلوبيولين. وقد خلصت النتائج الى أن الأملج والقسط الهندي يعززان القدرة المناعية وذلك بزيادة مستوى CD4, CD8, CD16, CD19, IgM and IgA في الدم وزيادة الألبومين والجلوبيولين في السيرم. وقد أظهرت مجموعة القسط الهندي عند جرعة ٥٠٠ ملليجرام /كجم أفضل النتائج لتعزيز المناعة مقارنة بالمجموعات التجريبية الأخرى. وقد استخلص البحث الحالي أن المستخلصات المائية للأملج والقسط الهندي تمتلك فعالية مناعية لدى الحيوانات المثبطة مناعيا.