# EFFECT OF APPLE PEEL EXTRACT ON THE PRODUCTION OF IgG IMMUNOGLOBULINS IN IMMUNOSUPPRESSED MICE

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Email: ikdoc@outlook.com
Date Received:
December 03, 2018
Date Revised:
August 18, 2019
Date Accepted:
August 28, 2019

## ABSTRACT

**Objective:** To determine the effect of apple peel extract on serum IgG levels in corticosteroid induced immunosuppression in mice.

**Methodology:** Twenty four Swiss albino mice were divided into four groups, with six animals in each group. Group A was given distilled water. Group B was given prednisolone 4 mg/kg, dissolved in distilled water. Group C and D received prednisolone, followed by 25 mg/kg and 100 mg/kg apple peel extract, respectively, half an hour later. All the doses were given orally as a single morning dose for 14 days. On 15th day, serum IgG levels were measured by mouse ELISA kit.

**Results:** An increase in serum IgG levels was observed in both of the extract-treated groups. However, there was no significant difference between the low dose and high dose extract-treated groups.

**Conclusions:** Apple peel extract has a stimulatory effect on humoral immunity and can be used in adjunct with other drugs in immunocompromised patients.

**Key Words:** Immunosuppression, Antibody, Humoral immunity, Apple peel extract

This article may be cited as: **Kamal I, Zaffar S, Malik JA, Ahmad M. Effect of apple peel extract on the production of IgG immunoglobulins in immunosuppressed mice. J Postgrad Med Inst 2019; 33(3): 189-93.**

## INTRODUCTION

Immune system is a network of cells, tissues and organs that work in collaboration to protect the body against attacks by "foreign" invaders. The immune system comprises of thymus, lymph nodes and lymphatic ducts. Continuous maturation and degradation of T lymphocytes occurs at these sites. Maturation of B lymphocytes occurs in the bone marrow. Upon stimulation, B cells give rise to large numbers of plasma cells which, in turn, produce antibodies such as IgG, IgE and IgM to fight against the invading organisms, mainly bacteria, viruses, parasites and fungi. IgG works by coating microbes and speeding their uptake by other cells of the immune system. Although immune system is the protection system of the body, but when the immune system hits a wrong target, it can unleash an array of diseases including allergies[1]. A properly functioning immune system is one of the most important components in staying healthy. Immuno-compromised individuals are more prone to infections, especially by opportunistic microbes such as *Pneumocystis jirovecii, nocardia, aspergillus and cryptococcus*. Reactivation of varicella zoster, herpes simplex, cytomegalovirus, hepatitis B and C as well as tuberculosis is common in these patients. Individuals receiving immunosuppressive therapy are inevitably exposed to an increased risk of infections and malignancy[2,3].

Researchers are exploring the effects of diet, exercise, age, psychological stress and other factors on the immune response, both in animals and in humans. Every part of the body, including immune system, functions better when protected from bolstered environmental assaults and by healthy living strategies such as not smoking, consuming sufficient fruits and vegetables and adequate sleep[4,5]. Many health disorders are triggered by disturbance in the normal redox state within the human body caused by oxidative stress. Oxidants increase the risk of de-oxy ribonucleic acid (DNA) damage and damage the defense mechanisms of the body. Fruits and vegetables are rich sources of antioxidants and therefore, are potential immunity boosters[6].

Amongst fruits and vegetables, apples are well known for their antioxidant abundance and health benefits. Mostly, apple flesh is consumed and the peel is discarded. It is, however, noteworthy that the apple peel has more antioxidant and phenolic content in comparison

to the flesh[7-9]. Phenols present in apple peel include flavones, flavanols, anthocyanidins, hydroxycinnamic acids and dihydrochalones[10]. The phytochemicals present in apple peel have complex, overlapping mechanisms which can play role in treatment and prevention of various diseases. It can modulate cholesterol synthesis and thus decreases the risk of cardiovascular disorders[11].

Apple peel has been found to have a role in altering the immune response. Multiple studies have documented the stimulatory effect of apple peel on proliferation of T cells and IL-2Rα expression, attributed to the procyanidins and tannins present in the peel[12,13]. Similarly, flavonoids in the peel have exhibited inhibitory effect on generation of extracellular reactive oxygen species (ROS)[14]. Keeping in view, this research was designed to evaluate the pro stimulatory effects of apple peel on glucocorticoid induced immunosuppression in mice.

## METHODOLOGY

Red apple was chosen for study because it is very rich in polyphenol components. Fresh apples with no apparent physical damage were obtained from local market in Lahore. All of the fruit was of eating quality and selected on basis of uniformity in shape, size and weight. Fruits were identified from Botany department of Punjab University, Lahore. They were washed with tap water, weighed and peeled off. The peel was cut into small pieces and dried. The dried peel was then soaked in 80% of ethanol (1:10 w/v) with daily shaking for 3 days. The residue was separated from the extract by filtration, using Whattman filter paper No.1. The excess solvent was evaporated and the concentrated extract was stored at $40^0$ C temperature[9].

Twenty four male Swiss albino mice were purchased from University of Veterinary and Animals Sciences, Lahore and kept in animal house of Postgraduate Medical Institute, Lahore. The animals were provided with rat chow and water ad libitum. They were acclimatized for one week and provided humane care according to the criteria outlined in the "Guide for the care and use of Laboratory Animals"[15]. All the animal handling protocol was approved by the institutional ethical committee before commencement of the study. Animals were randomly divided into four groups, with six animals in each group. Group A (normal control) was given distilled water orally, in volume equal to that given to experimental groups, daily as a single morning dose for 14 days. Group B (immunosuppressed control) received prednisolone (Pfizer Inc.) 4mg/kg[16] dissolved in distilled water by oral route daily, as a single morning dose, for 14 days. Animals in group C and D (experimental groups) were given prednisolone 4mg/kg dissolved in distilled water, followed by apple peel extract in a dose of 25mg/kg/d and 100 mg/kg/d respectively, half an hour later. All the doses were administered by oral route daily, as a single morning dose for 14 days.

On 15th day, mice were anesthetized with intraperitoneal dose of 100 mg/kg ketamine[17] (Indus Pharma Pvt. Ltd). Blood samples were taken by cardiac puncture, using a 3 ml disposable syringe and collected in clot activator tubes. Serum was collected by centrifuging the blood sample at 3000 rpm for 20 minutes. IgG levels were estimated with mouse ELISA kit (Glory Science Co. Ltd), according to the manufacturer's instructions, using Stat Fax 2100 Microplate reader. Data were transcribed into GraphPad Prism 5.0. After checking normality by Kolmogorov-Smirnov test, data was presented as Mean ± SD. One way ANOVA, followed by post hoc Tukey's test, was applied to test the significance of difference between the study groups.

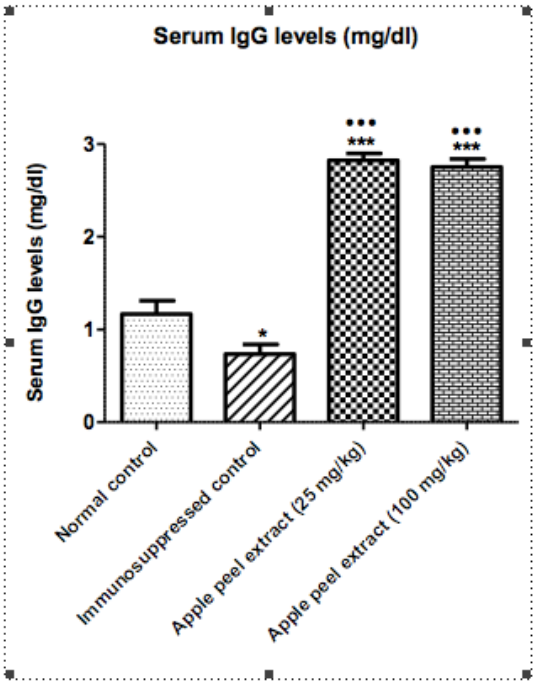
## RESULTS

Mean serum IgG levels ± standard deviation of all the groups is represented in Table 1 and Figure 1. To analyze the difference between the group means, post hoc Tukey's test was applied. It was observed that prednisolone treatment decreased IgG levels as compared to the normal control. Apple peel extract significantly increased the IgG levels in both the experimental groups (C and D), as compared to the normal control (A) as well as the immunosuppressed control (B), with p value <0.001 each. However, there was no statistically significant difference between the group that received low dose (25 mg/kg) apple peel extract (C) as compared to the group that received high dose (100 mg/kg) of extract (D).

**Table 1: Effect of different doses of apple peel extracton serum IgG levels in mice (n=6)**

| Groups | Serum IgG Level (mg/dl) | | | |
|---|---|---|---|---|
| | Mean | SD | Minimum | Maximum |
| Normal Control (A) | 1.17 | 0.35 | 0.83 | 1.80 |
| Immunosuppressed Control (B) | 0.73 | 0.26 | 0.43 | 1.07 |
| Apple Peel Extract 25 mg/kg (C) | 2.82 | 0.19 | 2.50 | 3.00 |
| Apple Peel Extract 100 mg/kg (D) | 2.75 | 0.21 | 2.45 | 3.01 |

**Figure 1. Effect of different doses of apple peel extract on serum IgG levels in mice (n=6)**




*= p < 0.05 (vs. normal control)
*** = p < 0.001 (vs. normal control)
••• = p < 0.001 (vs. immunosuppressed control)

## DISCUSSION

Humoral immunity plays a pivotal role in protecting the body against microbial infections. Production of IgG antibodies leads to activation of complement cascade and facilitates the uptake of the invading organism by other cells of the immune system[1]. Immunocompromised states markedly increase the risk of microbial invasion and opportunistic infections. Therefore, multiple plants and herbs are being investigated for their proposed immunostimulatory effect[18]. It was observed that both the doses of apple peel extract significantly raised the serum IgG levels, with respect to the normal as well as immunosuppressed control, with a p value <0.001, each. However, there was no dose dependent increase in IgG levels.

Apple peel has captured enormous interest from researchers, owing to its antioxidant potential and various therapeutic benefits. Oligomeric procyanidins (OPCs), one of the polyphenols found in apple peel, have exhibited immunostimulatory and antiviral activity, possibly by affecting gene transcriptions in immune cells[12]. Tannins derived from the apple peel have demonstrated stimulatory effect on gamma delta-1 and gamma delta-2 T cells, resulting in increased expression of IL-2Rα and cellular proliferation[13]. Flavonoids present in the apple peel have shown anti-H. pylori activity due to inhibition of extracellular reactive oxygen species (ROS) production[14]. However, currently, there is no study demonstrating the effect of apple peel extract on humoral immune response and antibody production. Therefore, studies performed on other compounds, with documented effects on serum IgG levels, were used as reference for comparison of results of the present study.

The results of this study were comparable to the work conducted by Sforcin et al[19] which demonstrated that administration of propolis led to enhanced antibody production in rats (p <0.05). In another study, Tridax procumbens was observed to boost IgG titers (p <0.05) and enhance humoral immunity in experimental animals[20]. Similarly, onion extract has also demonstrated to have protective effect on serum IgG levels (p <0.05) against dexamethasone induced immunosuppression in rats[21].

## LIMITATIONS

The study had a few limitations because of limited resources. Firstly, only one variety of apples was chosen for immunostimulatory effects. Secondly, only IgG antibody levels were measured due to scarcity of funds and personnel.

## CONCLUSION

Apple peel extract increases IgG levels in corticosteroid induced immunosuppression and can be used in adjunct with other drugs in immunocompromised patients.

## RECOMMENDATIONS

It is recommended that the apple should be eaten as a whole including the peel to get the maximum health benefits. Apple peel extract enhances antibody production in immunosuppressed individuals and can be used as an inexpensive source of immunity booster. This immunostimulatory effect might be linked to genetic transcription and increased proliferation of B lymphocytes. However, there is no current evidence to support this and further studies are required to support the results of this study.

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**CONTRIBUTORS**

IK conceived the idea, designed the study, and acquired the data. SZ analyzed the data, wrote and critically revised the manuscript. JAM did literature search, wrote and edited the manuscript. MA supervised the study and carried out expert revision of the manuscript. All authors contributed significantly to the submitted manuscript.