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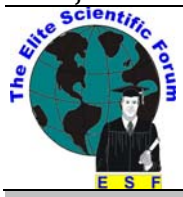

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RESEARCH ARTICLE

Comparative Evaluation of Indigenous Herbal Drugs for their Effects on Non-specific and Cell Mediated Immunity in Rabbits

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ABSTRACT

The present study was conducted to evaluate the effect of indigenous herbal drugs i.e. Immunol (Syrup), Gen-Xing (tablet) and Mufarreh Yakuuti (semi-solid) on non-specific and cell mediated immunity in rabbits. A total of 50 healthy rabbits were divided into four groups i.e. 1, 2, 3 and 4. First three groups consisted of 15 rabbits each and five rabbits in group 4 which served as control. Rabbits in group 1, 2 and 3 were further divided into three sub-groups (a, b and c). Drugs were administered orally at three increasing dose levels (normal, 25% and 50%) for 29 days. Effect on non-specific immunity and cell mediated immunity was observed by % Neutrophil Adhesion (% NA) and delayed type hypersensitivity (DTH) response against Sheep Red Blood Cells (SRBC). Results showed significant ($P<0.05$) increase in % NA (92.58±2.36) at 50% increased dose level of Immunol at 14th day whereas at 29th day Immunol and Gen-Xing showed significant increase in % NA at all dose levels and Mufarreh Yakuuti at normal and 25% increased doses with respect to control. The DTH response was also statistically significant in all treated groups in terms of mean increase in skin thickness in mm with both 1% and 2% SRBC in comparison to control. The study revealed that the all three herbal products did stimulate the immune system of rabbits both non-specifically and specifically in terms of significantly higher ($P<0.05$) values of % NA and mean increase in skin thickness in comparison to control. However, Immunol was found to be the best among three drugs used.

INTRODUCTION

Immunity is the body's natural resistance that protects against attacking microbes and alleviates the disease caused by them (Mazumder et al., 2012). It has two main branches namely innate (non-specific) and adaptive (specific or acquired) immunity (Bhatia and Ichhpujani, 2008). Innate immunity is the natural resistance of the body at birth time not caused by microbial exposure time and again (Uthaisangsook et al., 2002). Two components of innate immunity are first line and second line of defense. Skin and mucous membranes are the anatomical barrier or the first line of defense. Second line protection is provided by defensive cells like phagocytes (Neutrophils and blood monocytes), NK cells and defensive responses like fever and inflammation.

Two main branches of the adaptive immunity are Humoral and cell mediated immunity (CMI) (Tortora et al., 2013). Hypersensitivity can be defined as the altered response of the immune system when body comes across to a notorious substance and is a protective body mechanism. Delayed type hypersensitivity (DTH) is the measure of CMI as T cells are involved in it. It develops with subsequent exposure to the same antigen to which it was previously exposed (Marc and Olson, 2009).

The use of herbs as medicine is increasing day by day and majority of population in developing countries depend upon traditional medicinal herbs for their basic health needs. The allopathic medicines have many side effects and herbs are considered as relatively safe, efficacious, easily available and cost effective (Ali, 1995). The use of these herbs and herbal products is more popular in developed countries and many Asian countries like India and Pakistan because of reliable treatment (WHO, 2005).

There are number of Herbal products in Pakistan which are being claimed as immunostimulant by their manufacturers; however, least studies are available in support of their claims. Therefore, it is the need of the time to evaluate their immunomodulatory activity in order to rationalize their use. Keeping in view the importance of the subject, Immunol, Gen-Xing and Mufarreh Yakuuti were selected as indigenous herbal drugs representing three dosage forms i.e. syrup, tablet and semi-solid, respectively to study their effects on Non-specific and cell mediated immune response of rabbits.

MATERIALS AND METHODS

Animals

A total of 50 male rabbits weighing between 1 to 1.60 kg were procured from local market and kept under the standard condition of temperature (23±2°C) and 12 hours light/dark cycle in Laboratory Animal House of College of Pharmacy. Feed and water were given to each rabbit *ad libitum*.

Herbal drugs

Three indigenous herbal drugs representing three different pharmaceutical dosage forms namely Immunol (syrup), Gen-Xing (tablet) and Mufarreh Yakuuti (semi-solid) were procured from dawanakhana Chiniot Bazar Faisalabad Pakistan.

Preparation of antigen

Fresh sheep blood was obtained from University of Agriculture Faisalabad and Sheep Red Blood Cells (SRBCs) were collected by centrifugation of blood aseptically in Alsever's solution. The SRBCs were washed with pyrogen free 0.9% normal saline three times and 1% suspension was prepared for immunization and challenge (Fulzele et al., 2003).

Treatment

A total of 50 rabbits were divided into four groups i.e. 1, 2, 3 and 4. First three groups consisted of 15 rabbits each and five rabbits in group 4 which served as control. Rabbits in group 1, 2 and 3 were further divided into three sub-groups (a, b and c). Total period of experiment was 30 days which was equally divided in to two phases i.e. Pre-immunization and Post-immunization phase. Doses of herbal drugs for individual rabbits were calculated equivalent to human being according to their body weight. All three herbal products i.e. Immunol, Gen-Xing and Mufarreh Yakuuti were fed orally at normal, 25% and 50% increased dose levels in sub-groups (a, b and c) of group 1, 2 and 3, respectively for 29 days.

Neutrophil adhesion test

On 14^{th} day of treatment with herbal drugs, blood samples were collected with the help of sterile disposable syringes (BD UK). Blood sample from each rabbit was subjected to Total Leukocyte Counts (TLC) and Differential Leukocyte Counts (DLC). After that these samples were incubated with 80 mg/ml of nylon fibers at 37°C for 15 minutes and again analyzed of TLC and DLC. The TLC and % neutrophil values were used to determine the Neutrophil Index (NI) and finally the Percent Neutrophil Adhesion (% NA) was calculated by using following formula as described by Sathianarayanan and Rajasekaran (2012).

% Neutrophil Adhesion (% NA) = NI_u - NI_t / NI_u x 100

NI_u = Neutrophil Index of untreated blood samples

NI_t = Neutrophil Index of fiber treated blood samples

Immunization of rabbits

All the rabbits in treated and control groups were immunized on 14^{th} day of drug treatment by intravenous administration of 1mL dose of 1% Sheep Red Blood Cells (SRBCs) suspension into the marginal ear vein. Herbal products were fed for further 14 days and blood samples were again collected aseptically on 29^{th} day. All the parameters were repeated on un-treated and fiber treated blood samples to calculate % NA Post-immunization (Fulzele et al., 2003).

Delayed type hypersensitivity (DTH) response

Loin area of each rabbit was shaved with the help of scissors. All the animals in treated and control groups were challenged on 29^{th} day by intradermal administration of 0.1 ml 1% SRBCs suspension into shaved loin area with sterile insulin syringes (BD UK). DTH response was measured in terms of mean increase in skin thickness in mm with the help of Vernier Calipers at 0, 2, 4, 24, 48, 72 and 96 hours after post-challenge (Sajid et al., 2007). After 96 hours, rabbits were administered with 0.1 ml of 2% SRBCs suspension intradermally and again DTH response was measured for 4 days post-challenge at different time intervals.

Statistical analysis

The observed data were analyzed statistically through one way Analysis of Variance (ANOVA) and represented as Mean ± SE followed by Dunnett multiple comparisons test at 5% level of significance (Steel et al., 1997).

RESULTS

The rabbits of each group were closely observed for any behavioral changes post-treatment with indigenous herbal drugs. All the rabbits were found normal and no such changes were observed in any of the group post-treatments.

Neutrophil adhesion test

All three drugs showed significant increase ($P<0.05$) in Percent Neutrophil Adhesion (% NA) at different dose levels in comparison to control after 14 days of treatment. Immunol and Gen-Xing did show a dose dependent response with the highest values of % NA at 50% increased dose level as 92.578±5.284 and 84.604±4.956, respectively, whereas, the % NA values were higher at normal and 25% doses of Mufarreh

Yakuuti. The results of Neutrophil Adhesion in different groups before immunization are shown in Table 1. The % NA values were also significantly higher ($P<0.05$) in all treated groups in comparison to corresponding value for untreated control group after 29 days of treatment. An increase in % NA after 14 days of further treatment and post-immunization was observed in all three herbal drugs at a similar pattern as observed before immunization. The results of Neutrophil Adhesion in different groups post-immunization are shown in Table 2.

Delayed type hypersensitivity (DTH) response

For Cell Mediated Immunity (CMI), DTH response in rabbits with 1% and 2% SRBCs was observed at different dose levels of three herbal products. A significantly higher ($P<0.05$) DTH response in terms of mean increase in skin thickness was observed with 1% SRBCs in treated groups as compared to untreated control group. The maximum DTH response was 2.16±0.153 and 2.23±0.139 with normal dose of Immunol and 50% increased dose of Mufarreh Yakuuti in comparison to the corresponding value of 1.26±0.036 in control. The results of DTH response in different groups with 1% SRBCs are shown in Table 3. Similarly DTH response with 2% SRBCs was statistically significant ($P<0.05$) in herbal treated groups in comparison to untreated control group. The highest DTH response was 2.15±0.095 with normal dose of Immunol in comparison to the corresponding value of 1.47±0.052 in control. However, the increase of DTH response among treated groups with 2% SRBCs was none-significant in comparison to 1% SRBCs. The results of DTH response in different groups with 2% SRBCs are shown in Table 4.

DISCUSSION

The major task of immune system is to boost immunity and to protect the physiological system from infections. Leukocytes are the major fraction of the blood cells involved in immunity. Particularly among leukocytes, neutrophils are the first line of defense against diseases and first cells to reach at the site of inflammation too. Similarly, lymphocytes are the central cells which play major role in specific immunity as they have the attributes of specificity, selectivity, memory and self & non-self recognition (Kuby, 1996). Immunomodulatory agents from plants and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. However,

Table 1: Pre-immunization Neutrophil Index (NI) of Fiber untreated and treated blood samples and % Neutrophil Adhesion in different groups at 14th day of drug treatments

Groups/ Treatments	Subgroups	Dosage	NI (10^3) Untreated Blood	NI (10^3) Fiber Treated Blood	Percent Neutrophil Adhesion (% NA)
1 Immunol	1a	Normal	623±127.4	117.68±23.84	80.968±4.206
	1b	25% increase	569.8±77.9	122.20±17.25	78.616±1.796
	1c	50% increase	728±46.8	39.40±13.27	92.578±5.284
2 Gen-Xing	2a	Normal	435.4±272.1	125.24±77.05	72.524±8.415
	2b	25% increase	485.4±164.4	121.98±39.76	74.060±9.333
	2c	50% increase	402.4±99.1	58.32±18.63	84.604±4.956
3 Mufarreh Yakuuti	3a	Normal	532±132.8	108.74±58.84	79.912±6.535
	3b	25% increase	461.6±183.3	87.60±19.90	79.782±6.347
	3c	50% increase	550±110.8	152±50.42	72.303±4.774
4	Control	---	390±152.5	165±75.02	57.69±8.223

The values are mean ± SE of 5 rabbits in each group. One-way ANOVA followed by Dunnett multiple comparisons test ($P<0.05$ Vs Control).

Table 2: Post-immunization Neutrophil Index (NI) of Fiber untreated and treated blood samples and % Neutrophil Adhesion in different groups at 29th day of drug treatments

Groups/ Treatments	Subgroups	Dosage	NI (10^3) Fiber Untreated Blood	NI (10^3) Fiber Treated Blood	Percent Neutrophil Adhesion (% NA)
1 Immunol	1a	Normal	711.8±126.4	81.24±24.29	87.99±5.18
	1b	25% increase	601±79.2	43.68±8.56	92.32±0.59
	1c	50% increase	614±43.4	12.86±7.44	97.87±1.31
2 Gen-Xing	2a	Normal	323±56.5	47.70±15.65	85.38±4.20
	2b	25% increase	445.8±53.3	71.68±36.31	83.65±9.00
	2c	50% increase	553.2±33.8	42.98±18.71	91.18±4.91
3 Mufarreh Yakuuti	3a	Normal	686±167.5	96.24±31.57	85.97±3.25
	3b	25% increase	560.8±160.5	67.70±27.44	86.82±6.61
	3c	50% increase	579.6±101.2	118.64±34.40	76.98±6.74
4	Control	---	358±74.8	145.26±21.31	59.42±6.58

The values are mean ± SE of 5 rabbits in each group. One-way ANOVA followed by Dunnett multiple comparisons test ($P<0.05$ Vs Control).

Table 3: Mean Delayed Type Hypersensitivity response with 1% sheep Red Blood Cells in different groups

Sub-Groups	Dosage	Groups / Treatments			
		1	2	3	4
		Immunol (syrup)	Gen-Xing (capsule)	Mufarreh Yakuuti (semi-solid)	Control
A	Normal	2.16±0.153	1.91±0.088	1.80±0.107	
B	25% increase	2.07±0.053	1.95±0.150	1.80±0.043	1.26±0.036
C	50% increase	1.79±0.065	1.91±0.099	2.23±0.139	

The values are mean ± SE of 5 rabbits in each group. One-way ANOVA followed by Dunnett multiple comparisons test ($P<0.05$ Vs Control).

Table 4: Mean Delayed Type Hypersensitivity response with 2% sheep Red Blood Cells in different groups

Sub-Groups	Dosage	Groups / Treatments			
		1	2	3	4
		Immunol (syrup)	Gen-Xing (capsule)	Mufarreh Yakuuti (semisolid)	Control
A	Normal	2.15±0.095	1.76±0.155	1.99±0.195	
B	25% increase	1.93±0.070	1.99±0.086	1.81±0.048	1.47±0.052
C	50% increase	1.85±0.121	1.85±0.058	1.97±0.058	

The values are mean ± SE of 5 rabbits in each group. One-way ANOVA followed by Dunnett multiple comparisons test ($P<0.05$ Vs Control).

these agents and the polyherbal formulations must be subjected to systematic studies to substantiate their therapeutic claims for clinical use (Fulzele et al., 2003).

The use of herbal drugs has tremendously increased in the recent years because these are considered as an alternate to allopathic medicines with fewer side effects. For instance, the diverse role of ginseng has been described in physiological processes such as cancer, neurodegenerative disorders, insulin resistance, and hypertension. In particular, ginseng has been extensively reported to maintain homeostasis of the immune system and to enhance resistance to illness or microbial attacks through the regulation of immune system. Ginseng contains various pharmacological components including a series of tetracyclic triterpenoid saponins (ginsenosides), polyacetylenes, polyphenolic compounds, and acidic polysaccharides (Kang and Min, 2012). Similarly, a number of other herbal drugs are being marketed in the Country with active ingredients reported to have Immunomodulatory activity. In this scenario the present study was successfully completed to evaluate the immunomodulatory activity of three selected indigenous herbal products representing solid, liquid and semi-solid dosage forms. The Percent Neutrophil Adhesion (% NA) and DTH response were measured to see their effect on non-specific and cell mediated immune response of rabbits.

The overall results of TLC, % neutrophils, NI and % NA in Phase-1 i.e. Pre-immunization phase were highly significant ($P<0.05$) and encouraging in all herbal drug treated groups as compared to control group. All the herbal products at normal, 25% and 50% dose level stimulated the immune system of rabbits non-specifically. However, Immunol at 50% dose level was proved to be the best in this regard, followed by the other two. A gradient dose response was observed with Immunol whereas, variable results were found with respect to different dose levels in Mufarreh Yakuuti and Gen-Xing. Following incubation with nylon fibers, all three herbal products significantly increased neutrophil adhesion to fibers *in-vitro* except un-treated control group which clearly shows their immunostimulatory effect on non-specific immune response of rabbits and correlates to the process of margination of cells in blood vessels *in-vivo.*

Fulzele et al. (2003) also performed similar experiment with a single herbal product namely Haridradi Ghrita (HG) at four doses (50, 100, 200 and 300mg/kg/day) in rats and observed NI and % NA for only one herbal product at different doses. They noticed that drug enhanced neutrophils adhesion to nylon fiber significantly at dose rate of 300mg/kg/day. The results of present study are in line with their work. However, rabbits were used as experimental animal in present study instead of rats because of their easy handling and benefit of multiple blood sampling.

Similarly, overall results of TLC, % neutrophils, NI and % NA in Post-immunization phase were also statistically significant ($P<0.05$) as compared to control. The treatments for 14 more days significantly increased neutrophil adhesion to nylon fibers except in control. The overall trend of increase in neutrophil adhesion was similar in both phases of study which showed that the herbal drugs were generally equally good in potentiating the rabbit's immune system non-specifically both in healthy conditions as well as post challenge with antigen. Immunol again showed dose dependent immune response post-immunization with maximum neutrophil adhesion at 50% increased dose level whereas, variable results were obtained with respect to the dose levels of other two drugs. The results of Immunol are in agreement with Fulzele et al.

(2003) who also found dose dependent response with maximum neutrophil adhesion with 300mg/kg/day dose. The results of neutrophil adhesion in the present study are also in line with the work of Banji et al. (2012) who studied the immunomodulatory effects of different extracts of *Moringa olifera* Lam leaves in Wistar rats.

Delayed Type Hypersensitivity (DTH) response is directly linked to Cell Mediated Immunity (CMI). It develops on second exposure to the same antigen and involves non-specific (macrophages) and specific cells (T lymphocytes) (Solanki and Jain, 2010). To measure DTH response, rabbits were hyper sensitized with 1% and 2% suspensions of SRBCs on 29th day of drug treatment and 14th day post-immunization. The SRBCs were injected intradermally in shaved loin area of each rabbit in treated and control groups. The DTH response was measured in terms of mean increase in at different time intervals post-challenge.

The overall results of DTH revealed that all three herbal products showed significantly higher response in terms of mean increase in skin thickness as compared to control. With 1% SRBCs, higher value of skin thickness was noted at 4 & 24 hour in group 1, at 24 hour in group 2 and at 4 hour in group 3 as compared to control which showed high skin thickness at 2 hour, however, skin thickness returned to normal at 96 hours. Likewise with 2% SRBCs value of skin thickness was high at 4 and 24 hours in group 1. In group 2, high value was observed at 24 hour in 2a & 2b but at 4 hour in 2c. The 3a at 24 hour, 3b & 3c at 4 hour showed high skin thickness in group 3. With 2% SRBC skin thickness remained high till 96 hour than normal skin thickness and also with respect to control. However there was non-significant difference in DTH with respect to mean increase in skin thickness when results of two different concentrations i.e. 1% and 2% SRBC were compared. The results are in line with Sajid et al., (2007) who determined DTH in rabbits with Ivermectin (200, 400 and 600 mg) using 2% dinitrocholobenzene (DNCB) as antigen. They found that increase in skin thickness was significant with respect to control which showed that Ivermectin stimulated CMI.

It was concluded that all three tested indigenous herbal drugs (Immunol, Gen-Xing and Mufarreh Yakuuti) showed an immunostimulatory effect on both non-specific and specific wings of immune system as expressed by enhanced percent Neutrophil Adhesion and response, respectively. Furthermore, the effect was equally good in both healthy rabbit as well as post-challenge with antigen. However Immunol syrup was proved to be the best than the other two drugs. It may be recommended for use as immunostimulatory drug in human however, people with diabetes and high blood pressure must use it only after consultation with some physician.

REFERENCES

Ali M, 1995. Textbook of Pharmacognosy. 2nd Edition. CBS publications, New Delhi, India, pp: 112-115.

Banji V, OJF Banji and R Kavitha, 2012. Immunomodulatory effects of alcoholic and hydro alcoholic extract of *Moringa olifera* Lam leaves. Indian Journal of Experimental Biology, 50: 270-276.

Bhatia R and RL Ichhpujani, 2008. Essentials of Medical Microbiology. 4th Edition. Jaypee Brothers Medical Publishers, India, pp: 413-422.

Fulzele SV, PM Satturwar, SB Joshi and AK Dorle, 2003. Study of the immunomodulatory activity of *Haridardi Ghrita* in rats. Indian Journal of Pharmacology, 35: 51-54.

Kang S and H Min, 2012. Ginseng, the immunity boost: the effects of Panax Ginseng on immune system. Journal of Ginseng Research, 36: 354-368.

Kuby J, 1996. Immunology. 2nd Edition. W.H. Freeman and company, New York.

Marc D and K Olson, 2009. Hypersensitivity reactions and methods of detection. Neuroscience, 6: 1-4.

Mazumder PM, S Pattnayak, H Parvani, D Sasmal and RP Velusamy, 2012. Evaluation of immunomodulatory activity of *Glycyrhiza glabra* L roots in combination with zinc. Asian Pacific Journal of Tropical Biomedicine, 2: 15-20.

Sajid MS, Z Iqbal, G Muhammad, MA Sandhu, MN Khan, M Saqib and MU Iqbal, 2007. Effect of Ivermectin on the cellular and humoral immune responses of rabbits. Life Sciences, 80: 1966-1970.

Solanki YB and M Jain, 2010. Immunomodulatory activity of Ayurvedic plant Aparajita (*Clitoria ternatea* L.) in male albino rats. Global Journal of Science Frontier Research, 10: 2-8.

Steel RGD, JH Torrie and DA Dickey, 1997. Principals and procedures of Statistics: A biometrical approach. 3rd Edition. McGraw Hill Book Publications, New York, USA.

Tortora GJ, BR Funke and CL Case, 2013. Microbiology: An Introduction. 9th Edition. Dorling Kindersley Private limited, India, pp: 503-518.

Uthaisangsook S, NK Day, SL Bahna, RA Good and S Haraguchi, 2002. Innate immunity and its role against infections. Annals of Allergy, Asthma and Immunology, 88: 253-264.

WHO, 2005. WHO monographs on selected medicinal plants. AITBS Publishers, India.