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Pharmacological Evaluation of Rizer Syrup- A Poly Herbal Formulation by Anti-Oxidant and Immunomodulatory Activity

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ABSTRACT

The present study was aimed at evaluating the therapeutic effect of Rizer Syrup-an Ayurvedic polyherbal formulation indicated in oxidative stress, low immunity and General Debility like condition. The Anti-oxidant activity was performed with 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) assay and Hydrogen peroxide(H_2O_2) assay by using a methanolic extract of Rizer Syrup and Ascorbic acid as the reference standard. The IC_{50} Value was 19.82µg/ml for Ascorbic acid and 56.52µg/ml for alcohol extract of Rizer syrup respectively in DPPH assay. The IC_{50} values in H_2O_2 scavenging assay is 24.70µg/ml for ascorbic acid and 57.50µg/ml for alcohol extract of Rizer syrup. The *in vivo* immunomodulatory activity was performed using Neutrophil Adhesion Test. The % neutrophil adhesion in the control group animals was 15.025±0.73. While in Rizer Syrup treated an animal group of three different doses i.e. 1ml/kg, 3ml/kg, 5ml/kg were shows 18.170±0.90, 20.365±0.97 and 21.190±1.38 of % neutrophil adhesion. All studies showed that Rizer Syrup possesses antioxidant activity and immunomodulating activity. Rizer Syrup may explain its Rasayana effect and justify its use as a medicine for Age-associated diseases.

INTRODUCTION

The main aim of Ayurvedic therapeutics is the prevention of disease i.e capacity of the body to resist disease condition (1). Several herbs traditionally employed in the Indian system of medicine i.e. Ayurveda have yielded positive results towards various diseases conditions. The herbs referred to as rasayanas in Ayurveda, in sync with a complete and balanced diet improve vigor and longevity to individuals. This is called as Rasayana therapy. This Rasayan therapy plays a crucial role in enhancing the body's resistance towards various diseases, memory, and energy, which ultimately balances the health of the individual as a whole. Indian medicinal literature also emphasizes the synergistic effect of the polyherbal drug in restoring and rejuvenating the immune system (2). The concept of Rasayanas is more likely towards the modern concept of an adaptogenic agent which are known to afford protection of the human physiological system against diverse stressors (3).

It is well known that Oxidative stress has emerged as a key role player in the pathogenesis and pathophysiology of several diseases in humans. Oxidative stress occurs when there is an imbalance between the generation of ROS and antioxidants defence mechanisms of a cell or a tissue. Experimental evidence suggests that free radicals and reactive oxygen species (ROS) can be involved in a high number of disorders such as arthritis, connective tissue disorders, liver disorders, neurodegenerative disorders, diabetes, chronic inflammation, cancer and in the process of Aging (4). Excess generation of these reactive species can be taken care of by the defences in the form of antioxidants. Natural compounds from many medicinal plants having antioxidant and immunomodulatory activities have potential as therapeutic agents.(5)

Thus, it is very essential to subside the oxidative stress and thereby improving the body resistance power by enhancing the immunity of the body system. With this aim, the present study was conducted to evaluate Rizer Syrup (A marketed Polyherbal formulation of Vital Care Pvt. Ltd.) by performing antioxidant and Immunomodulatory activity.

MATERIALS AND METHODS

Test drug

A Polyherbal formulation with Brand Name Rizer Syrup (RS) is an Ayurvedic proprietary medicine of Vital Care Pvt Ltd, Vadodara, Gujarat. This formulation is prepared from Ashwagandha *(Withania somnifera)*, Shatavari *(Asparagus racemosus)*, Amla *(Emblica*

Officinalis), Haritaki *(Terminalia chebula),* Bibhitaki *(Terminalia bellirica),* Gokshura *(Tribulus Terrestris),* Bala *(Sida cordifolia),* Varahikand *(Dioscorea bulbifera),* Vidarikand *(Pueraria tuberose),* Bhringraj *(Eclipta alba),* Kaucha *(Mucuna Pruriens)* as an active ingredients. All the active ingredients are procured from approved vendors of the manufacturer. The final syrup formulation is also collected from routine manufacturing batch.

Antioxidant activity

Antioxidant constituents of plant materials are important in the maintenance of health and protection from the coronary disease because they possess the ability to protect the body from damage caused by free radical-induced oxidative stress.

Instruments: UV spectrophotometer (Shimadzu-UV-1601), Centrifuge Machine (Eltek-research centrifuge-TC-4100D).

Chemicals: All chemicals used for the study are purchased from SD-fine chemicals; India and all other reagent used were of analytical grade.

Preparation of Rizer Syrup solution: Alcoholic extract of the formulation was prepared at the concentration of 1000 µg/ml in methanol. From the stock solution, different concentrations were prepared in methanol and used for antioxidant studies.

Preparation of Standard stock solution of Ascorbic acid: Ascorbic acid used as a reference standard for the study and its stock solution was prepared in the concentration of 1000 µg /ml in methanol. It was prepared freshly and used immediately for the study. From the stock solution different concentration viz.10, 20, 40, 60, 80, 100µg/ml were prepared in methanol & used for antioxidant studies.

DPPH radical scavenging activity (6-8)

Chemicals: α- α diphenyl β picryl hydroxyl (DPPH) and Methanol.

Principle: The antioxidant reacts with stable free radical, DPPH and converts it to 1,1-Diphenyl-2-Picryl Hydrazyl. The free radical scavenging activity of the product extract, based on the scavenging activity of the stable (DPPH) free radical was determined.

Procedure: Product extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80, 100µg/ml are added to 3 ml of a 0.004% methanol

solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from [(A0-A1)/A0] ×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with an average value of three observations.

H_2O_2 scavenging activity (9)

Chemicals: Hydrogen peroxide (H_2O_2) and Phosphate buffer saline.

Principal: The ability of extract of product to scavenge hydrogen peroxide was determined.

Procedure: A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of product extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80, 100 µg/ml in methanol (1ml) where added to hydrogen peroxide solution (2 ml). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for subtraction. The percentage inhibition activity was calculated from [(A0-A1)/A0] x 100, where A0 is the absorbance of the control and A1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC_{50}. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

Immunomodulatory activity

Immunomodulation is the process that alters the immune system of the host resulting in either immunostimulation or immunosuppression thus regulating or normalizing it. Hence, immunomodulators referred to as biological response modifiers, improve the host defense mechanism against diseases by striking a balance between regulatory and effector cells [2,3]. Using this quality of biological mediators, various alternative Ayurvedic formulations have been developed for various diseases where they either activate the host defense mechanism e.g. in case of an impaired immune response or can selectively suppress it in conditions like autoimmune disorders and hypersensitivity. Such immunomodulatory properties of various medicinal plants provide an alternative to conventional synthetic drug therapy, which causes

side effects, allergic reactions, tolerance to drugs and increased resistance of microorganisms to antibiotics.

Animals: Healthy albino rats of wistar strain, weighing 150-200 gm of either sex were used for the study. The animals were housed in a two rat per polypropylene cages, maintained under the controlled condition of temperature (25±1°C), relative humidity: 30-70%, and 12-hr/12-hr light/dark cycle. Animals had free access to a standard pellet diet and purified drinking water ad lithium. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of B.M.C.P.E.R, Modasa and with permission from Committee for Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Protocol for the study of Immunomodulatory activity. (10-12)

The experimental animals were divided into four Groups, six animals in each group and drug were given in the following order:

Neutrophil adhesion test was performed as per the method described previously by Wilkonson (1978). Rats were treated as Group 1: Control (1% CMC, 2ml/kg p.o), Group 2: Rizer Syrup (1 ml/kg p.o), Group 3: Rizer Syrup (3.0 ml/kg p.o), Group 4: Rizer Syrup (5 ml/kg p.o). On the 14th day of drug treatment, blood samples were collected (before challenge) by puncturing the retro-orbital plexus into Heparinized vials and analysed for total leucocytes counts (TLC) and differential leukocyte counts (DLC) by fixing blood smears and staining with Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37^{0}C. The incubated blood samples were again analysed for TLC and DLC. The Product of TLC and % neutrophil gives neutrophil index (NI) of the blood sample, NI = (TLC × % neutrophil).

Percent neutrophil adhesion was calculated as shown below:

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NIu - NIt}}{\text{NIu}} \times 100$$

Where

NIu = Neutrophil index of an untreated blood sample

NIt = Neutrophil index of a treated blood sample

Statistical Analysis

Results are presented as Mean ± SEM of six animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett test using graph pad prism software. The significance difference if any among the groups at $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Antioxidant activity of Rizer Syrup

The antioxidant activity of the alcoholic extract of the formulation was carried out by *in vitro* antioxidant models. In the models tested, the antioxidant activity of the formulation was studied about a known antioxidant, Ascorbic acid.

1,1-Diphenyl-2-picryl hydroxyl Method

Antioxidant activity of Rizer syrup was carried out using DPPH model comparing with ascorbic acid as standard antioxidant. Results are given in table-1 and graphical presentation is given in Figure No.1.

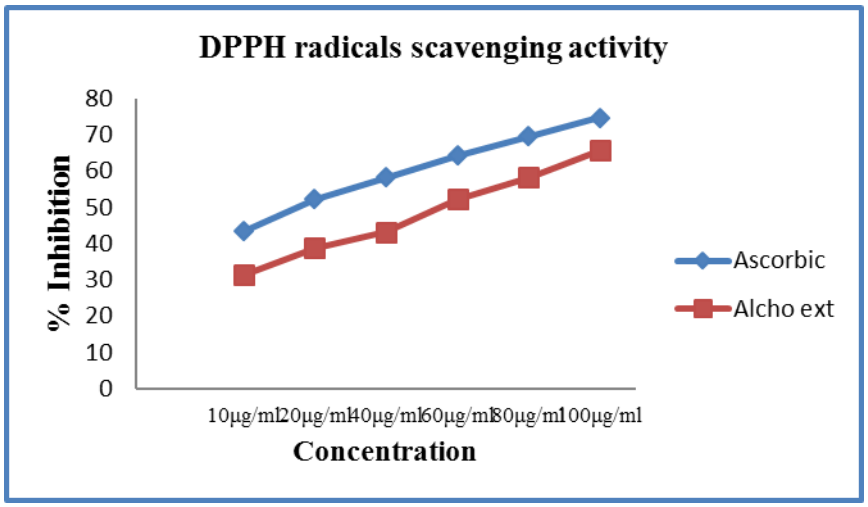

Figure No. 1: DPPH radical scavenging activity alcoholic extract of Rizer Syrup(ARS)

Table No.1: DPPH radicals scavenging activity of alcoholic extract of Rizer Syrup(ARS)

Sr. No.	Concentration (µg/ml)	Ascorbic acid	ARS
1	IC_{50} value	19.82	56.52
2	Regression equation	Y=0.3248x+43.56	Y=0.3650x+29.37
3	R^2	0.9652	0.9888

There was a significant reduction in the concentration of DPPH radicals due to the scavenging ability by increasing the dose of alcohol extract of Rizer syrup and Ascorbic acid, as a reference standard. Maximum inhibition of DPPH radicals scavenging ability with 100µg/ml of Ascorbic acid and alcoholic extract of Rizer Syrup was exhibited 74.62% and 65.67% respectively. The IC_{50} values in DPPH radical scavenging model were 19.82µg/ml for Ascorbic acid and 56.52µg/ml for alcohol extract of Rizer syrup respectively.

Scavenging of Hydrogen Peroxide

Antioxidant activity of Rizer syrup was carried out using Hydrogen peroxide model comparing with ascorbic acid as standard antioxidant. Results are given in table-2 and graphical presentation is given in Figure No.2.

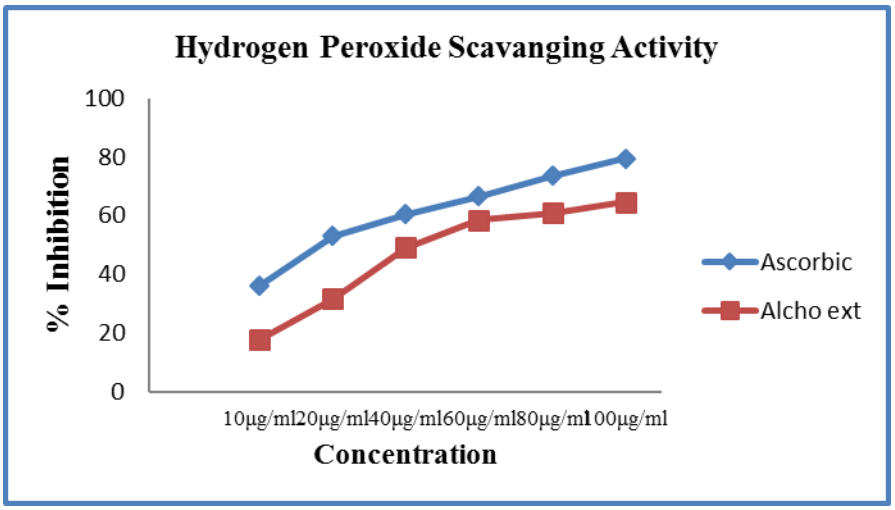

Figure No. 2: H_2O_2 scavenging activity of alcoholic extract of Rizer syrup(ARS)

Table No. 2: H_2O_2 scavenging activity of alcoholic extract of Rizer Syrup(ARS)

Sr. No.	Concentration (µg/ml)	Ascorbic acid	ARS
1	IC_{50} value	24.70	57.50
2	Regression equation	Y=0.4262x+39.48	Y=0.4979x+21.37
3	R^2	0.9110	0.8681

There was a significant reduction in the concentration of H_2O_2 due to the scavenging ability by increasing the dose of alcohol extract of Rizer syrup and ascorbic acid, as a reference standard. Maximum inhibition of H_2O_2 scavenging ability with 100µg/ml of Ascorbic acid and alcoholic extract of Rizer syrup were exhibited 79.41% and 64.70% respectively. The IC_{50} values in H_2O_2 scavenging model is 24.70 µg/ml for ascorbic acid and 57.50 µg/ml for alcohol extract of Rizer syrup. Results indicated that Rizer syrup had shown Dose-dependent antioxidant activity. Antioxidant activity of Rizer syrup may be due to the presence of phytoconstituents ingredients.

Table No. 3: Effect of the Rizer Syrup(RS) on Neutrophil adhesion test in rats

Group	Treatment	Neutrophil index (NI)		%Neutrophil adhesion
		UB	FTB	
I	Control	174.2±2.6	148.1±2.8	15.025±0.73
II	RS 1.0 ml/kg, p.o	230.2±1.2	188.3±2.8	18.170±0.90
III	RS 3.0 ml/kg, p.o	291.2±2.4	232.0±2.0	20.365±0.97**
IV	RS 5.0 ml/kg, p.o	351.1±2.9	275.6±3.3	21.190±1.38**

[All values represented as mean±S.E.M. of six animals. One-way ANOVA followed by Dunnett multiple comparisons test; comparisons were made between control and treated animals. * denotes significance at the level of $p \leq 0.05$. ** denotes the significance at the level of $p \leq 0.01$. UB= Untreated Blood; FTB= Fiber Treated Blood]

This test is indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation as shown in Table 3 and graphically depicted in Figure No. 3 and 4.

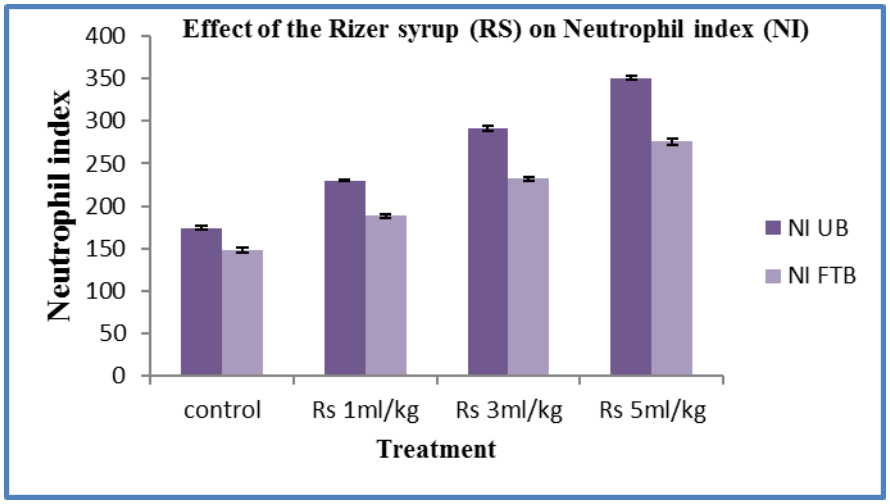

Figure No. 3: Effect of the Rizer syrup (RS) on Neutrophil Index(NI)

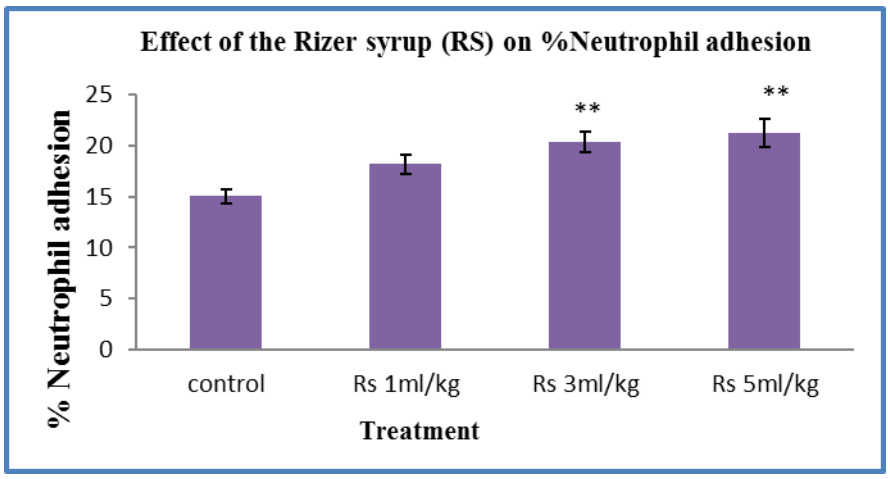

Figure No. 4: Effect of the Rizer syrup (RS) on %Neutrophil adhesion

The % neutrophil adhesion in control group animals was 15.025±0.73 while in Rizer syrup at a dose of 1ml/kg was 18.170±0.90, at dose 3 ml/kg was 20.365±0.97 and at dose 5 ml/kg was 21.190±1.38. As it is evident from the results of the neutrophil adhesion test, a Dose-dependent increase in neutrophil adhesion was observed after administration of Rizer syrup ($p \leq 0.01$). The result indicated that Rizer syrup had shown a dose-dependent immunomodulatory effect.

CONCLUSION

The role of plant extracts and Ayurvedic polyherbal preparations in treating various ailments has been acknowledged since time immemorial. Studies based on the effect of these extracts in the treatment of different diseases have also been well documented. They also possess the

restorative and rejuvenating powers as they act on the immune system and positively affect the response of the body towards infection (13).Use of these traditional medicines for improving immunity and treating Various diseases has been approved by WHO.(2)

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