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Immunomodulatory Activity of Methanolic Extract of Drypetes Roxburghii Leaves

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Abstract

The immunomodulatory potential of methanolic extract of leave extract of *Drypetes roxburghii* leaves was evaluated. For this models like estimation of serum Immunoglobulin level and carbon clearance test were used All the Data was analyzed by one way ANOVA followed by Dunnet's test at *p<0.05, **p<0.01, ***p<0.001. Serum immunoglobulin level was raised from normal 61.4±1.47 NTU up to 69.083±1.549** and 101.416±1.367*** NTU in Drypetes roxbhrghii low dose (DHLD) (100mg/kg) and Drypetes roxbhrghii high dose (DRHD) (200mg/kg) respectively. Phagocytic index of animals in control group was found to be 1.8±0.186 and was raised up to 2.98±0.069^{ns} and 3.97±0.690** Drypetes roxbhrghii low dose (DHLD) (100mg/kg) and Drypetes roxbhrghii high dose (DRHD) (200mg/kg) respectively. This study showed that methanolic extract of *Drypetes roxburghii* leaves possessed immunostimulatory activity.

Introduction

An immunomodulator may be defined as biological or synthetic substance can modulate any of the components of innate and adaptive immune system. Immunomodulators may be classified as immunoadjuvant, immunostimulants and immunosuppressant [1]. The immune system is continuously balancing between quiescence and preparedness for activation and attack [2]. The active agents of immunotherapy are collectively called immunomodulator. Immunotherapy is defined as the "disease treatment by inducing, enhancing, or suppressing an immune response". Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases [3]. A range of synthetic, natural and recombinant immunomodulatory compounds are available. levamisole, isoprinosine, pentoxifilline, and thalidomide are some of the most significant used synthetic immunomodulators. These synthetic immunomodulating drugs possess numerous benefits but their general deliberate use is limited by its adverse side effect profile and demands the search for a more safer and effective agents exerting immunomodulatory activity [4]. Several herbal preparations are used to enhance the body's immune status. Many plants constituent like saponins, glycosides, polysaccharides alkaloid, flavonoids, sterols and sterolins have unique ability to modulate immune system [5].

Putranjiva roxburghii Wall (syn. Drypetes roxburghii Wall.) is an evergreen tree of tropical region belonging to family Euphorbiaceae family [6]. The leaves are reported to contain β-amyrin and its esters, putrone, putrol, putranjivic acid, methyl putrajivate, stigmasterol and hydrocarbons, triterpene roxburghonic acid and biflavones [7].

Material and Methods

Chemicals

Ethanol, CMC, zinc sulphate, barium chloride and sulphuric acid (7664-93-9) were purchased from CDH, pvt ltd, New Delhi.

Plant material

Leaves of *Drypetes roxburghii* was procured and authenticated from NBRI, Lucknow

Extraction and fractionation

The leaves were dried in shade at room temperature. The dried leaves were powdered by using grinder to coarse powder, packed into Soxhlet column and the extracted 70% ethanol for

48hrs. The excess of solvent was removed using rotatory flash evaporator. The obtained crude extract was stored in airtight container in refrigerator below 10 °C for further studies [8].

Acute oral toxicity Study (LD50)

Animals were fasted prior to test drug administration. Following the period of fasting animals was weighed and then the test substance administered in a single dose of 2000mg/kg to animals by oral gavage. After the test drug administration, food was withheld for next 3-4hours. Following administration, animals were closely observed for next 4hours to see any clinical symptom, any change in behavior or mortality. After 6 hours of test administration the animals weighed again. A careful clinical examination was made once in each day for next 14days (OECD 4/26, 2006) [9].

In-vivo Carbon clearance test

Animals (Wistar albino rats) were divided into three group. Each group contained six animals. Group I served as control and received 1% CMC (p.o), group II and group III received DHLD (100mg/kg, p.o) and DRHD (200mg/kg, p.o). On day 7, all the animal of entire group was treated with intravenous injection of Indian ink dispersion (0.3ml per 30g). 50µl of Blood samples were withdrawn in EDTA solution (125mM, 5µl) by retro orbital puncture at interval of 0 and 15minutes. Blood samples were added to 2ml of 0.1% sodium carbonate to lyse the erythrocytes. Absorbances of samples were taken at 660nm. After 15min of blood collection animals were sacrificed and livers and spleen were collected and weighed.

Rate of carbon clearance (K) and Phagocytic index (α) were calculated by using following formula.

$$Rate\ of\ carbon\ clearance\ (K) = \frac{logOD_0 - log\ OD_{15}}{T_2 - T_1}$$

$$Phagocytic\ index\ (\alpha) = \frac{K^{1/3} \times body\ wt\ of\ animal}{Liver\ wt + spleen\ wt}$$

Where OD_0 is the log absorbance of blood at 0min; OD_{15} is log absorbance of blood at 15min; T2 is the last time point of blood collection; T1 is the first time point of blood collection. Rate of carbon clearance and phagocytic index of treated group animals were compared with the control group animals [10,11].

Effect on serum immunoglobulins

Animals (Wistar albino rats) were taken for the study. They were divided into three group, each group contained six rats. Group I was taken as control group and animals in this group were treated with 1% CMC (p.o). Animals in group II and group III received DHLD (100mg/kg, p.o) and DRHD (200mg/kg, p.o). All the treatments were done up to 21 days. Blood samples were collected after 6hours of the last dose and serum was separated. Serum was than analyzed for immunoglobulin level.

For each serum sample to be analyzed, a control tube containing 6ml of distilled water and a test tube containing 6ml of zinc sulphate solution were prepared. To each, 0.1ml of serum was added from a pipette. They were inverted to enable complete mixing of the reagents and left to stand for 1 hr at room temperature. The first tube served as blank and the second tube was taken as sample. The turbidity developed was measured using a digital nepheloturbidity meter. The turbidity obtained (sample-blank) was compared with that obtained with standard barium sulphate ($BaSO_4$) solution. The standard $BaSO_4$ solution was prepared by adding 3ml of barium chloride solution (1.15% w/v) to 97ml of 0.2 N sulphuric acid. The turbidity was expressed in NTU [12].

Result

Acute oral toxicity

Acute oral toxicity No sign of mortality was found at the dose of 2000mg/kg. Hence two doses were selected 100mg/kg as lower dose and 200mg/kg as higher dose to check the effectiveness of test drug (Table 1 & 2).

Table 1: Effect of Drypetes roxburghii extract on phagocytic index.

Treatment	Dose (mg/kg body weight)	Phagocytic index
Control (1% CMC)	-	1.8±0.186
DRLD	100mg/kg	2.98±0.069^{ns}
DRHD	200mg/kg	3.97±0.690**

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. *P<0.05, **p<0.01, ***p<0.001 when Arthritic control group compared with other treated groups

Table 2: Effect of Drypetes roxburghii on serum immunoglobulin level of animals.

Treatment	Dose (mg/kg of body weight)	Serum Ig level (NTU)
Control (1% CMC)	-	61.4±1.47
DRLD	100	69.083±1.549**
DRHD	200	101.416±1.367***

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. * P<0.05, **p<0.01, ***p<0.001 when Arthritic control group compared with other treated groups.

Discussion

Two models were used to evaluate the immunomodulatory activity of leaves of *Putranjiva roxburghii*. Zinc sulphate turbidity test was used for rough estimation of Immunoglobulins present in the serum. . When a zinc sulphate is added to serum containing immunoglobulin it causes precipitation of the same imparting cloudy appearance of the solution. The carbon clearance assay was used to evaluate the effect of extract on reticuloendothelial cell mediated phagocytosis. When colloidal carbon (in ink) is injected intravenously, the macrophages from reticulo endothelial call engulf the carbon particles of the ink. Rate of clearance of carbon particles from blood is known as phagocytic index.

The immunostimulatory activity of *Putranjiva roxburghii* may be contributed to presence of flavanoids. Also, previously

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leaves of Putranjiva roxburghii were reported to have anti-oxidant potential and anti-oxidants act as immunomodulator [13].

Conclusion

Estimation of serum Ig level, phagocytic index. The findings suggested that extract of leaves of Putranjiva roxburghii possess the capacity to modulate immune system.

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