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STUDY OF IMMUNOMODULATORY ACTIVITY OF NATURALLY OCCURRING AND RELATED SYNTHETIC 1-PHENYLNAPHTHALENE LIGNANS

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Abstract- Modulation of immune response to alleviate diseases has long since been of interest.1-Phenylnaphthalene lignans were investigated for their possible immunomodulatory activity by the assay of Immunoglobulin (IgM) production-stimulating activity. The plant samples containing 1-Phenylnaphthalene lignans as well as their synthetic derivatives were found as promising immunomodulatory agents. Thus the present investigation can fulfill our aim of HIV treatment, where 1-Phenylnaphthalenes lignans can assist the immune system in driving out the HIV virus and are also expected to lower the incidence of opportunistic infections in several immunodeficiencies.
Keywords- Immunomodulatory activity, 1-Phenylnaphthalene lignans, spleen cell proliferation, immunoglobulin stimulation, Ruta graveolens, Jatropha gossypifolia, plant extracts.

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Introduction

Immunomodulation is a therapeutic approach in which we try to intervene in auto regulating processes of the immune system which is known to be involved in the etiology and pathophysiological mechanisms of several diseases [1].Immunomodulators do not tend to boost immunity, but to normalize it [2].

1-Phenylnaphthalene lignans are of considerable pharmacological and clinical interest in the treatment of antibacterial [3] antioxidant [4], anticancer [5], antiinflammatory [6], CNS depressants [7], HIV and other diseases [8-9]. The present study was therefore undertaken to explore the immunomodulatory potential of 1-Phenylnaphthalene lignans by the assay Immunoglobulin (IgM) production-stimulating activity [10]. Synthetic derivatives were prepared in the laboratory whereas the study of natural Phenylnaphthalene lignans was carried out by choosing two popular medicinal plants - *Ruta graveolens* and *Jatropha gossypifolia* consisting of lignans showing structural similarity with the synthetic compounds.

The objective of the present study was to discover a new approach in which human immunodeficiency virus (HIV) can be eradicated from an infected individual by intensified antiretroviral treatment coupled with immunomodulation. The hypothesis is that eradication is possible only if very potent antiretroviral drugs are delivered in conjunction with an immunomodulatory agent that simultaneously attacks the viral reservoirs [11].

Material and Methods

Synthetic 1- Phenylnaphthalene derivatives

Perkin condensation of aromatic aldehydes with β-benzoyl propionic acid produced α-arylidine-γ-phenyl-Δ-β-γ-butenolides [12] followed by α-arylidine-β-benzoyl propionic acids [13]. Cyclization of the above keto acids and their formylated and ester derivatives ultimately led to synthetic 1-Phenylnaphthalene lignans [14].

Plant material

The plants *Ruta graveolens* (L) and *Jatropha gossypifolia* (L) collected from plant nursery and authenticated from the Deptt. of Botany, RTMNU, Nagpur. The specimen voucher numbers are 9605 & 9606 for *Ruta* and *Jatropha* respectively. The whole plant materials were shade dried and powdered separately with mechanical blender.

Extraction Methodology

The plant material of *Ruta graveolens* (about 750g) was defatted with petroleum ether and subjected to extraction with methanol using soxhlet apparatus; whereas for *Jatropha gossypifolia* (about 500g) petroleum ether was taken as a solvent for extraction, for about 30–35 complete cycles.

Both the extracts were concentrated by rotary vacuum dryer and kept at 4°C until use.

Experimental animals

In the experiments, 8 to 10 week old male rats were used. The animals had free access to pelleted feed and purified water *ad libitum.*

In vitro Assay

Assay of IgM production-stimulating activity

Separate group of rats (n=3) were treated with daily single dose of synthesized lignan derivatives (50, 100, 200 mg/kg, ip) or *Ruta graveolens* extract (MeOH) (100, 200, 400 mg/kg, ip) or *Jatropha gossypifolia* extract (Petroleum ether) (100, 200, 400 mg/kg, ip) for 5 days. Blood withdrawn from retro-orbital vein aseptically was centrifuged and serum was used for the determination of IgM levels. The IgM production-stimulating activity was examined by measuring the amount of secreted Igs by ELISA method [15].A goat anti-human IgM antibody solution at 1 µg/ml (Cappel, Durham, NC. USA) in a 50 mM sodium carbonate-bicarbonate buffer (pH 9.6) was added to a 96-well microplate (Nunc, Roskilde, Denmark) at 100 µl/well and incubated for 2 h at 37°C.After washing the wells three times with 0.05% Tween 20-PBS (PBS-T-Phosphate buffer saline -T), each well was blocked with PBS containing 1% (w/v) BSA for 2 h at 37°C. Following the blocking reaction, each well was treated with 50 µl of the culture supernatant for 1 h at 37°C. After washing with PBS-T each well was then treated for 1 h at 37°C with 100 µl of the horseradish peroxidase (HRP)-conjugated anti-human IgM or IgG antibody (Biosource International, Camarillo, CA, USA) diluted at 1:1000 in PBS containing 1% BSA(Bovine serum albumin).After washing again 0.6mg/mL of 2,2'-azino-bis(ethylbenzothazoline-6-sulfonic acid) (ABTS) dis-solved in a 0.03% H_2O_2 - 0.05 M citrate buffer (pH 4.0) was added to each well at 100 µl and the absorbance at 415 nm was measured after adding 100 µl of 1.5% oxalic acid to terminate the coloring reaction.

Result and Discussion

About synthetic lignans

In the assay of IgM production-stimulating activity, synthetic 1-Phenylnaphthalene compounds significantly accelerated the IgM production at 50 µg/ml dose levels and acceleration rates were increased with increased concentration of the compounds. As shown in Fig. (**1**) & depicted in Table **1**, the IgM production was facilitated 2-fold by treatment with almost all the synthesized compounds in a concentration dependent manner.

It was observed that the lignan derivatives possessing dimethoxy and/or methylenedioxy substituent were more potent in producing immune stimulation.

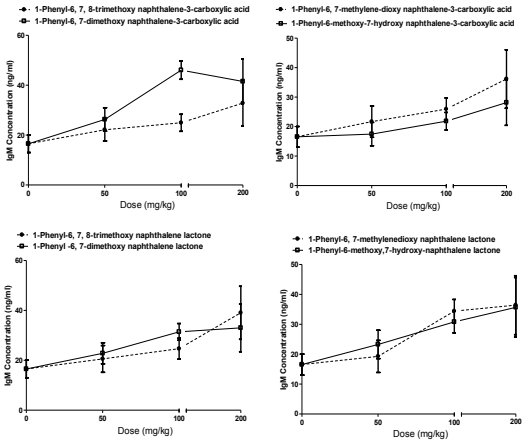

Fig. 1- Effect of lignan derivatives on IgM production

About plant lignans

Maximum immunomodulatory activity was evaluated in the methanolic extract of *Ruta graveolens* and petroleum ether extract of *Jatropha gossypifolia.* The reason is given by the phytochemical study of *Ruta graveolens* [16] and *Jatropha gossypifolia* [17] which showed the presence of lignans in these particular extracts. Thus it can be concluded that each plant extract showing immunomodulatory activity is due to presence of lignans.1-Phenylnaphthalide lignan *Helioxanthin* is present in *Ruta graveolens* and *Arylnaphthalene* lignan in *Jatropha gossypifolia*.

In the assay of IgM production-stimulating activity, the oral administration of the plant extracts at doses of 100,200 and 400 µg/ml, accelerated the IgM production as shown in Table **2** & Fig.(**2**)in a concentration dependent manner.

Although the activity shown by the extracts, at the given doses was less significant as compared to synthetic lignan compounds.

All over findings suggested that both synthetic and naturally occurring 1-Phenylnaphthalene lignans positively modulate the immunity of the host.

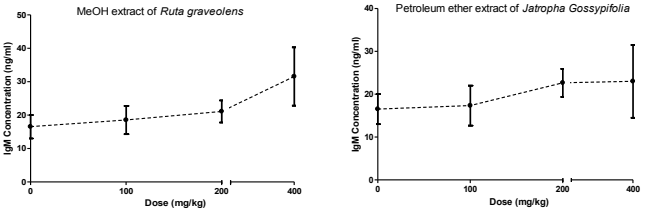

Fig. 2- Effect of plant lignans on IgM production

Conclusion

In summary,1-Phenylnaphthalene lignans have protective effects on host against immune diseases. They can clear HIV-like infection by boosting the function of the cells vital to the immune response. They can reinvigorate the immune response to chronic viral infection, allowing the host to completely clear the virus. Furthermore, immunostimulation may be a prerequisite for efficient therapeutic vaccination strategies to work effectively. More importantly, the herbal formulations do not cause any notable derogatory effects and are readily available at affordable prices.

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Abbrevations

- g = gm
- MeOH =methanol
- Igs = Immunoglobulins
- IgM =Immunoglobulin M
- PBS-T= Phosphate buffer saline –T
- w/v =weight / volume
- BSA = Bovine serum albumin
- HRP = Horseradish peroxidase
- H_2O_2 = Hydrogen peroxide
- PHA = Phytohaemagglutinin
- DMSO =Dimethylsulphoxide
- HCl = Hydrochloric acid
- NH_4Cl =Ammonium chloride
- CO_2 = Carbon dioxide
- µl = micro litre
- µg = microgram
- ml = milliliter
- IgG = Immunoglobulin G
- ELISA = enzyme-linked immunosorbent assay
- Pet. ether = Petroleum ether
- RTMNU = Rashtrasant Tukroji Maharaj Nagpur University
- Deptt. = Department

Table 1- Effect of synthesized lignan derivatives on IgM production

Sr No.	Compound	50 mg/kg	100 mg/kg	200 mg/kg
1	1-Phenyl-6, 7, 8-trimethoxy naphthalene-3-carboxylic acid	22.12 ± 4.46	24.94 ± 3.41	32.83 ± 9.12
2	1-Phenyl-6, 7-dimethoxy naphthalene-3-carboxylic acid	26.29 ± 4.70	46.05 ± 3.66	41.50 ± 9.05
3	1-Phenyl-6, 7-methylene-dioxy naphthalene-3-carboxylic acid	21.68 ± 5.31	25.92 ± 3.82	36.15 ± 9.91
4	1-Phenyl-6-methoxy-7-hydroxy-naphthalene-3-carboxylic acid	23.26 ± 4.74	30.81 ± 3.68	35.68 ± 9.96
5	1-Phenyl -6, 7, 8-trimethoxy naphthalene lactone	17.46 ± 3.94	21.84 ± 2.98	28.15 ± 7.72
6	1-Phenyl -6, 7-dimethoxy naphthalene lactone	20.55 ± 5.38	24.65 ± 4.19	39.06 ± 10.63
7	1-Phenyl-6, 7-methylenedioxy naphthalene lactone	22.83 ± 4.23	31.41 ± 3.35	33.02 ± 9.57
8	1-Phenyl-6-methoxy-7-hydroxy-naphthalene lactone	22.26 ± 5.37	32.93 ± 3.93	36.40 ± 9.87
9	Vehicle (PBS and/or DMSO)	16.53 ± 3.51		

Table 2 - Effect of Ruta graveolens and Jatropha gossypifolia on IgM production

Sr. No.	Compound	100 mg/kg	200 mg/kg	400 mg/kg
1	MeOH extract of Ruta graveolens	18.53 ± 4.23	21.10 ± 3.30	31.57 ± 8.73
2	Pet.ether extract of Jatropha gossypifolia	17.34 ± 4.66	22.65 ± 3.28	23.00 ± 8.50