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Effect of Aqueous and Alcoholic Extracts of *Phyllanthus fraternus* Linn on Platelets Aggregation and Nitric Oxide Production in Chicken Lymphocytes.

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ABSTRACT

Nitric oxide (NO) is generated in many mammalian tissues and is an important mediator of both physiological and pathological responses. The present study investigated the effect of alcoholic and aqueous extracts of whole plant of *Phyllanthus fraternus Linn* in NO production and platelets aggregation. Splenocytes were separated and NO production was measured. Both alcoholic and aqueous extracts showed significant increase in NO production and inhibition of platelets aggregation in chicken lymphocytes at a dose of 600 µg/ ml. The study reveals that *P. fraternus* may be used in asthma or in cardiovascular implications.

KEY WORDS: Nitric Oxide (NO) , Platelets aggregation, Chiken lymphocytes

INTRODUCTION

The plant *Phyllanthus fraternus* is a well known drug of Ayurveda (Indian System of Medicine) and extensively used for the treatment of various liver and, cardiac disorders, obesity and asthma (1). It is widely distributed in India and is a well known natural remedy for a number of illnesses like viral infection [(2-4) and hepatic disorder (5).

Simultaneous administration of *Phyllanthus species* extract along with carcinogen has been reported to inhibit the hepatocellular carcinoma development induced by N –nitrosodiethylamine (NDEA) (6). In a previous study Chattopadhyay *et al*. (7) showed that *Phyllanthus amarus* increased mitotic division with karyomegali, anisocytosis against alcohol induced liver cell injury in partially hepatectomised albino rats.

It is believed that endothelium derived relaxing factor (EDRF) produced by vascular endothelium cell is NO (8).Increase in NO production and decreased platelets aggregation is common mechanism of any anti-asthmatic drugs (9). As no extensive work on anti-asthmatic activity of *P. fraternus* has been done, the present study was undertaken to investigate its anti-platelet aggregation and NO production activities.

MATERIALS AND METHODS

Well identified plants of *P.fraternus* were collected from the campus of College of Pharmacy,IFTM, Moradabad, dried, powdered and extracted separately with water and alcohol. The extracts were concentrated and dried in vacuum. Spleens of healthy poultry birds were collected under aseptic conditions. Splenocytes were separated as according to the methods described by Klien (10). The lymphocytes count was adjusted to 10^7 cells/ml in media by trypan blue (0.5%) dye exclusion test. The cell cultures were divided into four groups with 6 cell culture tubes in each group, each containing 2 ml of suspension of lymphocytes and incubated for 2h. Group I served as untreated group, Group II served as alcoholic extract of *P. fraternus* (AFA) treated, equivalent to 600 µg/ ml by direct inoculation, Group III received aqueous extract of *P. fraternus* (WFA) equivalent to 600 µg/ ml. Group IV received extract of salbutamol equivalent to 600 µg/ ml by direct inoculation.

NO was measured as described by Hibbs (11). Briefly, lymphocytes were seeded (5 X 10^6/ml) in RPMI 1640 phenol red free medium supplemented with 10% fetal calf serum in petri dishes and incubated at 37° C in 5% CO_2 for 4 h. WFA, AFA and salbutamol were added and incubated for additional 48 h. At the end of 48h, 5 ml of Griess reagent [mixture of 1:1 of napthlethlenediamine dihydrochloride (0.1% in water) and sulphanilamide (1% in phosphoric acid)] was added

and incubated in the dark at 30 ^{O}C. Finally, the absorbance was measured at 546 nm and a standard curve using sodium nitrite was used to calculate the concentration of nitrite.

Platelet aggregation was initiated by exposure to ultraviolet light at 366 nm. Alcohol treated platelets were used as positive control group and aggregation was determined as per methods of Hedengue (12).

The experimental data were expressed as mean ± standard error of means. The significance of difference among the WFA and AFA treated groups and control analyzed by means of one way ANOVA followed by Tukey's post-hoc test.

RESULTS

Fig 1 and 2 show the NO production and percentage platelet aggregation effects, respectively, in the presence or absence of extracts. Each value is the mean ± SEM of six separate experiments. Treatments with alcoholic extract conferred significant protection against UV induced platelet aggregation and induce production of NO.

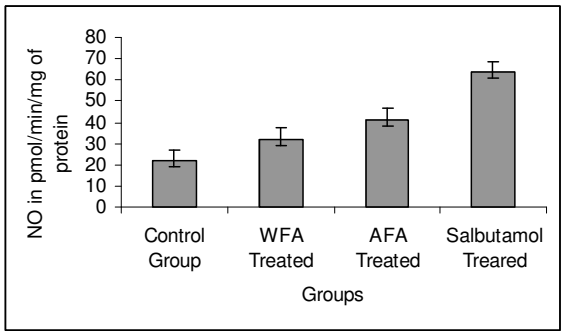

Fig 1. Induction of NO synthase in lymphocytes *in vitro*.

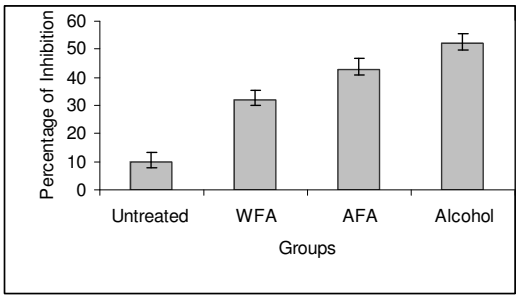

Fig 2. Percentage of platelets inhibition by *P. fraternus* in lymphocytes *invitro*.

DISCUSSION

NO synthase is present in a number of cells including vascular endothelial cells of brain and platelets (13). NO synthesized by the constitutive enzymes in these cells serves as a transduction mechanism for the simulation of soluble guanylate cyclase and therefore, plays a role in the regulation of cell function and communication. NO has numerous role in biological system including vasodilatation (14), regulation of blood (14) and neuromodulator in the CNS (15), and as second messenger of Insulin (16). In our present study the NO production was significantly increased by alcoholic extract of *P. fraternus*. Incubation with alcoholic extract also gave protected against platelet aggregation.

Biological membranes consist mainly of proteins and lipid (16) Present study revealed the efficacy of alcoholic extract of *P. fraternus* against platelet aggregation and possible mechanism of action may be an increase in membrane fluidity.

The study also correlates between the NO production and platelet aggregation. NO is reported to protect against platelet aggregation and adhesion (17) and is antithrombic (18). Same phenomena have been exhibited by P. *fraternus* by increasing NO production and inhibiting platelets aggregration.

The study work revealed an important physiology event in pathology, and haemorheology, where alcoholic extract of *P. fraternus* generated NO and prevented platelet aggregation.

CONCLUSION: The findings indicate that alcoholic extract may be used in asthma or in cardio-vascular implications. However, further study to establish it as an anti- asthmatic or cardio-protective drug at cellular and molecular level is in progress.

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REFERENCES

1. K.C. Chunekar and N.P.Hota in: *Plants of Bhav Prakash* (ed Sharma S.K.) National Academy of Ayurveda, New Delhi., 1999 pp 1-322.
2. S.A.Thyagarajan , P. Subramaniam, S.M. Tirunalasundari, P.S. Venkateshawaran, and B.S. Blumberg. Effects of *Phyllanthus fraterrnus* on chronic carriers of hepatitis B virus. *Lancet.* **2 (8614):** 746-766 (1988).
3. J.B.Calixto, A.R. Santos, V. Cechinel Filho and R.A. Yunes. A review of the plants of the genus *Phyllanthus* : their chemistry, pharmacology, and therapeutic potential. *Med. Res. Rev.* **84(2):**274-278 (1998).
4. S. Jayaram, S.P. Thyagarajan, S, Sumathi , S. Manjula, S. Malathi, and N. Madangopalan. Efficiency of *Phyllanthus* treatment in acute viral hepatitis A,B and non A and Non B; an open clinical trial. *Ind. J.Virol.* **13(1):** 59-64 (1997).
5. S.S. Agarwal, A. Garg and S, Agarwal. Screening of *Phyllanthus and Ricinus communis Linn* on alcohol induced

liver cell damage in non-hepatectomised and partially hepatectomised rats. *Ind. J. Pharmacol.* **18(4):** 211-214 (1986).

6. N.V. Rajeshkumar and R. Kuttan. *Phyllanthus amarus* extract administration increases the life span of rat hepatocellular carcinoma. *J Ethnopharmacol.* **73(12):**215-219 (2000).
7. P. Chattopadhyay, S.S. Agrawal and A.Garg. Liver regenerative effects of *Phyllanthus amarus* Linn against alcohol induced cell injury in partially hepatectomised albino rats. Int. J. Pharmacol**. 2(4):**426-430. (2006).
8. J.S. Beckman and J.P.Crow. Pathological implication of nitric oxide, superoxide and peroxynitrile formation. *J. Biochem. Soc.* **21(2):** 330-334 (1993).
9. Tufvesson E, Aronsson D, Ankerst J, George SC, Bjermer L.Peripheral nitric oxide is increased in rhinitic patients with asthma compared to bronchial hyperresponsiveness. *Respir Med.* 101(11):2321-2326 (2007).
10. A.B. Kelin, S.G. Witonsky , S.A. Ahmed, S.D. Holladav, R.M. Gogal Jr., L. Link, and C.M. Reilly. Impact of different cell isolation techniques on lumphocyte viability and function. *J. Immunoassay and Immunochemistry.* **27(1):** 61-76 (2006).
11. J.B. Hibbs Jr., R.R. Taintor, Z. Vavrin and E.M. Rachlin. Nitric Oxide: A cytotoxic activated macrophage effectors molecule. *Biochem. Biophys. Res. Comm.*. **157(1):** 87-94 (1988).
12. A. Hedengue, S.M. Razavian, M, Del-Pino, A. Simon, J. Levenson. Influence of sialic acid on erythrocyte aggregation in hypercholesterolaemia. *Thromb. Haemost.* **76(6):** 944-949 (1996).
13. R.G.Knowles , M. Salter, S.L. Brooks and S. Moncada Anti-inflammatory glucocorticods inhibit the induction by endotoxin of nitric oxide synthase in the lung, liver and aorta of the rat. *Biochem.Biophys.Res.Commun*. **172(3):** 1042-1048 (1990).
14. L.J. Iganrro 1996. Nitric Oxide as a communication signal in vascular and neuronal cells, In: J.Lancaster eds. **Nitric Oxide Principles and Actions**,4 th ed. New York, Academic Press:111-138 (1996).
15. P.L. Feldman, O.W.Griffith and D.J.Stuehr. The surprising life of nitric oxide. *Chem. Eng. News.* **71:**26-38 (1993).
16. N.N. Khan, K. Acharya, S. Bhattacharya, R. Acharya, S. Mazumdar, W. Barman and A.K. Sinha. Nitric Oxide: The second messenger of Insulin. *IUBMB Life.* **49(5):** 441(2000).
17. B. Mc Donald, B. Reep, E.G Lapetina and L.M Vedia. Glyceraldehyde -3- phosphate dehydrogenase is required for the transport of nitric oxide in platelets. *Proc. Natl. Acad. Sci. USA.,* **90(6) :** 11122-26 (1993).
18. A.K. Sinha, K. Acharya, S. Bhattacharya , S Mazumder, W Baumann and N.N Khan . Prevention of thrombosis *in vivo* by nitric oxide . *Indian Physiol. Allied Sci.,* **52 (3):** 148-61 (1998).