Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 1 (1): 17-20, 2007
ISSN 1819-3455

Increase Insulin Activity by *Phyllanthus amarus* Linn on Liver Cell Regeneration in Partially Hepatectomised Albino Rats

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Abstract: The hydroalcoholic extract of whole plant of *Phyllanthus amarus* Linn showed significant increase in activity of insulin at 200 mg kg^{-1} dose in regenerative hepatocytes against alcohol induced liver cell injury in partially hepatectomised albino rats. The blood sample were collected from the abdominal aorta and the serum insulin estimated by radio immuno assay and regenerative capacity measured by thymidine kinase assay by ^{3}H thymidine incorporation into hepatic DNA which showed that *Phyllanthus amarus* Linn has a potential role in insulin action during liver cell regeneration.

Key words: *Phyllanthus amarus* Linn, thymidine kinase, insulin

Introduction

Phyllanthus amarus linn (Euphoriaceae) is widely distributed in India. It is known as a natural remedy for a number of illnesses like Viral infection (Thyagarajan *et al*., 1988; Calixto *et al*., 1998; Jayaram *et al*., 1997) and hepatic disorder (Agrawal *et al*., 1986). Simultaneous administration of *Phyllanthus amarus* extract along with the carcinogen has been reported to inhibit the hepatocellular carcinoma development induced by N-nitrosodiethylamine (NDEA) (Rajeshkumar and Kuttam, 2000). We observed in our previous study (Chattopadhyay *et al*., 2006) that *Phyllanthus amarus* increased mitotic division with karyomegali, anisocytosis against alcohol induced liver cell injury in partially hepatectomised albino rats. Insulin-like growth factor-I (IGF-I) is an anbolic growth factor required for development chondrocytes (Vincent and Feldman, 2002; Lo and Kim, 2004) and cooperates with membrane ion transport system to modulate epithelial cell motility and proliferation (Shen *et al*., 2004). No liver regenerative property of IGF-I has been so far reported. The present study was, therefore, undertaken to substantiate the claim as a liver regenerative activity and to provide an understanding of the regulatory role of insulin in the activity of TK in regenerating liver.

The [Methyl-3H] Thymidine (2 mci) was purchased from Board of Radioisotope Technology, Mumbai, India and Durapore membrane filter, from Milipore, USA. Diphenyl amine and TRIS purchased from SRL, Mumbai, India. ATP, Marcaptoethanol and Phenyl Ethyl Sulphonyl Fluoride were purchased from Sigma (Louis, MO, USA). Optiphase Hi safe scintillation cocktail LKB scintillation was purchased from FSA Laboratory (Lough Boorough, England LE 110 RG). Insulin determination RIA kit was purchased from Midicrop Inc. (Chez Republic). *Phylanthus amarus* was collected at month of September from the Delhi Institute of Pharmaceutical Sciences and Research campus. It was identified according to the description of the Wealth of India (Council of Industrial and Scientific Research, Government of India, New Delhi, 1985) and macroscopic microscopic comparison

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to authentic samples at laboratory of Delhi Institute of Pharmaceutical Sciences and Research where a voucher specimen is deposited. Dried and coarsely powered whole plant of *Phylanthus amarus* were extracted with a mixture of 50% ethanol and 50% water using Soxhlet extractor and the extract concentrated at water bath at bellow 60°C and evaporated to dryness in rotary vacuum evaporator. Radio isotope study was carried out at Nuclear Research Laboratory, Indian Veterinary Research Institute, Izatnagar, India.

Male Wister rats weighing 200-250 g obtained from Laboratory Animal Resources, Indian Veterinary Research Institute, Izzatnagar, U.P., India and were maintained under temperature-controlled rooms with 12h alternating light and dark cycles were given adequate nutrition and water *ad libitum* at Division of Physiology, IVRI, Izatnagar. All experimental protocols using animals were performed according to the Principles of Laboratory Animal care (NIH publication 85-23, revised 1985) adopted with Institutional Animal Ethical Committee Permission. Two-third Partial Hepatectomy (PH) was performed under diethyl ether anesthesia following the procedure of Higgins and Anderson, 1981. The rats were divided into three groups with 6 animals each and studies were made at 24 h, 48 h and on 7th day post operation intervals. Group I served as vehicle control, Group II served as only partial hepatectomy (PH) and Group III received extract of *Phyllanthus amarus* equivalent to 200 mg kg^{-1} body weight daily by oral route. At 24, 48 and on 7th day liver samples were collected from each group and used for the determination of enzyme activity of TK. Serum was separated from blood and used for insulin determination. Insulin levels were measured in serum of the rats by radio immune assay by using radioimmuno assay kit. Excised liver was homogenized with 5 volumes of 50 mM Tris-HCl buffer (pH 7.3) containing 0.25 mM sucrose, 10 mM β-mercaptoethanol, 1 mM phenylmethylsufonyl fluoride and 1 mM EDTA. The supernatant fractions of liver homogenate centrifuged at 36000 g for 30 min at 4°C, was used for determining the enzymatic activity. Activity of TK was measured (Nakata *et al*., 1985a) and expressed as pmol of product formed/min/mg of protein at 37°C. Insulin levels were measured in serum of the rats by radio immune assay by using radio immune assay kit (Medicrop Inc. Chez Republic) according to the manufacture's instruction at Nuclear Research Laboratory, Indian veterinary Research Institute, Izatnagar, U.P., India and expressed as p mol L^{-1} blood serum.

Measured values were expressed as the mean±standard error of mean. Kruskal-Wallis test and Mann-Whitnly test were used to compare the means of each group using the SPSS for Window release 10.0 packages. A p-value 0.05 was considered statistically significant

The results of TK and insulin are shown in Table 1. The TK actions increased significantly when compared with normal activity (resting in Go state) at 24 h in all groups (Table 1).

At 24 h, the TK activity was found to be at peak level when compared with 48 h and on 7th day. Insulin activity increased significantly ($p<0.05$) at 24 h in *Phyllanthus amarus* treated group (Group III) as compared to PH group (Group II). T.K and insulin activity in down stream towards 48 h and 7th day in all groups.

Table 1: Effect of *Phyllanthus amarus* in TK and Insulin level on regenerating liver after partial hepatectomised rats at 24, 48 h and on 7th day

Groups	Time	TK (p mol min^{-1} mg^{-1} of protein)	Insulin level (p mol L^{-1} blood serum)
Non	24 h	33.84±3.01*	987.88±5.97*
hepatectomise	48 h	30.11±14.12	948.94±7.01*
(Group I)	Day 7	34.57±7.96*	1002.83±5.10*
Partial	24 h	245.09±7.63*	2269.50±0.89*
hepatectomy	48 h	174.22±5.43*	1485.90±4.10*
(Group II)	Day 7	97.952±0.72*	1499.12±4.63*
Partial hepatectomy	24 h	197.06±1.45	2813.33±12.98
Plus *phyanthus amarus*	48 h	117.13±6.46*	1743.17±3.57*
(Group III)	Day 7	64.603±8.64	1183.85±1.96*

Values as mean±SEM, n = 6, Where *indicate significant differences from the control ($p<0.05$)

Thymidine kinase, the enzyme responsible for phosphorylation of thymidine before incorporation into DNA has been used in cell proliferation studies and changes in the enzyme have been correlated with DNA synthesis as measured by labeled thymidine incorporation and thymidine kinase activity (Morley and Royse, 1981). Thymidine kinase is almost undetectable in non-proliferating tissues, such as normal liver cells but after partial hepatectomy the activity increased markedly after a short lag period. It has been shown that this enzyme is closely correlated with DNA biosynthesis. Its activity increases dramatically in rapidly proliferating cells, such as those of regenerating liver and cancer cells (Khan *et al*., 1980). Insulin stimulates the protein synthesis by stimulating messenger RNA (mRNA) transcription, increasing translation and protein synthesis. Thus insulin takes part in stimulation of cell growth and maturation (Stanbury and Dumont, 1983). Activation of the type I insulin like growth factor receptor (IGFR), by binding of IGF-I superphysiological does of insulin result activation of downstream signaling pathways, including phosphodiyl- inositol 3 kinase (PI3 K), Akt, Ras, Raf and mitogen activated protein kinase. Activation of the IGFIR leads to proliferation and antiapoptotic signaling in variety of cell lines (Parrizas *et al*., 1997; Burtscher and Christofri, 1999). It is reported that IGF –I plays important role in IGF-I signaling promote growth and spread gynecological cancers (Shen *et al*., 2004) and increase cell proliferation of neural progenitor in focal ischemia (Yan *et al*., 2006). In our present study observed that insulin activity increase maximum at when liver cell are in maximum cell division at 24 h in Group III which supported that *Phyllanthus amarus* stimulate insulin activity during liver cell regeneration. Bonnette and Hadsell (2001), reported that inhibit cellular proliferation in mammilary terminal buds by disruption of IGF-I gene. Glucagon-like peptide 2 (GLP-2) induced intestinal growth are regulated by IGF-1 (Dube *et al*., 2006). In our present study also found that TK activity increased with increasing insulin concentration and which supported the previous described observation. DNA synthesis starts 12-16 h after pH and TK reaches peak at about 24 h (Khan *et al*., 1980) and this time insulin activity also found maximum. Insulin is known to modulate the responsive of many tissues to a variety of hormones, including α- adenergic agents (Nakata *et al*., 1985b) which are thought to regulate liver regeneration. In conclusion, this study shows that the 50% alcohol and water extract of *Phyllanthus amarus* whole plant possesses a significant liver regenerative effects with increases insulin activity during regeneration of the liver following PH. Further studies are required to elucidate the molecular mechanism of the action *Phyllanthus amarus* on the expression of insulin-regulating genes during liver regeneration.

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