Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2010, 1 (1) : 147-152
(http://scholarsresearchlibrary.com/archive.html)

Bio-evaluation of Antioxidant-Hepatoprotective Activity of Methanolic and Aqueous Extracts of *Azadirchata Indica* Leaves

Shivanand Pandy*, Viral Devmurari

Smt. R.B.Patel Mahila Pharmacy, College, Atkot. Dist Rajkot. Gujarat, India

---

Abstract

Liver is one of the most vulnerable organs for variety of insults such as chemical toxicants, viruses, drugs etc. Wide varieties of herbal formulations are in use to counter the hepatotoxicity due to such insults. However, there has always been a need for better agent, which can provide faster recovery. Drugs from herbal origin are the only treatment available for liver damage. Various herbs and their extracts are reported to possess hepatoprotective activity. Azadirachta indica commonly known as neem is claimed to be therapeutically useful to control leprosy, intestinal helminthiasis, blood morbidity, biliary affliction, skin infection, epistaxis, biliousness and anorexia.

Keywords: *Azadirchata indica,* Carbon tetrachloride; Hepatoprotective activity; Antioxidant activity; Silymarin;

Introduction

Azadirachta indica (Maliaceae) is known for its several medicinal values. The leaves, seeds, roots and bark of the plant possess bitter active principles in different constituents [1] The efficacy of *Azadirachta indica* extract against malarial [2] and bacterial and viral infections [3] had been reported. The plant also has insecticidal properties [1, 4]. Moreover, the antifertility [5, 6, 7] and hypotensive with minimal negative chronotropic [8] effects of *Azadirachta indica* had been reported. The bitter principles of *Azadirachta indica* are also known to increase the flow of saliva and gastric juice as a result of which the plant is used as stomachics. *Azadirachta indica* lowered blood glucose level and attenuated gastric ulcerogenesis [9]. These actions prompted us to investigate the effect of methanolic and aqueous extract of the plant's leaves on in-vitro hepetoprotective effect on rats.

Materials and Methods

Plant resources and preparation of crude drug extract

The leaves of *Azadirchata indica* were collected from botanical garden of K.B.Institute of pharmaceutical education and research center, Gandhinagar district of Gujarat state, India and

identified at the Department of pharmacognosy, K.B.Institute of Pharmaceutical Education and Research Center College, Gujarat, India. Leaves were shade dried and defatted with petroleum ether. The defatted material was extracted with methanol and then vacuum dried. One part of powdered leaves was decocted in boiling water and the other part was macerated for 7 days in water with occasional stirring. The decoction and maceration were filtered and vacuum dried [10].

Animal

Male Wistar rats (180–200 g) were obtained from Cadila Laboratory Animal Center, Ahmedabad. They were housed in animal care facility at the Faculty of Pharmaceutical Sciences, K.B.Institute Of Pharmaceutical Education and Research Center, Gandhinagar, Gujarat, Under controlled environmental conditions (room temperature 25±1 ◦C with 12-h light:12-h dark cycle, relative humidity of approximately 60%) with free standard rat pellets and tap water.

Chemical

Silymarin (15µg/ml, 30µg/ml, 90µg/ml), Methanolic extract (10µg/ml, 50µg/ml, 100 µg/ml) aqueous extract (10µg/ml, 50µg/ml100 µg/ml) and CCl_4 (0.01 ml/ml liquid paraffin for in vitro study) were used.

***In vitro* hepatoprotective activity studies**

In vitro studies involved isolation of hepatocytes and examination of the effect of toxicants along with the test samples. The rat hepatocytes were isolated according to Seglen (1975) with slight modifications (Visen et al., 1991a) by recirculating enzymatic perfusion technique (in situ). The hepatocytes thus isolated were kept in the medium in petridishes for 15 min at 37 ◦C. The petridishes were divided in five groups of three petridishes each. Group A was kept as normal, group B was given CCl4 treatment (10 µl), group C CCl4 + Aqueous extract (10, 50, and 100 µg/ml) and group D CCl4 + methanolic extract (10, 50 and µg/ml).group E CCL4+ Silymarin(15µg/ml, 30µg/ml, 90µg/ml). The viabilityof the cells was determined by trypan blue exclusion method (Visen et al., 1991b) [11-15].

Measurement of cell viability

The viability of cells to exclude trypan blue was determined by incubating the cell suspension (0.1ml) with 0.4% trypan blue (0.9ml). Viability of the isolated cells was determined by Trypan blue exclusion assay by counting the number of stained and unstained cells (viable cells). The concentration of the viable cells were adjusted to 1×10^6 cells per ml [16].

Assessment of antioxidant activity

Assessment of antihepatotoxic activity was done by determining the glutamyl pyruvate transaminase (GPT), reduced glutathione (GSH), Alkaline phosphate level (ALP), asparatate transminase activity (AST) enzyme activity. The enzyme assay was carried out by Reagent Kits maintained by Miles India Ltd. and the procedures were essentially those described in the literature available with kits. Estimations were made on Auto-analyser, Reitman and Frankel method (1957) was used for determining the enzyme activity in the supernatant of various groups [17].

Stastical Analysis

Results are presented as Mean ±SEM. and percentage degree of reversal against hepatotoxin by test. The percentage was calculated by considering enzyme level difference between CCl4 and normal rats as 100% degree of reversal. Total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). The estimated *F*-ratio has been tabulated along

with the critical value of *F*-ratio. Difference among means has been analysed by applying Dunnet's't' test at 99.9% (P <0.001) confidence level [18, 19]

Results

The hepatotoxicity induced by CCl4 is due to its metabolite CCl3 •, a free radical that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum [20]. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Although enzyme levels and barbiturate sleeping time are not a direct measure of hepatic injury, they show the status of the liver. The lowering of enzyme level is a definite indication of hepatoprotective action of the drug. Protection of hepatic damage caused by carbontetrachloride treatment was observed by recording GSH, GPT, ALP and AST levels in treated, toxin control and normal groups because serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of liver injury [21] .The disturbance in the transport function of the hepatocytes as a result of hepatic injury, causes the leakage of enzymes from cells due to altered permeability of membrane [22]. This results in decreased levels of GOT, GPT and alkaline phosphatase in the hepatic cells and a raised level in serum.

Table: 1 Antihepatotoxic activity of *Azadirchata indica* cultured rat hepatocytes

Group (n-6)	GPT(IU/ml)	ALP(IU/ml)	GSH(IU/ml)	AST(IU/ml)
NC	23.68±1.4	32.89±0.78	45.05±0.68	5.6±0.7
MC	85.26±3.4@	104.15±1.43@	1742±0.94@	21.26±2.18@
AAI10	44.33±3.16#	73.99±0.96#	25.98±0.78#	10.45±1.14#
AAI50	36.14±2#	61.88±0.68#	32.17±0.6#	8.53±0.83 #
AAI100	27.25±1.57#	78.19±1.00#	35.55±3.78#	6.43±0.83#
MAI10	44.28±1.87#	78.19±0.81#	24.08±1.03#	10.45±1.1#
MAI50	38.12±4.69#	65.66±1.08#	28.12±0.98#	8.99±1.1#
MAI100	35.39±3.02#	57.23±1.04#	35.69±0.80#	8.35±0.9#
SM15	34.33±1.27#	57.95±1.32#	32.27±0.67#	8.1±0.9#
SM30	31.07±0.68#	48.24±0.68#	35.68±0.99#	7.33±0.8#
SM90	26.08±0.56#	42.11±1.62#	41.19±0.94#	6.15±0.7#

@ Significant different from normal group at (P< 0.05), # Significant different from control group at (P< 0.05). Mean ±SEM of six observations

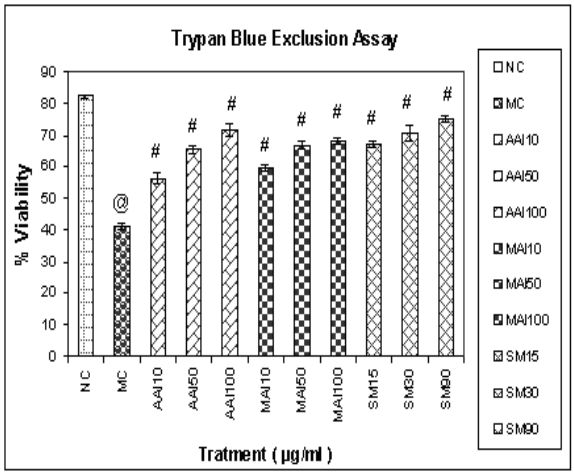

Fig 1: Effect of aqueous (AAI), Methanolic (MAI), extracts of *Azadirchata Indica* leaves and Silymarin on % viability of hepatocyte

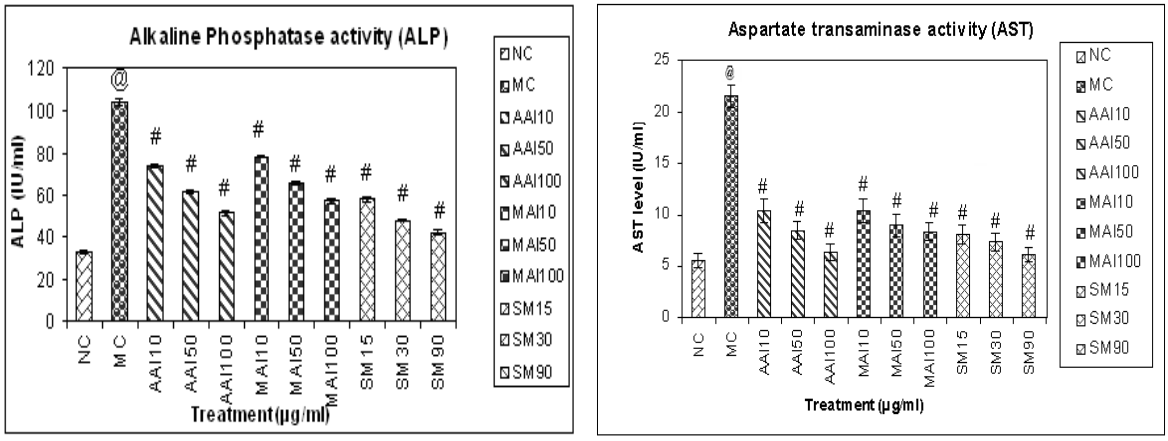

Fig 2: Effect of aqueous (AAI), Methanolic (MAI), extracts of *Azadirchata Indica* leaves and Silymarin on alkaline phosphatase activity and aspartate transminase activity of hepatocyte

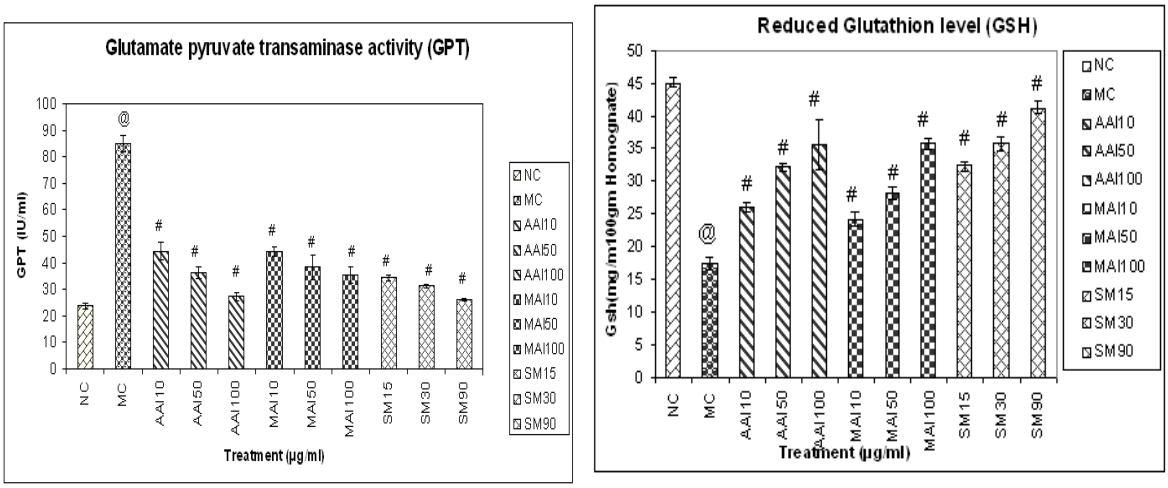

Fig 3: Effect of aqueous (AAI), Methanolic (MAI), extracts of *Azadirchata Indica* leaves and Silymarin on glutamate pyruvate transminase activity and glutathione level of hepatocyte

Discussion and Conclusion

Liver is one of the most vulnerable organs for variety of insults such as chemical toxicants, viruses, drugs etc. Wide varieties of herbal formulations are in use to counter the hepatotoxicity due to such insults. However, there has always been a need for better agent, which can provide faster recovery. Drugs from herbal origin are the only treatment available for liver damage. Various herbs and their extracts are reported to possess hepatoprotective activity. Azadirachta indica commonly known as neem is claimed to be therapeutically useful to control leprosy, intestinal helminthiasis, blood morbidity, biliary affliction, skin infection, epistaxis, biliousness and anorexia. (Biswas et al, 2002). There is also report showing hepatoprotective effect of aqueous extract of neem against paracetamol induced liver necrosis in rats. (Bhanwra et al.2000). Neem is a well known antioxidant. (Rao et al.1998). In present study we evaluated the hepatoprotective activity of aqueous, alcoholic, ethyl acetate and petroleum ether extracts of neem leaves against CCl4 induced hepatotoxicity in isolated rat hepatocytes.Carbon tetrachloride produces hepatotoxicity by formation of CCl3 free radicals in hepatocyte which generates cascade of inflammatory steps, also finally leading to cell necrosis. Lipid peroxidation also plays an important role in cell necrosis. (Recknagel et al. 1983) The endogenous antioxidant reduced glutathione (GSH) gets rapidly used up and the protective mechanism gets saturated. Excess accumulation of free radicals and peroxides thus causes cell lysis causing release of intracellular enzymes such as SGOT, SGPT, ALP etc. (Subramanium et al. 1998).Evaluation of hepatoprotective activity of drugs can be carried out using various other hepatotoxin such as paracetamol, alcohol, galactosamine, and isoniazid etc. such studies can be conducted in whole animal as well as using isolated or cultured hepatocytes. Isolated hepatocyte have distinct advantage of requiring lesser infrastructure and is more rapid. However major disadvantage is that the efficacy of drugs in conditions where severe morphological changes have already occurred cannot be studied. In our study we used to screen the various extracts of neem leaf. Isolated hepatocyte can therefore be a good tool to screen all these extracts without much usage of animals.

References

[1] Oliver-Bever B. Medicinal Plants in Tropical West Africa. Cambridge University Press, **1986**.
[2] Iwu, NM, Obiora O, Anazodo M. Biochemical mechanism of antimalarial activity of Azadirachta indica leaf extract. Proceedings of a Workshop on State of Medicinal Plant Research in Nigeria. If University Press, **1986**, In: Sofowora, A. (Ed.) :p. 369.
[3] Okpaniyi, SN, Ezeukwu GC. *Planta Medica*. **1981**; 41: 34–39.
[4] Nwude N. Veterinary Aspects of Medicinal Plant Research in Nigeria. Ife University Press, **1986**, In: Sofowora, A. (Ed.):pp. 101–103.
[5] Prakash, AO, Tiwari RK, *Journal of Ethnopharmacology* **1988**; 23: 3–9.
[6] Bardhan J, Riar SS, Sawhney RC, Kain, AK, Thomas P, IIavazhagen G. *Indian Journal of Pharmacology*. **1991**; 35: 278–280.
[7] Upadhyay SN, Dhawan S, Talwar GP. *Journal of Andrology* **1993**; 44: 27–281.
[8] Thompson EB, Anderson, CC, *Journal of Pharmaceutical Sciences* **1978**;67:1476–1478.
[9] Sen P, Mediratta PK, Ra A, *Indian Journal of Experimental Biology*. **1992**; 30: 1170–1175.
[10] Alkofahi A, Sallal AJ, Disi AM. *Phytotherapy research* .**1997**; 1: 15-40.
[11] Seglen, PO, *Methods in Cell Biology*. **1975**; 13: 29–83.
[12] Visen, PS, Shukla, B, Patnaik, GK, Kaul, S, Kapoor NK, Dhawan BN. *Drug Development Research.* **1991a**; 22: 209–219.

[13] Visen, PS, Shukla, B., Patnaik, GK, Chandra R, SinghV, Kapoor NK, Dhawan BN. *Phytotherapy Research.* **1991b**; 5: 224–227.
[14] visen, PKS, Shukla, B, Patnaik GK, Kaul S., Kapoor NK, Dhawan BN, *Drug Development Research.* **1991a**; 22: 209–219.
[15] Visen, PKS, Shukla B., Patnaik GK, Chandra R., Singh V, Kapoor NK, Dhawan, BN, *Phytotherapy Research*. **1991b**;5: 224–227.
[16] Frankel AS, *American Journal of Clinical Pathology*. **1957**; 28: 56–58.
[17] J S Frankel AS, *American Journal of Clinical Pathology*. **1957**; 28: 56–58.
[18] Kulkarni, S.K., **1993**. Handbook of Experimental Pharmacology, 2nd ed.Vallabh Prakashan, New Delhi, p. 82.
[19] Ostle, B., **1966**. Statistics in Research. Iowa State University Press, Iowa,USA, pp. 310, 361.
[20] Reitman S, Frankel AS, *American Journal of Clinical Pathology*. **1957**; 28: 56–58.
[21] Recknagael R. *Pharmacological Review*. **1967**; 19: 145–196.
[22] Molander, DW, Wroblewski, F, La Due JS. **1955**. *Clinical Research Proceedings* 3, 20–24.