Research Article

A Prelude *In Vivo* Evaluation of Ethno-hepato Curative Plants from South East Rajasthan

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ABSTRACT

The present study includes ethno-medicinal survey of tribal pockets of south east Rajasthan for the documentation of various hepato-protective and hepato-curative plants and their mode of usage. The reported plants were tabulated on frequency coefficient mode as per used by different tribes and localities. These documented plants were also sorted on the basis of secondary data and 20 plants were selected for preliminary screening. For initial screening pyridoxal phosphate (PLP) dependent SGOT/AST (EC 2.6.1.1) was used as a marker enzyme to indicate hepatocytic bio-chemical profile. The protocol included wistar rats of eithes sexes and was grouped as control vehicle, hepato-toxicated, reference and phyto-treated group. The observations were carried out after 24hrs, 48hrs and 72hrs of the last dose. Among screened plants *Acacia catechu, Aegle marmelos, Andrographis paniculata, Citrullus colocynthis* and *Phyllanthus amarus* were found to be at par as compared to reference group, treated with silymarin. Selection of such effective hepato-curative plants can be used for further investigations and will assist in opening new avenues for developing a new hepatic drug with low frequency of relapse and side effects.

Keywords: *Acacia catechu, Aegle marmelos, Andrographis paniculata, Citrullus colocynthis,* Hepato-protective, *Phyllanthus amarus,* Pyridoxal phosphate, SGOT/AST.

INTRODUCTION

History of human evolution and its civilization recalls the parallel evolution of culture and traditions. This evolutionary prefix was important for wellbeing state and has a greater impact socially as well as personally. Ethnographically among varied practices the consumption of liquor in nearly all civilizations has been reported since time immemorial. Ancient text speaks eloquently about adverse health impact of this drill. Among such cultural rhymes Rajasthan (India) finds leading positions. It is inhabited by various tribal communities and it is found that these communities hug routine liquor practices and /or the occasion of various ceremonies and festivals. Two major features are knocking down humans continuously first increasing liquor consumption leading to health detoriation and second scare availability of drugs to combat the alcohol generated ailments. Socio-cultural practices of alcohol consumption have caused several negative impacts socially as well as on health specifically on hepatic metabolism.

Hepatic system is a major organ system involved in the detoxification and excretion of various endogenous and exogenously administered/ingested substances like xenobiotics, pollutants, liquor etc. Physiologically it generates highly reactive free radicals, which covalently bonds with membrane lipids causing lipid peroxidation which in turn alters the membrane permeability and causes tissue damage. Since, the liver is involved in various biochemical reactions; it is prone to free radicals causing cell necrosis. However, inbuilt antioxidant system i.e. superoxide dismutase (SOD), tissue glutathione (GSH) etc. shield the tissues from free radicals. Inbuilt protective mechanisms or exogenous administration of antioxidants may be useful in antagonizing the necrosis and/ protecting the organ integrity.[1] Continuous and overdoses of hepato-toxicants checks and hinders recycling and regenerative capacity of hepatocytes. Excess accumulation of free radicals and hepato-toxicants require external source to oxidize the same with low metabolic side effects.

Despite of phenomenal growth of allopathic system of medicine, no drug can be desked on which may stimulate the hepatic functions, protect it from damage or help in the regeneration. The drugs available are corticosteroids and /or immuno – suppressive agents which are accompanied by serious side effects and several deaths are listed time to time. Hence, researchers are engaged in searching for liver protective i.e. hepato-protective drugs from herbal origin. Many combinations have been suggested and prescribed, which assist in retaining normal hepatic metabolic moiety with reduced side effects/risks.[2,3] Some of the formulations of AYUSH therapies that has been tested using animals models include Jigrine[4], Curcumin and APCL[5], Boswellic acids in herbal formulations[6], Livit-2, Curcumin 97-90, *Himoliv*[7], or food poly-formulations[8] etc. Effect of *Kumariasav, Kumari kalp, Arogyavardhinivati* and *Tamra bhasma* used in Ayurveda system has been screened for their alkaline and lipoprotein lipase activities.[9] In the same array Rajasthan is also enriched with vast arrays of tribes which utilize number of plants for hepato-protective / hepato-curative activity.[10,11] These plants are consumed as supplementary foods, infusion/decoction (kehwa) or as a masticatories.[12]

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In recent years, many researchers examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat disease of the liver.[13-15] Research confirmed traditional experience and wisdom by discovering the mechanism and mode of action of these plants as well as re-affirming the therapeutic effectiveness of certain plants or plant extract in clinical studies.[16,17]

In the present study ethno-medicinal survey of South-East Rajasthan was carried out to explore the hepato-protective/ curative plants used by tribal peoples and local healers. CCl_4 was used for the induction of hepatotoxicity and silymarin was used as a reference drug to compare the phyto-activity.[18] For the screening of reported plant wistar rats were used an animal models and marker enzyme SGOT/AST a test parameter. LD_{50} was determined as per OECD guidelines for dose confirmation and observation were made time dependent to ensure the effectiveness.

MATERIALS AND METHODS

Ethnobotanical studies

Field surveys were carried out during different seasons and different localities, interacting with varied tribal peoples with special reference to hepato-protective & hepato-curative plants. It included recording and documentation of relevant information followed by preparation of herbarium sheets and taxonomical authentication of reported plants. The respective herbarium sheets are deposited in Department of Biotechnology, BNPG College, Udaipur (Raj.) India. During survey nearly 60 plants were reported to be aligned with hepatic metabolism in either way. On the basis of preliminary survey, secondary data and frequency coefficient method 20 plants were selected for present study (Table-1).

Experimental Animal

White albino rats of Wistar strain weighing between 100-150g of either sex were used in the present screening. They were housed in well ventilated stainless- steel cages at room temperature and acclimatized for 15 days (Rthymic light and dark period of 12hr, 25± 5°C, and 35-60% humidity) and were given *ad libitum* (Free feeding). The litter in the cages was renewed thrice a week to ensure hygiene and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the institutional animal's ethical committee wide letter No. - 90/AA/BNCP-11/IAEC. dated- 10.12.2011.

Extract preparation

Fresh Plants in flowering and fruiting stages were collected from different localities of the South-East Rajasthan. Aqueous extract was prepared by cold maceration of respective shade dried parts by soaking 100g in 500ml of distilled water for 5 days and followed up by filtration and the respective extract was stored at 5-8°C.

Induction of hepatic damage

Dose selection was based on acute toxicity studies (100ml/kg bw.). For inducing *in vivo* hepato-toxicity, carbon tetrachloride (CCl_4) was injected at a dose of 0.7 ml/kg bw. every alternate day for a tenure of 7 days.

Experimental protocol

For evaluation of the hepato-metabolic activity white albino rats of wistar strain were divided randomly in 23 groups with 3 rats in each group and protocol was designed as-

Group 1: Control vehicle-It received normal saline (5 ml/kg).

Group2: Hepato-toxicated group- It was administered CCl_4 /olive oil (1:1), v/v, 0.7 ml/ kg, ip on alternative days for a period of week.

Group 3: Reference group- It was administered drug silymarin (100mg/kg) with toxicant CCl_4.

Group 4-23: Phyto-treated hepato-toxicated groups- Rats received aqueous extract of documented ethno plant (plant part as reported) as 150-200ml/kg. (According to 1/10 of LD_{50} values) for 7 days.

Assessment of SGOT/AST (E.C.2.6.1.1) activity

On the seventh day of the respective treatment the blood samples were collected from the post anal tail region and were allowed to coagulate for 30 mins and serum was separated by centrifugation at 2500 rpm. Marker enzyme SGOT/AST was assessed by UV kinetic method as recommended by International federation of clinical chemistry (IFCC).[19]

The result was presented as the mean ± SEM for 3 animals in each observation and result was statically analyzed using ANOVA followed by Tukeys test.

RESULTS AND DISCUSSION

Determination of SGOT/AST level in the blood serum is a good indicator for the disintegration of hepatic moiety.[20] Utilizing this parameter the preliminary screening of documented ethno-plant reveals that after 24 hrs a slight reduction of SGOT values was observed among the phyto-treated group except group 15 and 17 which were treated by *Glycyrrhiza glabra* & *Leucas aspera*. These two plants didn't revealed any change in sgot concentration. After 48 hrs moderate declining of marker enzyme was observed in group treated with *Acacia catechu*, *Aegle marmelos*, *Andrographis paniculata*, *Citrullus colocynthis*, *Digera muricata*, *Phyllanthus amarus* and *Wrightia tinctoria*. While in other groups no significant alteration was there. After 72 hrs a significant declining at 1% and 5% freedom was observed in groups treated with *Acacia catechu*, *Aegle marmelos*, *Andrographis paniculata*, *Citrullus colocynthis* and *Phyllanthus amarus*. The decline in transaminase activity was compatable to the reference drug silymarin but in case of *Digera muricata* and *Wrightia tinctoria* the further declining ceased. *Leucas*

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aspera & *Mimusops elengi* the sgot levels retained the level of difference less than 25%, while in other it was moderate and most significant being in groups treated with *Acacia catechu*, *Aegle marmelos*, *Andrographis paniculata*, *Citrullus colocynthis* and *Phyllanthus amarus* (Table 2; Figure 1).

Table 1: List of 20 ethno hepatocurative plants selected for prelude *in vivo* screening

Gr. No.	Hepato-protective / Hepato-curative plants	Family name	Plant part/s used	Herbarium Ac. no.
4	*Abutilon indicum Linn.*	Malvaceae	Bark, Leaves	BNC/11-12/022052
5	*Acacia catechu (Roxb.) Willd.*	Mimosaceae	Heart wood	BNC/11-12/022155
6	*Aegle marmelos* (Linn.) Corr.	Rutaceae	Fruit pulp	BNC/11-12/022965
7	*Andrographis paniculata* Nees.	Acanthaceae	Whole plant	BNC/10-11/02011
8	*Cassia occidentalis* Linn.	Caesalpiniaceae	Leaves	BNC/11-12/020819
9	*Caesalpinia bonducella* Linn.	Caesalpiniaceae	Leaves	BNC/11-12/020817
10	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	Root	BNC/11-12/021123
11	*Commelina benghalensis* Linn.	Commelinaceae	Whole plant	BNC/11-12/020920
12	*Digera muricata* (L.) Mart.	Amaranthaceae	Leaves	BNC/10-11/02037
13	*Dioscorea bulbifera* Linn.	Dioscoreceae	Tuber	BNC/11-12/021326
14	*Enicostemma axillare* Raynal	Gentianaceae	Whole plant	BNC/11-12/021643
15	*Glycyrrhiza glabra* Linn.	Fabaceae	Root	BNC/11-12/021540
16	*Indigofera tinctoria* Linn.	Fabaceae	Whole plant	BNC/11-12/021539
17	*Leucas aspera* (Will.)Link.	Lamiaceae	Leaves	BNC/11-12/021744
18	*Mimusops elengi* Linn.	Sapotaceae	Leaves	BNC/11-12/023067
19	*Phyllanthus amarus* Schum. & Thonn.	Euphorbiaceae	Whole plant	BNC/10-11/021431
20	*Portulaca oleracea* Linn.	Portulacaceae	Leaves	BNC/11-12/022762
21	*Solanum nigrum* Linn.	Solanaceae	Leaves	BNC/11-12/023270
22	*Tephrosia purpurea* Linn.	Fabaceae	Leaves	BNC/11-12/021541
23	*Wrightia tinctoria* (Roxb.) R.Br.	Apocynaceae	Leaves	BNC/10-11/02048

Table 2: Prelude *in vivo* biochemical screening of documented 20 ethno-hepato-protective/curative plants

Group 1: Control vehicle: (SGOT/ AST)- 176.60±1.05 (IU/L); **Group 2:** CCl_4treated: (SGOT/AST)-281.57±1.61 (IU/L) (SV: 10-20%); **Groups 3:** CCl_4+Silymarin: (SGOT/ AST)- 182.06±1.15 (IU/L) (SV:10-15%)

Groups: 4 to 23: Hepatotoxicant (CCl_4) + Aqueous Phyto -extract as 150/200ml/kg bw.		SGOT/AST (IU/L)		
Gr. No.	**Phyto source**	**After 24 hrs**	**After 48 hrs**	**After 72 hrs**
4	*Abutilon indicum*	254.73±1.34*	248.34±1.90*	234.90±1.00**
5	*Acacia catechu*	229.66±2.00*	214.15±0.87*	183.99±1.85*
6	*Aegle marmelos*	211.90±2.12*	209.66±2.66**	180.00±1.50*
7	*Andrographis paniculata.*	214.75±1.34**	212.34±0.69*	187.80±2.20*
8	*Cassia occidentalis*	265.90±1.00*	264.78±1.85*	256.34±1.50**
9	*Caesalpinia bonducella*	268.34±1.89*	258.34±1.67*	242.10±1.67*
10	*Citrullus colocynthis*	240.67±2.15*	230.34±1.15*	200.09±2.05*
11	*Commelina benghalensis*	269.89±1.50*	261.35±0.57**	244.00±1.00*
12	*Digera muricata*	244.10±1.35*	230.64±1.00*	216.88±2.15*
13	*Dioscorea bulbifera*	256.84±1.76*	248.43±1.35*	230.33±1.85*
14	*Enicostemma axillare*	260.00±1.39*	253.45±1.90*	243.75±1.39**
15	*Glycyrrhiza glabra*	281.57±1.15**	280.00±1.39*	238.78±3.25***
16	*Indigofera tinctoria*	273.65±1.85*+	267.24±1.50*	256.77±1.67*
17	*Leucas aspera*	280.00±1.45*	278.44±1.85*	266.78±1.50*
18	*Mimusops elengi*	271.34±1.15*	263.89±1.15**	246.10±0.45**
19	*Phyllanthus amarus*	234.33±1.00*	226.48±1.13*	178.10±1.90*
20	*Portulaca oleracea*	254.88±2.10**	247.89±1.67*	234.45±1.39*
21	*Solanum nigrum*	271.00±1.15*	267.00±0.55*	223.56±1.35*
22	*Tephrosia purpurea*	277.89±2.89	272.55±0.35*	238.90±1.90*+
23	*Wrightia tinctoria*	256.89±1.15*	231.90±2.15**	222.89±1.35**

Values are expressed as mean ± SEM for 3 animals in each observation, P*<0.05; **<0.01; ***<0.001; as compared with CCl_4 induced group; +< 0.001 as compared with normal and reference groups; SV= Standard Variance over time from 24hrs to 72hrs.

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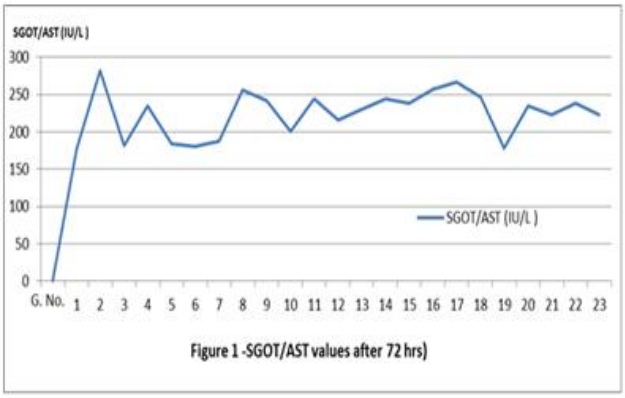

Figure 1 -SGOT/AST values after 72 hrs)

Liver serum enzymes can be grouped into three categories (1) Enzymes whose elevation in serum reflects damage to hepatocytes. (2) Enzymes whose elevation in serum reflects Cholestasis and (3) Enzymes that do not fit precisely into either pattern. When experimental rats are intoxicated with CCl_4 the level of serum enzymes rises in a short tenure. This elevated level of marker enzymes in hepatocytes when compared to normal cells indicates hepatotoxicity. This may be a result of leakage from the cells through peroxidative damage of the membrane. CCl_4 toxicity is dependent on one of its highly reactive product, the trichloromethyl radical (ccl_3^*) this radical binds covalently to neighboring proteins and lipids and initiates lipid peroxidation that causes severe membrane alterations. Transaminases leak out through damaged membrane, elevating serum level and inhibition of CCl_4 bioactivation could reduce this toxic effect. Many compounds exhibit liver protection against Ccl_4 either by decreasing the production of ccl_3 free radical or by impairment of ccl_4 induced lipid peroxidation. Phytoconstituents like flavonoids, triterpenoids, saponins and alkaloids have the ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or inhibition of lipid peroxidation induced by CCl_4[21]. The phyto-extract of *Acacia catechu*, *Aegle marmelos*, *Andrographis paniculata*, *Citrullus colocynthis* and *Phyllanthus amarus* decreased the CCl_4 induced elevated enzyme levels in experiment models indicating recovery of hepatocytes cell membrane or regeneration of damaged liver cells by the extract.[22-27] As in transaminases, pyridoxal phosphate the prosthetic group of the enzyme forms a schiff base intermediate which remains tightly bound to the enzyme by multiple non covalent interactions. It gets disrupted through CCl_3 in intoxicated animals but when these animals are exposed to herbs the phytoconstituents have might quenched these free radicals or have reduce lipid peroxidation resulting regeneration of hepatocytes or capping of signal proteins to block the release of the enzymes viz. SGOT/AST through plasma membrane.

CONCLUSION

The five plants viz.- *Acacia catechu*, *Aegle marmelos*, *Andrographis paniculata*, *Citrullus colocynthis* and *Phyllanthus amarus* indicate *in vivo* hepato-curative property. For the formulation of mono source plant drug/s or poly herb drug these plants can be investigated further for the complete *in vivo* biochemical profile and histological parameters, so that a drug with low risk magnitude can be obtained from immediate vicinity to heal the liquor addicts. Along with pharmacological aspects it will also assist to conserve and promote heritable biodiversity through promotion of ethno-medicinal plants.

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