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Impact of *Triphala* on kupffer Cell Regeneration : A Possible Mechanism

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Abstract **-** As we humans are far too aware, all mammals have a finite lifespan. Apart from basic longevity, age is also a major risk factor for most of the diseases. Since stem cells are responsible for maintaining tissue homeostasis throughout an organism's lifetime, an attractive theory is that ageing-related phenotypes might be, at least in part, due to an alteration in the number and/or function of tissue stem cells. Here, we have focused on kupffer cell regeneration after ethanol cytotoxicity and possible role of cytokines and oxidative stress enzymes during the cascade along with genopreventive effect of *Triphala* in mouse bone marrow cell.

Keywords *- Triphala, Ethanol, Kupffer cells, oxidative stress.*

I. INTRODUCTION

The ''disposable soma'' theory of ageing describes a potential compromise between the damage that occurs throughout life and the effort expended to cope with this damage. This theory predicts that damage will accumulate throughout life in a stochastic fashion until eventual organismal senescence at a time point dependent on the ability of the soma to cope [7].

Somatic stem cells (SSCs) are responsible for the continual regeneration of lost and damaged cells throughout life (especially in high-turnover tissues); they are the longest-lived cells in any system and hence may be exposed to the most damage. Damage to SSCs may be particularly dangerous as they already possess many of the characteristics of malignancy, such as self-renewal, telomerase activity and chemoresistance [6].

In recent decades, many antihepatotoxic drugs have been assayed, although the development of liver fibrosis, cirrhosis and its complications (liver failure, portal hypertension and hepatocellular carcinoma) were not significantly reduced in many clinical situations.

Alcoholic liver disease is a major cause of morbidity and mortality worldwide [1]. Chronic consumption of ethanol is the major cause of liver injury and the development of serious liver diseases [2;4]. Ethanol-induced liver disease is linked with an increase in oxidative stress. Acute and chronic ethanol treatment increases the production of reactive oxygen species (ROS), lowers cellular antioxidant levels, and enhances oxidative stress in many tissues, especially the liver [3]. Oxidative stress induced by ethanol is known to play an important role in the pathogenesis of liver injury. Numerous plants have demonstrated free radical scavenging and/or antioxidative potential due to the flavonoids and other polyphenolic compounds they contain [5].

Triphala' is a commonly used Ayurvedic powdered preparation in Indian systems of medicine. This well known formulation is made by combining Amala or Amalaki, (Emblica officinalis), Bibhitaki or Behada (Terminalia belarica) and Harada or Haritaki (Terminalia chebula) in equal proportions based on the observation of Ayurvedic Formulary of India, 2002. It is considered a 'tridoshic rasayana', having balancing and rejuvenating effects on the three constitutional elements that govern human life: Vata, Pitta, and Kapha by Charaka (1,500 B.C.) in the Charaka Samhita [8] which supports nervous system, metabolism and structural integrity respectively.

II. MATERIALS AND METHODS

A. Plant Material

Fruits of *Emblica officinalis* (EO), *Terminalia belerica* (TB) and *Terminalia chebula* (TC) were purchased from local vendor and the voucher specimen (EO: Bot/Her./1130; TB: Bot./Her./892; TC: Bot./Her./8934) of the individual fruits has been deposited in Depatment of Botany, Dr. H.S. Gour Central University, for further references.

Triphala formulation (TL) was prepared by mixing the power of these three fruits in equal proportions (1:1:1) as prescribed in Ayurvedic Formulary of India [AFI].

B. Methanolic extract preparation:

The mixed powder was extracted with methanol by Soxhlet method. The alcoholic extract was evaporated to dryness by rotary vacuum evaporator, yielding semi-solid residue. This semi-solid residue was lyophilized to fine powder and the yield was 6.3% (w/w).

C. Aqeous extract preparation:

One hundred grams of *Triphala* in equal proportion was boiled in 1000 mL distilled water till the volume was reduced to one fourth of the original (250 mL). The extract was cooled; centrifuged using a cold centrifuge and the supernatant was collected and was concentrated by evaporating its liquid contents (Jagetia et al., 2002). An approximate 20% yield of the extract was obtained.

D. Animals:

Mice (F1 Hybrid) of either sex, weigh around 25±3 gm were obtained and housed in good condition in the department's animal house and given standard mouse pellet and water ad libitum. All the mice were kept at controlled light condition (light: dark, 12:12 hr) and temperature 22 ± 1º C. The use of animal was as per CPCSEA norms (CPCSEA Registration No. PBRI/DES/Ph.D/10/122).

E. Preparation of tissue sample:

Preparation of tissue sample: In the experiment, the mice were sacrificed after ethanol treatment. The blood was collected, and the liver was quickly removed at 250C. Then, the collected blood was placed 30–40 min for clot formation, and then the serum was separated by centrifugation at 4000 g for 15 min at 40C. Each liver tissue was immediately rinsed with saline, blotted on filter paper, weighed and homogenized in 1:5 volumes of PBS (KCl 0.2, KH2PO4 0.2, NaCl 8.0, Na2HPO4·12H2O 2.08 g/l, pH 7.4). One part of liver was cut and put into a flasket containing 10% buffered formalin solution for the histopathology analysis and the other for antioxidant parameters.

F. Experimental proceedure:

The animals were administered ethanol at a dose of 2g/kg BW, 20% w/v intraperitoneally. Aqueous and methanolic extract of *Triphala* (AET & MET) was given once daily for five days. Ethanol was given after half an hour of last dose of plant extract and all mice were sacrificed after 24 hrs.

- Group 1: Control (vehicle only).
- Group 2: Ethanol (2g/kg, i.p.).
- Group 3: Silymarin (25 mg/kg BW orally) + Ethanol (as in group 2).
- Group 4: MET (100mg/kg BW orally) only.
- Group 5: AET (100mg/kg BW orally) only.
- Groups 4: MET (100mg/kg BW orally) + Ethanol (as in group 2).
- Group 5: AET (100mg/kg BW orally) + Ethanol (as in group 2).

G. Liver function test:

Serum enzymes: aspartate aminotransferase (AST), alanine transaminase (ALT), serum alkaline phosphatase (ALP) were determined according to manufacturer's instructions.

H. Measurement of oxidative stress:

Assessment of oxidative stress in liver content such as lipid peroxidation (LPO), reduced glutathione (GSH) assay, superoxide dismutase (SOD) assay and catalase (CAT) assay was done by Sapakal method, 2008 [9].

I. Assessment of cytokines:

TNF-α and IL-6 level in plasma of control and experimental mice were determined by enzyme-linked immunosorbent assay (ELISA, Cayman Chemicals, USA), according to manufacturer's instructions.

J. Kupffer cells isolation [11]:

The phagocytic ability of kupffer cells was assessed by mixing liver homogenate to the incubating medium (0.1 ml of minimum essential medium (MEM), 0.1 ml of inactivated fetal calf serum and 0.1 ml of heat inactivated yeast was added and incubated at 37^0C for 15 min, followed by centrifugation. From the sediment, thin smears were made and stained with Geimsa.

K. Bone marrow Chromosomal Assay [10]:

After the dosing schedule all mice were sacrificed after 22hrs; by intraperitoneal injection of 0.025% Colchicine (2 hr incubation) by cervical dislocation. Both femurs were dissected out and metaphase plates were prepared by Air Drop Method. Briefly, bone marrow from the femur was aspirated, washed in saline, with 0.056% KCl and fixed in cornoy's fixative stained with 4 % Giemsa.

III. RESULTS AND DISCUSSION

The effect of *Triphala* was studied on serum marker enzymes in ethanol intoxicated animal. Activity of liver enzymes in mice were significantly higher than in control (ALT: 3.82±0.48vs. 17.92±1.09; AST:

31.54±2.34 vs. 44.45±1.42; ALP: 6.18±0.32 vs 10.72±0.74) as shown in Table 1. It is confirmed that *Triphala* can ameliorate hepatic function of hepatic injury induced by ethanol. In mice pretreated with *Triphala* followed by hepatotoxins; liver enzymes significantly decreased in all the doses of *Triphala* however MET-100mg was found to be more potent than the AET.

The effect of *Triphala* on oxidative stress in liver was shown in Table 1. SOD activity of the liver homogenate in ethanol was lower than that in control ($p < 0.05$) group. SOD, CAT, and GSH activities of MET and AET groups in liver homogenate were significantly decreased respectively. The observed increase of enzymes activity suggested that *Triphala* had an efficient protective mechanism in response to ROS. However AET does not produces any significant effect against ethanol toxicity. (Figure 1)

The concentration of MDA, an end product of lipid peroxidation, in the ethanol-treated mice was increased significantly to the normal control mice. On the other hand, the pretreatment of MET and AET in mice lead to lower MDA levels than the ethanol dosed group significantly ($p < 0.05$)

Following the ethanol treatment, TNF-α and IL-6 levels were significantly increased (200±0.001 pg/ml and 116.66±0.003pg/ml), while this ethanol- induced rise in serum was reduced with prior treatment of *Triphala* extract significantly. Also the kupffer cells capability of phagocytosis was significantly increased after *Triphala* consumption indirectly suggest that it is having immunostimulant activity which may be the reason of regeneration of kupffer cells. (Figure 2)

Genomic protection is the biggest task because a single or double break plays a major role to change the genomic status of an individual. However *Triphala* does not produces any significant aberration when compared to control group. However *Triphala* protect the individual aberration significantly as compare to the ethanol dosed group. (Fig.3)

Table 1. Effect of *Triphala* on oxidative stress enzymes Value expressed as ± S.E.M., n = 6. *: $P < 0.05$ compare to ethanol group, ns: non significant, # $P < 0.05$ compare to control group. (Figure 3)

SNo	Groups	LPO (nM/mg)	GSH(mM/g)
1	Control	2.39±0.50	18.73±0.98
2	Ethanol	4.99±1.60$^{\#}$	11.84±4.25$^{\#}$
3	Sly+Ethanol	2.46±0.32*	18.26±0.84*
4	MET	2.75±0.73*	16.81±6.3^{ns}
5	AET	3.94±0.87*	12.77±3.9^{ns}

SNo	Groups	CAT (U/mg)	SOD (U/mg)
1	Control	34.5±1.01	1.59±0.04
2	Ethanol	22.61±5.61$^{\#}$	0.57±0.02$^{\#}$
3	Sly+Ethanol	33.51±1.54*	1.61±0.04*
4	MET	29.18±2.93*	1.25±0.07*
5	AET	25.04±7.21^{ns}	1.14±0.13^{ns}

Table 2. Effect of *Triphala* on % Yeast digestion of kupffer cells.

SNo	Groups	% Yeast Digestion
1	Control	3.79±0.57
2	Ethanol	4.06±0.52
3	MET	4.65±0.8
4	AET	4.47±0.71
5	Sly+Ethanol	10.58±0.29
6	MET+Ethanol	9.2±1.25
7	Aet+Ethanol	8.0±1.09

Figure 1**.** Effect of *Triphala* on biochemical parameters. on methanolic and aqueous extract of Tripahla (MET/AET). Value expressed as ± S.E.M., n = 6. *: $P < 0.05$ compare to ethanol group, ns: non significant, # $P < 0.05$ compare to control group.

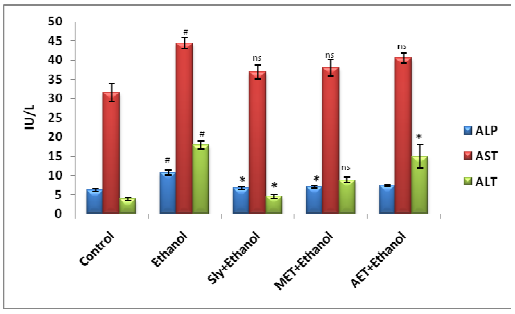

Fig. 2 : Effect of *Triphala*

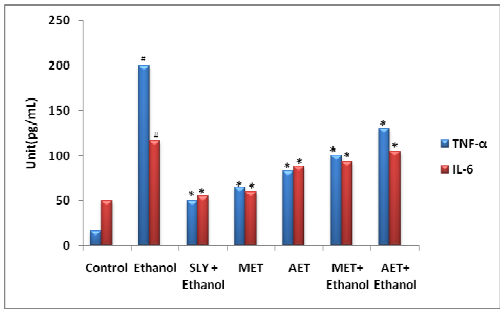

Fig. 3 : Bone Marrow chromosomal Assay

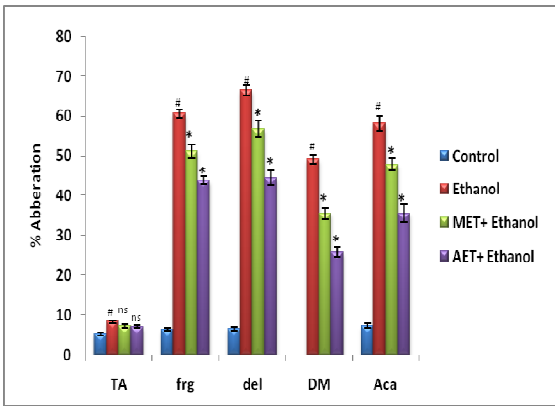

Fig. 4

IV. CONCLUSION

The liver is considered the largest and one of the most critical organs in the body and plays a crucial role in the body's metabolism. Review of literature reveals that, *Triphala* is credited with diverse beneficial properties like anti-stressor, antioxidant and immunostimulant activities which combine both the nutritional as well as detoxifying actions (blood and liver cleansing). The nutritional aspect is partly in the form of its bioflavonoids, high vitamin C content, gallic acid, linoleic oil, phospholipids and other important nutrients makes it immunomodulator and liver protective.

Approximately 80% of ingested alcohol is metabolized in the liver, so excessive alcohol consumption can lead to acute and chronic liver disease. During the metabolism of ethanol to acetaldehyde in the body, a state of oxidative stress is created by excessive ROS generation, which plays a vital role in the development of alcoholic liver disease. Therefore, this study focused on determining changes in the hepatic antioxidation defense system in association with oxidative stress, using animal models challenged with ethanol.

The present investigation indicates that AET and MET exert significant protection against ethanol-induced toxicity by its ability to ameliorate the oxidative stress enzymes system through the free radicals scavenging activity, which enhanced the levels of antioxidant defense system. Our study also showed that MET in the dose of 100mg/kg has greater effect than AET at the same dose level. Therefore MET appears to be useful in the attenuation of ethanol induced oxidation and showed more prominent effect than AET. Both the extract showed significant activity against liver damage when compared with that of standard drug silymarin. Further investigation is underway to determine the exact phytoconstituents in the extracts that are responsible for its hepatoprotective effect.

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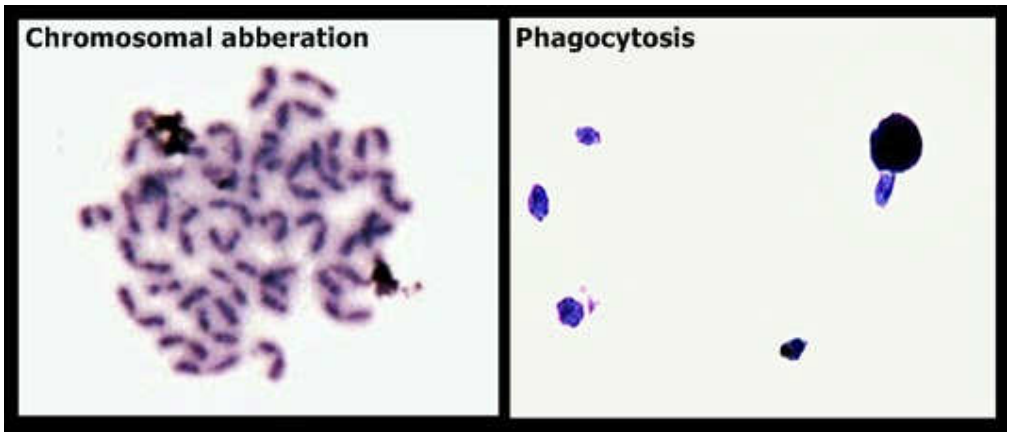

Plate No.1 Chromosomal abberaton and phagocytosis of kupffer cells.

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