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Modulatory effect of aqueous extract of amla on liver function markers of albino rats exposed to Environmental tobacco smoke

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ABSTRACT

The present investigation was carried out to study the ameliorative effect of aqueous extract of amla on liver function markers Viz., AST, ALT and ALP in Environmental Tobacco Smoke exposed albino rats. The rats were exposed to Environmental Tobacco Smoke (4 beedi/hrs/day for 28 days) shows alterations in the liver enzymes. While, Exposed to Environmental Tobacco Smoke along with supplementation of aqueous extract of amla (2mL/rat)shows improvement in the alterations of liver function markers

Key words : *Environmental Tobacco Smoke, Albino rats,Amla (aqueous extract), AST,ALT and ALP.*

Introduction

Environmental Tobacco Smoke is one of the main source of indoor air pollution. Tobacco smoking is very common in developing countries including India with beedi smoking is the main type and is harmful to smokers as well as those who are exposed to smoking. Chemical found in the beedi smoking are known for its toxicity. Nicotine, the principle pharmacological agent common to all form of tobacco is powerful addicting drug. Nicotine in beedi smoking put smokers at risk for addiction. Smoking also cause harmful effect on those children whose parents are smokers (Baker *et al*., 2006). Environmental Tobacco Smoke also contain respirable suspended particle all these enter in our body and cause adverse effects on our organs. One of the main organis liver which is not in direct contact with smoking but affected greatly. Liver is important for the process of eliminating the harmful compounds, alcohol, toxic compounds and drugs from the human body. *Emblica officinalis* commonly known as amla is a member of small genus, *Emblica* (Euphorbiaceae) is taken as an antioxidant. The fruit extract has been found to inhibit mutagenicity induced by smoking. Amla also used for the treatment of liver disorders. It has also been reported to have potent antidiabetic, hepatoprotective (Jose and Kuttan, 1995) and antibacterial properties owing to its antioxidative nature. The aim of the present study was to evaluate protective effect of amla against the hepatotoxicity caused by Environmental Tobacco Smoke in albino rats.

Materials and Methods

Preparation of aqueous extract of amla

Amla extract can be prepared according to the method given by Elobeid and Ahmed (2005).

Experimental animals

15 albino rats of equal size and weight 90-130g were used for the present study. The animal were fed

with standard laboratory chow and had free access to water under well ventilated conditions of 12hrs day and 12 hrs dark cycles. The animalswere acclimated to laboratory condition prior to experiment. The animal were divided into three set (A, B and C) each comprising five rats.

Control set A– Unexposed

Experimental set B – Exposed to Environmental Tobacco Smoke for 1 hr. / day for 28 days
Experimental set C – Exposed to environmental Tobacco Smoke (for 1 hr. /day for 28 days) along with supplementationof aqueous extract of amla (2mL/rat) with the help of gavage tube.

Albino rats of set B and C were kept in an isolated chamber for the exposure to Environmental Tobacco Smoke. The rats were subjected to whole body exposure for 1hr/day for 28 days. The smoke is dispersed into the chamber through the suction side of circulation fan. The oral administration of the amla extract was done 30 min. after the Environmental Tobacco Smoke exposure in set C. After the exposure period, i.e. 28 days the animals were sacrificed from each group and the blood was taken for serum liver enzyme analysis.

Separation of Serum

The centrifuge tubes containing blood sample were allowed to stand in slanting position for about 1hr at room temperature and were centrifuged at 2500rpm for 30 minutes.

The supernatant serum was then transferred successfully to a sterilized plain glass vials with the help of fine glass dropper for the estimation of serum enzymes viz. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP).

Serum Alanine Aminotransferase (ALT)

ALT was determined by modified UV (IFCC) kinetic assay kit method (Span diagnostic Ltd, Sachin) described by Schumann *et al*. (2002)

Serum Aspartate Aminotransferase (AST)

AST was determined by modified UV (IFCC) kinetic assay kit method (Span diagnostic Ltd. Sachin) described by Schumann *et al*. (2002).

Serum Alkaline Phosphatase (ALP)

ALP was determined by pNPP (p-Nitrophenyl phosphate) – AMP (2-amino-2 methyl -1propanol) (IFCC) kinetic assay kit method (span diagnostic Ltd, Sachin) described by Young (1997).

Results and Discussion

Data obtained in control and treatment group are summarized in Table 1. Treated group were averaged and analysed by students. 'T' test.

A number of differences were found between control rats and those in experimental rats. Statistically significant results were considered biologically relevant. AST, ALT and ALP show alternate changes in experimental rats as compared with those of control rats.

Discussion

In the present study, Environmental tobacco smoke exposure showed sign and symptoms of the hepatotoxicity in albino rats. Decrease in the serum enzyme activity nearly up to their normal range has been observed after the supplementation of aqueous extract of amla in the Environmental tobacco smoke

Table 1. Serum liver enzymes activity after exposure to Environmental Tobacco Smoke and supplementation with aqueous extract of amla after 28 days

Parameters	Control (5) Mean ±	Experimental set- B (5)	Experimental set-C (5)
Aspartate Aminotransferase (AST)	144.92 ± 1.94	159.18 ± 1.86 ↑**	159.75 ± 2.00 ↓**
Alanine Aminotransferase (ALT)	41.2 ± 2.37	56.56 ± 2.37 ↑**	52.52 ± 2.17 ↓*
Alkaline Phosphatase (ALP)	169.2 ± 3.01	177.48 ± 2.24 *	172.75 ± 1.64 **

S.Em = Standard error of mean Increase Decrease *non-significant (P>0.05)
**significant (P<0.05) ETS = Environmental tobacco smoke (5) = NO. of albino rats

exposed rats. In the present findings, Environmental tobacco smoke exposure shows significant increase in the activity of the serum liver enzymes Viz. ALT, AST and ALP in albino rat. Smoking produces chemical substances with cytotoxic potential which increase inflammation and promotes oxidative stress of hepatocytes which significantly associated with increased level of liver enzymes and liver tissue damage. Lipid peroxidation of biomembrane causes leakage of cellular components and damage of certain tissue and release of enzymes into the blood. The extent of elevation in the serum enzyme activity is proportional to damage and concentration of enzyme in the liver (Pant, 2004). Abdou *et al*. (2007) reported that in albino rats cadmium and lead increases the permeability of the cell membrane due to which the enzyme move into the blood and become elevated. Similarly, the elevation in the activity of ALT, AST and ALP associated with beedi smoke is mainly due to nitrostative stress in which the reactive oxygen species react together and damage the cells is also observed by Padmavathi *et al.* (2009) in human male.

Similar findings are also given by Wannamethee and Shaper (2010) who have reported that an increase in the level of AST, ALT and ALP is mainly due to inflammation and oxidative stress in cigarette smokers While, Yasmin *et al.* (2010) conducted a survey in which she reported that the women working in the beedi industry have high concentration of AST, ALT and ALP which is due to the presence of nicotine in the beedi that may enter from the finger and palm of the hand and may effect the liver.

Further, Supporting findings are given by Elameen and Abdrabo (2013) who have observed that the increase in the level of AST, ALT and ALP is due to combine effect of smoking and oxidative stress in the human. Farsalinos *et al.* (2013) have also reported that the elevation in the AST, and ALT in the smoker is due to effectiveness of the tobacco smoke and its harmful chemical compounds on liver cell that lead to over secretion of liver enzyme through inflammatory pathway

Present study suggests that the effects of Environmental tobacco smoke are counteracted by the supplementation of aqueous extract of amla due to antioxidant defence mechanism. Antioxidants have ability to loose electron without forming a chain reaction and they react easily with oxygen and protect the other neighbouring cell from damaging. Antioxidants reacts with oxidants in the cell cytosol and the blood plasma. While, lipid soluble antioxidant protect the cell membrane from lipid peroxidation.

The observations of the present study shows that the supplementation of aqueous extract of amla protect the cell membrane from lipid peroxidation thus mitigate hepatotoxicity in albino rats. Vitamin C is the most potent non enzymatic antioxidant as it directly scavenges the superoxide and hydroxyl radical and breakdown the hydrogen peroxide by the ascorbate peroxidase reaction which clearly explains a decrease in the lipid per oxidation in the liver of albino rats (Lykkesfeldt *et al.* (2000).

Similar to the present findings, Khandelwal *et al.* (2002) have reported that the ameliorative role of amla exert a stabilising action on cell membrane and reduce the leakage of liver enzyme into blood in rat. Perianayagam *et al*. (2004) have also reported antipyretic and analgesic activity of extract of amla. Hsu and Yen (2007) have observed that the Gallic acid present in amla significantly decrease the oxidative stress in albino rats.

Virk *et al.* (2013) also studied that the wistar rat which is exposed to cadmium along with supplementation of vitamin C show slightly better hepatic organization with more organised hepatic strand. The protective effects of amla against metal induced oxidative stress and related toxicityhave also given by Singh *et al.* (2015) in mice. Ghanwat *et al*. (2015) have also supports the present findings and stated that vitamin C have an activities of an endogenous antioxidant enzymes by scavenging the reactive oxygen species generated due to high lead level in human. Extract of amla inhibits reactive oxygen species production and retard the intracellular lipid accumulation in free fatty acid mixture generated due to high lead level observed by Lu *et al*. (2016). Similarly, Chaphalkar *et al.* (2017) stated that extract of amla restore abnormality of the liver enzymes AST, ALT and ALP.

Conclusion

Environmental Tobacco Smoke exposure are subjected to oxidative stress i:e like immunological, toxic, oncogenic effects and serum enzymes alterations, but parallel with supplementation of antioxidant aqueous extract of amla has mitigated the toxic impacts of Environmental Tobacco Smoke to a greater extent in both the sexes of albino rats.

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