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Protective Role of Aqueous Extract of Amla on the Liver Function Markers of Albino Rats Exposed To Environmental Tobacco Smoke

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ABSTRACT

The purpose of this study was to see if an aqueous extract of amla could improve liver function markers. AST, ALT, and ALP in albino rats exposed to tobacco smoke. Environmental Tobacco Smoke (4 beedi/hrs/day for 28 days) caused changes in the liver enzymes in rats. However, rats exposed to environmental tobacco smoke and given an aqueous extract of amla (2ml/rat) showed improvements in liver function indicators.

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

Introduction

Tobacco and the Environment One of the most common sources of indoor air pollution is smoking. Tobacco smoking is quite popular in developing nations, including India, with beedi smoking being the most common variety. Tobacco smoking is detrimental to both smokers and those who are exposed to it. The danger of the chemicals contained in beedi smoking is well established. Nicotine, the main pharmacological agent found in all types of tobacco, is a highly addictive substance. Smokers who smoke beedi are at danger of being addicted to nicotine. Smoking has also been shown to affect children whose parents smoke (Baker et al. 2003,). Tobacco and the Environment Smoke also contains respirable suspended particles, which enter our bodies and cause organ damage. The liver is one of the most important organs, however it is not directly impacted by smoking. The liver is necessary for the removal of hazardous chemicals such as alcohol, poisonous compounds, and medications from the human body. Emblica officinalis, often known as amla, is a tiny genus of plants in the Euphorbiaceae family that is used as an antioxidant. Smoking-induced mutagenicity has been reported to be inhibited by the fruit extract. Amla can also be used to treat liver problems. Due to its antioxidative nature, it has also been shown to have potent antidiabetic, hepatoprotective (Jose and Kuttan, 2000), and antibacterial activities. The purpose of this study was to see if amla could protect albino rats from hepatotoxicity caused by environmental tobacco smoke.

MATERIAL AND METHODS

SELECTION OF BEEDI

For Environmental tobacco smoke the beedi brand Ganesh 501 was selected for the present study. It is purchased from the local market (Fig. I).

Major toxic agents of beedi are given in Table-I (Hoffman *et al.* 2001)

Preparation of aqueous extract of amla

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

Experimental animals

For this investigation, 15 albino rats of similar size and weight (90-130g) were employed. The animals were fed conventional laboratory chow and had free access to water in a well-ventilated environment with 12 hours of light and 12 hours of darkness. Prior to the trial, the animals were habituated to laboratory conditions. The rats were split into three groups (A, B, and C), each with five rats.

Control set A – Unexposed

Experimental set B – Exposed to Environmental Tobacco Smoke for 1 hrs. / day for 28 days.

Experimental set C – Exposed to environmental Tobacco Smoke (for 1 hrs. /day for 28 days) along with supplementation of aqueous extract of amla (2ml/ rat) with the help of gavage tube.

Sets B and C albino rats were maintained in a separate chamber to be exposed to environmental tobacco smoke. For 28 days, the rats were exposed to whole body exposure for 1 hour per day. Through the suction side of the circulation fan, the smoke is spread into the chamber. The amla extract was given orally 30 minutes after the Environmental Tobacco Smoke exposure in set C. Animals from each group were slaughtered after the exposure period, which was 28 days, and blood was obtained for serum liver enzyme analyses.

SEPARATION OF SERUM

The centrifuge tube having blood sample were allowed to stand in a slanting posture for around 1 hour at room temperature before being centrifuged for 30 minutes at 2500 rpm.

With the use of a fine glass dropper, the supernatant serum was effectively transferred to sterilised plain glass vials for the estimation of serum enzymes such as 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-2methyl -1propanol) (IFCC) kinetic assay kit method (span diagnostic Ltd, Sachin) described by Young (1997).

Results and Discussion

Table III and Fig. 3,4 and 5 summarises the results from the control and treatment groups. Students averaged and analysed the treated group. The ‘T' test.

There were a number of variations between control rats and experimental rats. Biologically relevant results were those that were statistically significant. The levels of AST, ALT, and ALP in experimental rats differ from those in control rats.

Discussion

In this study, albino rats exposed to environmental tobacco smoke displayed signs and symptoms of hepatotoxicity. After feeding with aqueous extract of amla, serum enzyme activity in the Environmental cigarette smoke exposed mice decreased close to their normal range. Environmental tobacco smoke exposure causes a considerable rise in the activity of the serum liver enzymes ALT, AST, and ALP in albino rats, according to the data. Smoking produces chemical compounds with cytotoxic potential, which enhance inflammation and induce oxidative stress in hepatocytes, which is linked to elevated liver enzyme levels and liver tissue damage. Lipid peroxidation of the biomembrane results in the leakage of cellular components, tissue destruction, and the release of enzymes into the bloodstream. The extent of an increase in serum enzyme activity is related to the amount of damage and enzyme concentration in the liver (Pant, 2004). According to Abdou et al. (2007), cadmium and lead increase the permeability of the cell membrane in albino rats, causing the enzyme to migrate into the bloodstream and become increased. Padmavathi et al. (2009) found that the increase in the activity of ALT, AST, and ALP related with beedi smoke is primarily due to nitrostative stress, in which reactive oxygen species react together and harm the cells. According to Lerner et al. (2009), cigarette smoke produces reactive oxygen species, which cause oxidative damage in rats' livers.

Wannamethee and Shaper (2010) found similar findings, claiming that a rise in the levels of AST, ALT, and ALP in cigarette smokers is primarily attributable to inflammation and oxidative stress. While Yasmin et al. (2010) performed a survey and found that women working in the beedi sector have elevated AST, ALT, and ALP levels, which she attributes to the presence of nicotine in the beedi, which can enter through the finger and palm of the hand and affect the liver.

Elameen and Abdrabo (2013) provide additional evidence, stating that the increase in the levels of AST, ALT, and ALP in humans is due to a combination of smoking and oxidative stress. According to Farsalinos et al. (2013), the increase in AST and ALT in smokers is due to the impact of tobacco smoke and its toxic chemical compounds on liver cells, which leads to oversecretion of liver enzymes via the inflammatory pathway.

The current study reveals that supplementing with aqueous extract of amla can offset the effects of environmental cigarette smoke through an antioxidant defence mechanism. Antioxidants have the ability to lose electrons without initiating a chain reaction, and they react quickly with oxygen to protect the cells around them.

Vitamin C is the most powerful non-enzymatic antioxidant because it directly scavenges superoxide and hydroxyl radicals and breaks down hydrogen peroxide via the ascorbate peroxidase process, which explains why lipid peroxidation in albino rats' livers is reduced (Lykkesfeldt et al). (2000).

Khandelwal et al. (2002) found that amla has a stabilising effect on cell membranes and reduces liver enzyme leakage into the blood in rats, which is similar to the current findings. Perianayagam et al. (2004) discovered that amla extract had antipyretic and analgesic properties. Gallic acid found in amla was found to significantly reduce oxidative stress in albino rats by Hsu and Yen (2007).

Virk et al. (2013) also discovered that wistar rats exposed to cadmium and given vitamin C had somewhat improved hepatic organisation, with more organised hepatic strands. Singh et al. (2015) investigated the preventive benefits of amla against metal-induced oxidative stress and associated damage in mice. Ghanwat et al. (2015) agree with the current findings, claiming that vitamin C acts as an endogenous antioxidant enzyme, scavenging reactive oxygen species produced by elevated lead levels in humans. Lu et al. found that amla extract reduces reactive oxygen species formation and delays intracellular lipid buildup in a free fatty acid combination created by high lead levels (2016). According to Chaphalkar et al. (2017), amla extract restores abnormalities in the liver enzymes AST, ALT, and ALP.

Conclusion

Environmental Tobacco Smoke exposure causes oxidative stress, which causes immunological, toxic, and oncogenic effects, as well as changes in serum enzymes, but supplementing with an antioxidant aqueous extract

of amla mitigated the toxic effects of Environmental Tobacco Smoke to a greater extent in albino rats of both sexes.

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Table – III 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

Table I : MAJOR TOXIC AGENT OF BEEDI

Agents	**Concentration**
Tar	23-30mg
Carbon monoxide	18.9mg
Nicotine	2.61mg
Hydrogen cyanide	903µg
Phenol	249µg
Ammonia	284µ

m + p cresol	139µg
Benzoanthracene	117ng
Benzopyrene	78ng
p-ethyl phenol	41.6ng
Isoprene	533ng
Acrolein	67ng
Aerosol	55.3ng
2,4 dimethyl phenol	27.6ng
Heavy metals	15.6ng

Fig. 1 Beedi 501

Table II : CONTENTS OF AMLA (Charamkar and Singh, 2017)

Chemical components	**Concentration**
Fruit moisture	**0.2%**
Protein	**0.5%**
Fat	**0.1%**
Mineral matter	**0.7%**
Carbohydrates	**14.1%**
Fibre	**3.4%**
Vitamin C	**600mg/100mg**
Iron	**1.2mg/100mg**
Calcium	**0.85%**
Phosphorous	**0.02%**
Vitamin E	**14%**

Fig. 2 Amla Fruit

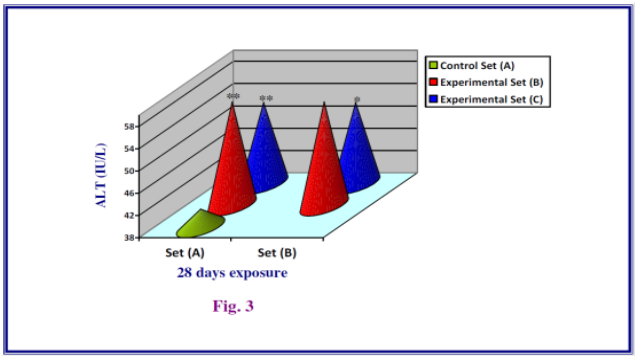

Fig. 3

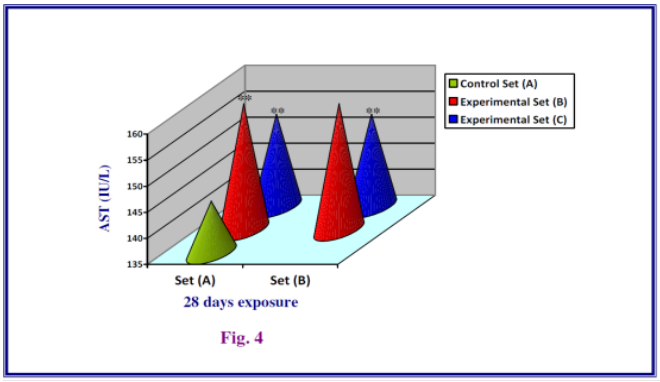

Fig. 4

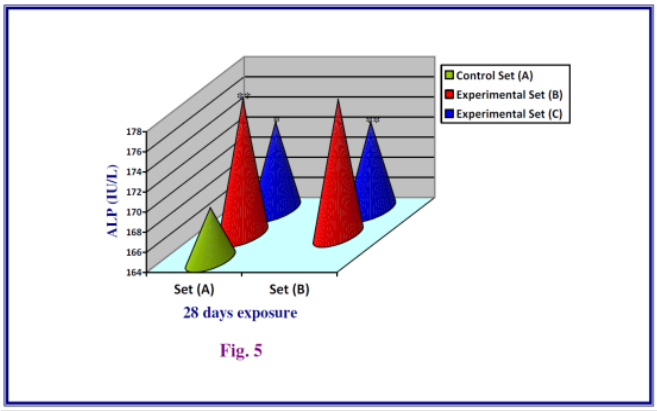

Fig. 5