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Hepatomodulation of Cigarette Smoke Toxicity by *Terminalia arjuna* Stem Bark in Rats

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Abstract: ***The aim of the present study was to analyze the effect of alcoholic extract of Terminalia arjuna (arjuna) on histological changes in liver of cigarette smoke exposed albino rats. The rats were grouped into three sets- control (I) and two experimental sets (II and III) consisting five rats each. Rats of set (I) was unexposed to cigarette smoke, set (II) was exposed to cigarette smoke for one hour/day for 30 days and set (III) was exposed to cigarette smoke with oral administration of alcoholic extract of Terminalia arjuna for 30 days. Histology of liver tissue in cigarette smoke exposed rats showed varying pattern of histological alterations such as hepatocytes degeneration, hepatocytes necrosis, centrilobular necrosis, expanded sinusoidal spaces and cellular debris. After oral administration of alcoholic extract of Terminalia arjuna, all of such histopathological alterations have been mitigated in comparison to cigarette smoke exposed rats.***

Keywords: albino rats, cigarette smoke, histology of liver, *antioxidant Terminalia arjuna*

1. Introduction

Cigarette smoke has enormous negative health consequences worldwide and the use of tobacco is still rising globally. Cigarette smoke produced by incomplete combustion of tobacco which generates a high free radicals load *in vivo* as reactive oxygen species (ROS). It can easily initiate the lipid peroxidation of the membrane of phospholipids, lipoprotein by propagating a chain reaction cycle [1]. Oxidative stress occurs when free radical formation exceed and the defense promoted by the antioxidant agents. Cigarette smoking increases the expression of several enzymes such as cytochromes and other enzymes involved in drug metabolism, especially in the liver [2]. Liver is responsible for detoxifying the poisonous substances in the body transforming and removing toxin and wastes [3]. Any injury to liver can result in many disorders ranging from transient elevation in liver enzymes to life threatening liver cirrhosis and hepatic failure [4].

Terminalia arjuna commonly known as arjuna belongs to the family combretacea, is a large evergreen tree. It has been used in Indian system of medicine for the cure of number of diseases from thousands of years. The bark of this tree has been commonly used in ayurvedic preparations to bring hepatoprotective effect in drug [5]. Therefore the present study was designed to investigate the effect of alcoholic extract of *Terminalia arjuna* bark on histology of liver in cigarette smoke exposed albino rats.

2. Materials and Methods

The wistar albino rat *Rattus norvegicus* (Berkenhout) of both the sexes have been selected for the present study. Healthy and adult albino rats (150-200g) were kept in polypropylene cages and maintained at standard laboratory conditions of temperature 21±0.5^{0}C and relative humidity 60±5% with a photoperiod 12 hours/day. The experimental protocol was in accordance with institutional ethics committee. The rats were fed on commercial food pellets (Golden feeds, New Delhi) and water *ad libitum*. The experimental animals were acclimatized for one week prior to experiment.

- ***Selection of cigarette***: The capston pilot (a filtered cigarette of 64mm length), ITC limited, Kolkata was selected for the present study.
- ***Plant material and extraction***: The fresh bark of *Terminalia arjuna* was collected from the botanical garden. The shade dried *Terminalia arjuna* bark was coarsely powdered and 200g of coarse powder was refluxed with 50% v/v ethanol for three hours using soxhlet apparatus. The extract was filtered and evaporated in a vacuum evaporator. The amount of residue remained (20%w/w) was obtained and stored in glass bottle at 4^{0}C [6] and was redissolved in distilled water when used.
- ***Experimental design***: The albino rats were grouped into three sets one control (I) and two experimental sets (II and III) containing five rats each.
- Control set (I) unexposed to cigarette smoke. Experimental set (II) exposed to 6 cigarette/hour in a day (1 cigarette/10 minutes) for 30 days. Experimental set (III) pre-treated with oral administration of alcoholic extract of *Terminalia arjuna* (5mg/100g b.w.t) before 30 minutes then exposed to cigarette smoke for one hour per day for 30 days.
- ***Exposure to cigarette smoke***: Mini exposure cabinet (60cmx30cmx30cm) manufactured by precision instrument, Varanasi is used for the cigarette smoke exposure. The experimental rats were kept in an isolated smoke chamber with their cages for whole body exposure to cigarette smoke of a filtered cigarette (6 cigarettes/hour in a day) for 30 days.
- ***Tissue collection***: The control and experimental rats were anesthesized and dissected. Liver was took out for the histopathological study.
- ***Histopathological study***: For histopathology, the liver tissue of rats were fixed in 10% formalin and dehydrated through a series of ethanol solution. The tissue were embedded in paraffin wax and sections (5µm) were cut. Sections were double stained and examined under light microscope for histological changes.
- ***Statistical analysis***: The results were expressed as Mean±S.Em. were signified by using student 't' test. The statistical calculations were carried out by using one way

ANOVA with the help of computer statistical programme KpKy plot (version 3.0).

3. Results and Discussion

Histological observations of liver tissue of control rats showed central vein, binucleated hepatocytes cells and sinusoids (Plate I). Exposure to cigarette smoke histopathological profile of liver showed degeneration of hepatocytes, hepatocytes necrosis, cellular debris, expanded sinusoidal spaces and centrilobular necrosis (Plate II) in comparison to control rats. After oral administration of alcoholic extract of *Terminalia arjuna* histological analysis of liver tissue showed prominent recovery in the form of liver architecture reduced centrilobular necrosis, degeneration of hepatocytes, normal sinusoidal spaces. Hepatocytes as restored to almost their normal texture (Plate III). Nicotine from cigarette smoke induced oxidative stress both *in vivo* and *in vitro* and depleted antioxidant defense mechanism through reduction of glutathione peroxidase in liver [7] and cause histopathological changes in liver [8]. This liver injury occurring from cigarette smoking is probably by enhancing lipid peroxidation [9]. Cigarette smoke induces lipid peroxidation in all the tissues, toxic compounds, including free radicals, occur mainly in liver and the metabolite from liver diffuses into various extra hepatic tissues causing lipid peroxidation and cell injury. Cigarette smoke increases the level of some enzymes such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase which are capable of inducing alterations in membrane permeability properties of the liver [10]. AST and ALT are markers of hepatocellular injuries [11]. Nicotine is a major component of cigarette smoke is mainly metabolized by the liver and induces lesions characterized by steatosis and focal or confluent necrosis [12]. The changes that occurred in the liver enzymes and endogenous antioxidant corroborated the histological observations of the liver that showed various degrees of alterations of hepatocytes. There were reduction in nuclear size, numerous vacuolation and area of necrosis and more damage of hepatocellular degeneration of liver parenchyma in rats [13]. Cigarette smoke generates partial containing heavy metals such as cadmium, zinc and lead. Lead toxicity are associated with necrosis, mild degree of hydropic degeneration and cytoplasmic swelling of hepatocytes [14].

The activities of GOT, GPT and ALP increased it could be release and leakout these enzymes from the liver cytosol in the blood stream which gives an indication of hepatotoxic effect [15]. Consequently, the biochemical perturbations seem to be correlated with the liver histological alterations such as presence of cellular debris and central vein [16]. Cadmium induced changes are characterized by enlarged nuclei, hepatocytes necrosis, hepatocytes vacuolization and hepatocytes with dilated central vein [17]. Smoke extract of tobacco nicotine may have acted indirectly through generation of high levels of ROS or directly as toxin to liver there affecting their cellular and disruption of hepatocytes [18]. *Terminalia arjuna* which is used as an antioxidant and also known as adaptogens. It has antioxidant and hepatoprotective activity. Its bark possesses glycosides, large quantities of flavonoids, tannins and minerals [19]. Falvinoids function as terminators of free radicals by donation of electrons to form stable products [20]. The bark extract treated through back cellular arrangement around the central vein and reduced necrosis. It helped to bring the blood vessels to normal condition [21]. *Terminalia arjuna* extract may exhibit its activity by blocking oxidative damage through lipid peroxidation and protein oxidation which must have enabled the prevention of the loss of membrane permeability and dysfunction of cellular protein and inturn, a decrease in the endogenous level of hydroxyl radical [22].

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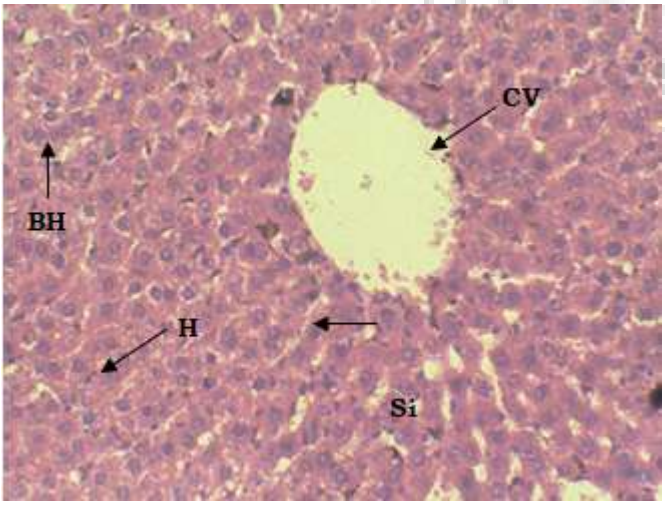

Plate I: control

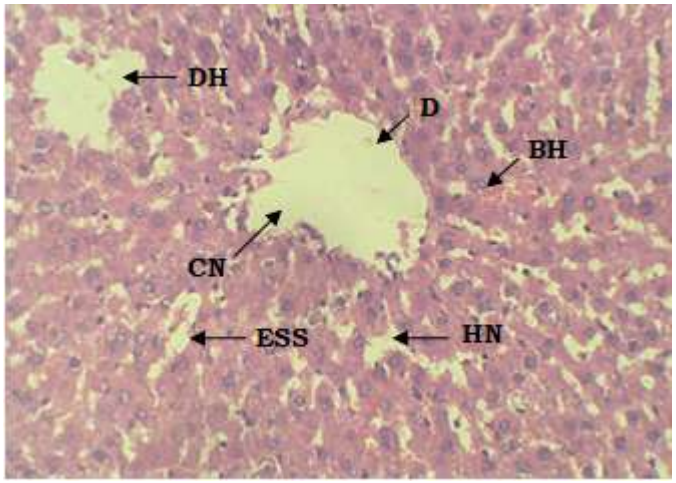

Plate II: exposure

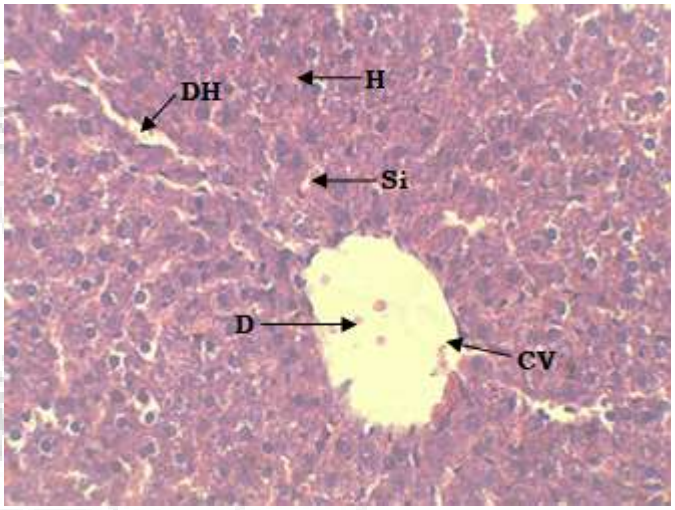

Plate III: Alcoholic+exposure

BH – Binucleated hepatocytes
H- hepatocytes
CV- central vein
Si- sinusoids
DH- degenerated hepatocytes
D- cellular debris
CN- central necrosis
ESS- expanded sinusoidal space
HN- hepatocytes necrosis