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EFFECTS OF ASHWAGANDHA (*WITHANIA SOMNIFERA*) ROOT EXTRACT ON ARCHITECTURE OF LIVER TISSUE AGAINST GENTAMICIN INDUCED HEPATOTOXIC RATS

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ABSTRACT

Prolonged use and higher doses of drugs, and some toxins produce hepatotoxicity. Ashwagandha (*Withaniasomnifera*) have free radical scavenging activity. It can be used as a hepatoprotective agent. This study was carried to observe the effect of Ashwagandha root extract on histology of liver against gentamicin induced hepatotoxic Wistar albino rats. Thirty-five rats were divided into control and experimental group. Control group was again subdivided into baseline control and gentamicin treated control group.Each of this group received standardized pellet for 22 consecutive days.In addition, gentamicin treated control and experimental group received gentamicin subcutaneously (100mg /kg body weight/day) for the last eight consecutive days. Experimental group also received Ashwagandha root extract (500mg/kg body weight/day; orally) for 22 consecutive days. All the animals were sacrificed on the 23rd day. Histology of liver revealed normal histological findings in 84.62% of experimental group. In conclusion, it was found that Ashwagandha may have protective effect against gentamicin induced hepatotoxicity.

KEYWORDS: **Histology, Hepatoprotective, Ashwagandha, Gentamicin.**

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INTRODUCTION

Liver is the main target of drug toxicity, because it is main site of metabolism and elimination of foreign substances[1]. Drug induced hepatotoxicity is a frequent cause of liver injury[2]. Drugs can cause hepatotoxicity through mitochondrial dysfunction or by hampering mitochondrial energy production. Gentamicin is a broad spectrum antibiotic belonging to the amino glycosides group, very effective in treating life threatening gram-negative bacterial and hospital acquired infection[4]. This drug causes generation of reactive oxygen species (ROS), which induces cell injury and necrosis via lipid peroxidation[5]. Gentamicin causes nephrotoxicity . Recently, Noorani *et al*. (2011),Esmatparast Mand Amniattalab A (2008) andKhan MR,Badarl and Siddiquah A (2011) studied the hepatotoxic effects of gentamicin[6,7,8]. *Withania somnifera*, known as ashwagandha / Winter cherry/ Indian ginseng, belongs to the family of Solanaceae widely used in the Ayurvedic medicine[9]. Leaves, fruits, seeds, shoots and roots of this plant have all been used traditionally as well[10]. The roots of *Withania somnifera* contained 35 chemical constituents[11]. Withaferin A and withanolides, the active ingredients contribute to the most of the biological actions of Withania[12]. Roots of this plant have some therapeutic effects[13]. Till today, no side effects have been found in ashwagandha[14]. The researchers found that root of *Withania somnifera* significantly inhibits the generation of free radicals and enhancing antioxidant system to provide protection against hepatotoxicity[15, 16, 17]. Liver failure is a medical emergency. It is a continuing challenge with consequence of high morbidity and mortality in the world. In absence of reliable hepatoprotective drugs in modern medicine, Ayurvedic medicinal preparation can be used as hepatoprotective agents. Therefore, the present study has been designed to observe the hepatoprotective role of ashwagandha (*Withania somnifera*) in experimental animals after inducing hepatotoxicity by gentamicin.

MATERIALS AND METHODS

Experimental animal:A total number of 35 healthy Wistar albino male rats, weighing between 150 to 200 grams, age range from 90 to 120 days were used. The animals were purchased from animal house of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Prior conducting the study, the animals were acclimatized for 14 days at 23 ± 2^0 C room temperature under 12 hour dark- light cycle. During this period, they had free access to food and water ad libitum.

Plant material;Ashwagandha is cultivated and harvested in the Ayurvedic nursery of Hamdard Laboratories, Meghna, Bangladesh.After collecting the roots of this plant, it was dried in sunlight for 2 days, crushed in an electric grinder into powder. Then the powder was extracted in methanol, filtered, evaporated by rotary evaporator and dried. After that, the dried root extract of ashwagandha was dissolved by propylene glycol (2ml/kg body weight) and finally mixed with distilled water for feeding. Methods: This experimental study was conducted between 1st July 2010 to 30th June 2011 in the Department of Physiology, SSMC, Mitford, Dhaka, Bangladesh. Ethical permission was taken from the Institutional Ethics Committee (IEC) of SSMC, Dhaka. After acclimatization animals were divided into two groups. , control group (Group A) consisted 20 rats and experimental group (Group B- Ashwagandha pretreated and gentamicin treated group) consisted 15 rats. Control group was again subdivided into group A_1 (baseline control) and group A_2 (gentamicin treated control group).Each of this group contained 10 rats. All groups of animals received standardized pellets for 22 consecutive days.Group A_2 also received gentamicin subcutaneously (100mg /kg body weight/day) for the last eight (15th to 22nd day) consecutive days. Again, experimental group received an ashwagandha root extract (500mg/kg body weight/day; orally) in the morning before giving food for 22 consecutive days and gentamicin subcutaneously (100mg/kg body weight /day) for last eight (15th to 22nd day) days. After giving gentamicin and ashwagandha all the animals, including baseline control rats, were anaesthetized with the help of chloroform and sacrificed on 23rd day. The liver samples were collected. Liver was washed with ice cold saline and preserved in 10% formalin for histological processing. Histopathology of liver was also being done by using standard laboratory procedure in the Department of Pathology, SSMC.[18] Statistical analysis were done by one way ANOVA, Bonferroni test, Fisher's exact test by using SPSS windows, version 16.

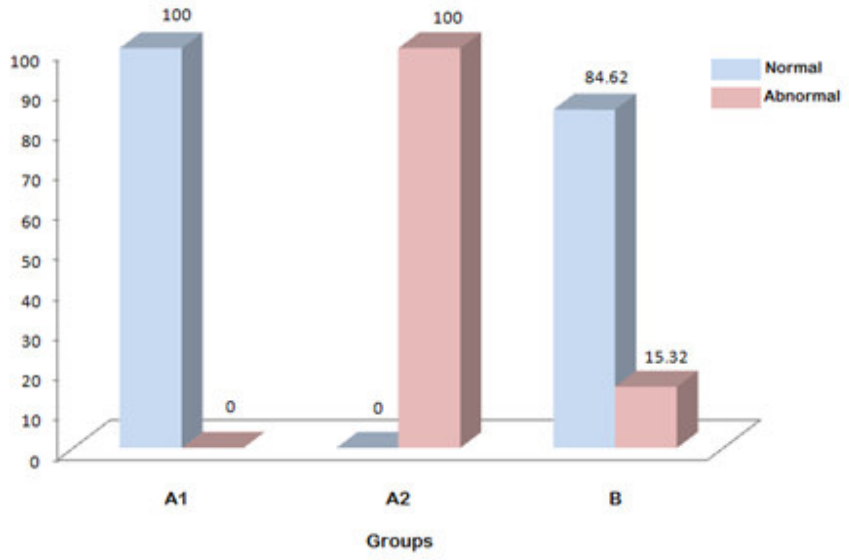
RESULTS

Histological examination of liver(Table I) revealed normal findings (Figure-1) in 100% of rats in group A_1, whereas moderate histological changes such as centrilobular necrosis (Figure-2), cellular infiltration (Figure-3) were observed in 100% of rats in group A_2. Again, 84.62%of rats in group B showed almost normal structure (Figure-4) whereas 15.38% of them showed mild histological changes in the liver. Though the percentage of histological changes were higher in group B than that of A_1, but the difference was not statistically significant (Figure 1).

Table 1
Histological observation of liver in different groups of rats (n=31)

Group	Observation	Result/findings
Group A_1 (n=9) (Baseline control group)	Architecture of - Hepatocytes - Central vein Orientation of - lining epithelium	Normal histological findings
Group A_2 (n=9) (Gentamicin treated control group)	- Centrilobular necrosis - Loss of lining epithelium. - Infiltration of lymphocytes. - Distension of central vein - Fatty changes	Moderate histological changes
Group B (n=13) (Ashwagandha pretreated and gentamicin treated group)	Restoration of normal architecture of hepatocytes and central vein. - Less/ absence of centrilobular necrosis. - Less/absence of loss of lining epithelium - Less/absence of lymphocytic infiltration. - Less/absence of fatty changes	Normal histological findings in 11 rats, but presence of scanty lymphocytic infiltration formation in 2 rats.

Graph 1
Distribution of different groups of rats by histological changes in liver (n=31)



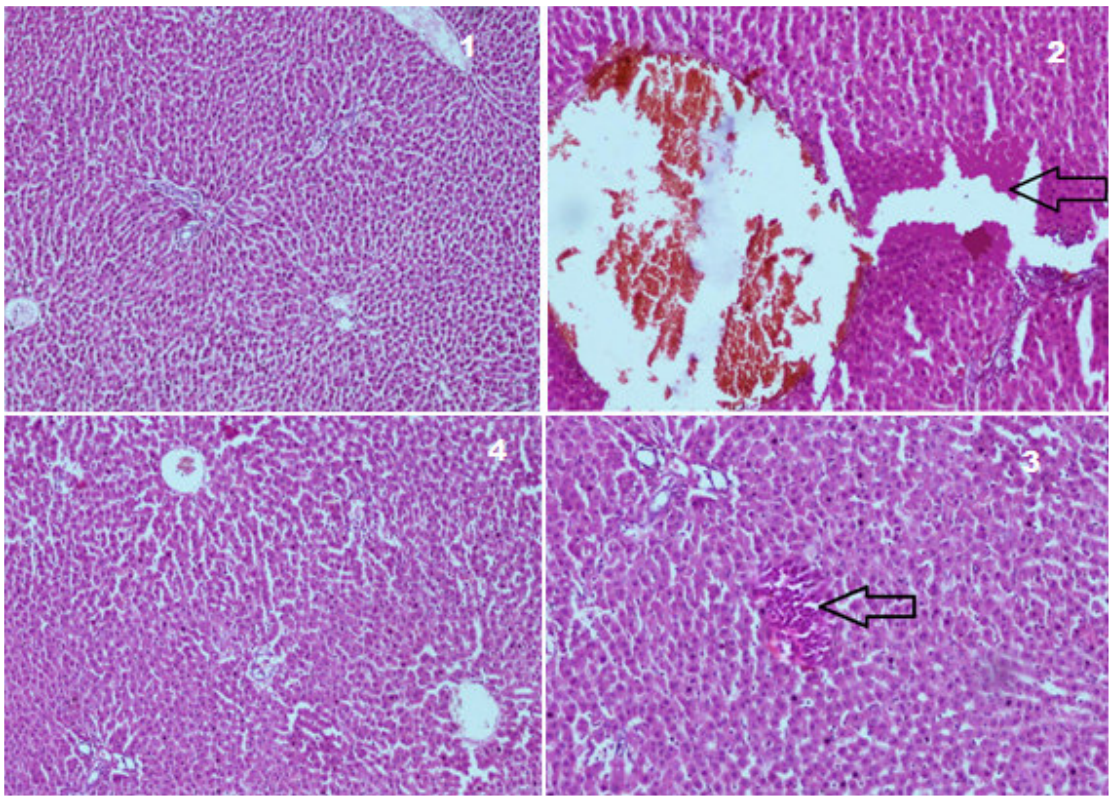


Figure 1

To :Histology slides showing haematoxylin and eosin stained hepatocyte. 1: Architecture of liver of baseline control rats (X 100);2:Centrilobular necrosis (arrow) in liver of gentamicin treated control rats (X 400),3: Cellular infiltration (arrow) in liver of gentamicin treated control rats, (X 400).4: Improvement of necrosis and other changes in liver of ashwagandha pretreated and gentamicin treated rats (X 100).

DISCUSSIONS

Moderate histological changes such as centrilobular necrosis, infiltration of lymphocytes, distension of central vein, fatty changes of liver were observed in this study in gentamicin treated control group. These changes in liver may be due to increased production of free radicals and are also in agreement with those of Noorani et al. (2011), Esmatparast Mand Amniattalab A (2008) and Khan MR,Badarl and Siddiquah A (2011). [6, 7, 8] Again,84.62% of the rats in experimental group were show almost normal liver architecture whereas, only minimal histological changes of liver were noted in 15.38% of rats in the same group of this study. Akbarsha MA et al. (2000),Mendhe MS(2009) and Elberry AA (2010) observed the similar type of finding.[15, 16, 17] It has also been suggested that, gentamicin causes alteration in cellular structure specially mitochondria, seem to enhance generation of free radicals and lipid peroxidation in liver.[8]The active principles of ashwagandha, sitoindosidesVII- X and withaferin A, have antioxidant activity by increasing the free radical scavenging enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).[9,12]So, it may improve the histological architecture of liver.[15,16,17] Ashwagandha root extract contained withanolides, which inhibit cyclooxygenase enzymes, lipid peroxidation and proliferation of tumor cells. Thus, it preferentially reduces inflammatory process by inactivating nuclear factor –kappa B (NF-KB) activation. This anti-inflammatory property may be helpful in protecting liver damage.[19] In the present study, hepatotoxicity was observed in rats treated with gentamicin as evidenced by moderate histological changes of liver. Restoration of architecture of liver cells in ashwagandha pretreated and gentamicin treated group provides a direct evidence of hepatoprotective effect of this root extract due to free radical scavenging activity of ashwagandha. However, the exact mechanism involved in the hepatoprotectiveactivity of ashwagandha (*Withania somnifera*) root extract against gentamicin induced hepatotoxicity in rats requires more detailed experiments.

CONCLUSION

Ashwagandha (*Withania somnifera*) root extract restored normal function and architecture of liver due to its inhibition of generating and scavenging free radicals. It is also expected that the result of this study would make the ashwagandha acceptable among the people as a rich source with medicinal value for the prevention of liver damages.

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