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Protective effect of *Withania somnifera* (L) against DEHP induced in hepatic tissues of *Channa punctatus*(BLOCH)

Swati Soren, Abhijit Dutta, Sweety Kumari, Musarrat Naaz and Abhijeet Ghosh

University Department of Zoology, Ranchi University, Ranchi, India

ABSTRACT

This study was designed to investigate the protective effect of *Withania somnifera*(L)(Ashwagandha)root extract on liver tissue damage caused by DEHP. Experimental animals were grouped into two i.e. Control and DEHP treated. Exposed group was treated with 0.4 ug/l of di-ethylhexyl phthalate for 3 months. After 3 months the changes were observed both histologically and ultrastructurally. *Channa punctatus* showed the toxic effects of the toxic agent DEHP causing complete dilation of hepatocytes.fish of DEHP group showed severe degenerative and necrotic changes in most hepatocytes. Necrosis detectable in the form vacuolated cytoplasm with pyknotic, karyorrhetic or karyolytic nuclei. Some hepatocytes showed nuclei with chromatin margination and dark eosinophilic cytoplasm which was seen in light microscope as well as ultrastructurally changes showed swollen mitochondria, degenerated nucleus and total loss of membrane integrity observed condition shows necrosis with loss of ER, nucleus ,mitochondria. However, Ashwagandha treated group(ART GR) showed signs of ameliorated condition with rejuvenation of nuclues , ribosomes , endoplsmic reticulum. The treated group was then fed with ashwagandha root extracts of 300mg/kg for 2 months. When ashwagandha was administered for 2 months damages seems to be recoverd showing quite normal mitochondria, nucleus in normal state.

Keywords: DEHP, Hepatocytes, Necrosis, Ashwagandha, Amelioration

INTRODUCTION

Phthalates, or phthalate esters, are a group of chemical compounds that are mainly used as plasticizers i.e. substances added to plastics to increase their flexibility . Phthalic acid esters are presently being used in amounts and products that can easily, although inadvertently, contribute to environmental pollution. Phthalates are not tightly bound into plastics and so can migrate into the environment during the life of the product or in the landfill sites after disposal .They are also released from numerous industrial sites. They have a tendency to accumulate in fatty tissue. When released into the wider environment, they are adsorbed onto sediments and may not breakdown for long time. 1,2-Benzenedicarboxylic acid esters, which are commonly denoted as phthalates, form a group of compounds that is mainly used as plasticisers for polymers such as polyvinylchloride (PVC). Other areas of application are adhesives, paints, films, glues, cosmetics, and so forth. The number of potential different phthalates is infinite. Despite only a few phthalates are produced at the industrial scale, the annual production of phthalates was estimated by the World Health Organisation (WHO) to approach 8 million tons [1] . The most important congeners are in that respect DEHP, which accounts for about 50 % of the world production of phthalates, DIDP, and DINP. Due to their widespread application phthalates have become ubiquitous in the environment, e.g. Hubert et al. estimated the release of DEHP to the environment to about 1.8 % of the annual production [2]. Estimated annual production in the United States (U.S.) of total dioctyl phthalates of which an estimated 90% was DEHP was 300 million pounds in 1985 to 1990, 340 million pounds in 1977 and 250 million pounds in 1982 (ATSDR, 1993, citing Mannsville Chemical Products Corporation and HSDB). Production facilities were described by ATSDR (1993) in Pennsylvania, New Jersey, Tennessee and Maryland; no production facilities in California were listed. Imports of six million pounds in 1988, and exports of 10 to 40 million pounds annually in 1980 to 1990 were also noted. To

define background levels in industrial, urbanized and rural regions numerous monitoring studies have been conducted in different parts of the world. The available monitoring data have been summarized within the EU RAR (ECB, 2008) [3]. Higher exposure levels were detected in samples of urban and/or industrial areas.

The liver plays many roles in whole body function, such as the control and/or synthesis of critical blood constituents including glucose, free-fatty acids, ketone bodies, amino acids, hormones, clotting factors, and inflammatory mediators. The liver is critical in immune function [4], and a first line of defense against certain infectious organisms and toxins entering from the gastrointestinal tract, to which it is intimately linked functionally, embryologically, and evolutionarily. DEHP has been observed to induce teratogenesis in rodents [5,6]. Moreover toxic effects on reproductive organs and liver of rats have been described after oral administration [7]

The liver is the largest internal organ in the human body, weighing three to four pounds. The rich supply of blood flowing through it gives it its dark red color and glossy appearance. Sometimes called "The Great Chemical Factory" the liver neutralizes harmful toxins and wastes, stores glycogen (a blood-sugar regulator), amino acids, protein, and fat. Environmental toxins and over-processed foods which are infused with many unnatural chemicals leave the liver at great risk for contamination. If the liver is not functioning well, a hazardous buildup of toxins may occur.

At the end of the treatment period the test animals were subjected to amelioration with ashwagandha.

Plants are one of the most important sources of medicines in world. According to the World health organization, traditional medicines are widely used in India. Approximately 80% of the populations of developing countries rely on traditional medicines for their primary health care needs[8-10].

Withania somnifera, also known as ashwagandha, Indian ginseng, and winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. Historically, the plant has been used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent, and more recently to treat bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia. Clinical trials and animal research support the use of ashwagandha for anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease. Ashwagandha's chemopreventive properties make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. Ashwagandha is also used therapeutically as an adaptogen for patients with nervous exhaustion, insomnia, and debility due to stress, and as an immune stimulant in patients with low white blood cell counts. to stress, and as an immune stimulant in patients with low white blood cell counts. The present review describes ashwagandha (*withania somnifera*) and its active compounds, mechanism of action and biological chemistry and classical beneficial applications of ashwagandha in biomedicine and veterinary sciences viz., immunomodulatory effects, activity against microbes and infection and usefulness as an alternative, chemotherapeutic agent, general health benefits, promoting vigour and vitality, stress reliever antidepressant, anti-inflammatory and adaptogenic property, effects on cardiovascular system, role in treating sexual disability, disease and disorders, potent anti-cancer effects, reducing poisoning due to toxins/chemicals/drugs, anti-aging activities, memory enhancer treating neurodegenerative disorders, role in development of drug tolerance and dependence.

MATERIALS AND METHODS

The experiment was cleared by the Ethical committee, Ranchi University, Ranchi, for conducting research on fish *Channa punctatus*.

Procurement of Experimental Animals

Live specimen of *Channa punctatus* were procured from local markets of Ranchi. They were allowed to acclimatize to the laboratory aquaria for 15 days. Fishes were grouped into control fishes (CF), DEHP treated (DT) respectively.

Treatment Protocol

The DT group was treated with 0.4mg/litre in tap water. In each group 30 fishes were examined. After 3 months of exposure, DT group were then continued with ashwagandha root extract of 300ml/2L for another 2 months to check its ameliorating property and it was grouped under ART group. The ashwagandha root was collected almost 30kg and it was left for sun drying for 2 days. Then it is was crushed and powdered. It was then boiled in 10ltrs of normal tap water. Further cooled and filtered with whatmann filter paper. The filtered was used for ART group. It was administered 300ml/2L of water.

At the end of the experiment, fishes from both the groups were sacrificed.

The fragments from harvested tissues were fixed in BOUIN'S fixative, embedded in paraffin, stained and observed for histological analysis under light microscope which was stained in Hematoxylin and Eosin (H&E). Another portion was fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer and processed to observe its ultrastructure by Morgagni 268D FEI company transmission electron microscope (Holland) at AIIMS, New Delhi.

RESULTS

In the present investigation *Channa punctatus* showed several alteration related to exposure of DEHP as compared to CF controlled fishes in all the parameters of experimental protocol e.i. Histologically as well as Transmission Electron Microscopically.

Histology is an essential tool of biology and medicine. It is commonly performed by examining cells and tissues under a light microscope.

Generally, no changes were detected in mice in the control group. Under light microscope Control Fish(GR1) Hematoxylin & Eosin (H&E) sections of liver showed normal polygonal hepatocytes with round nuclei and granular cytoplasm. Blood sinusoids and Kupffer cells were detected (Fig.1), portal vein and central vein was also observed.

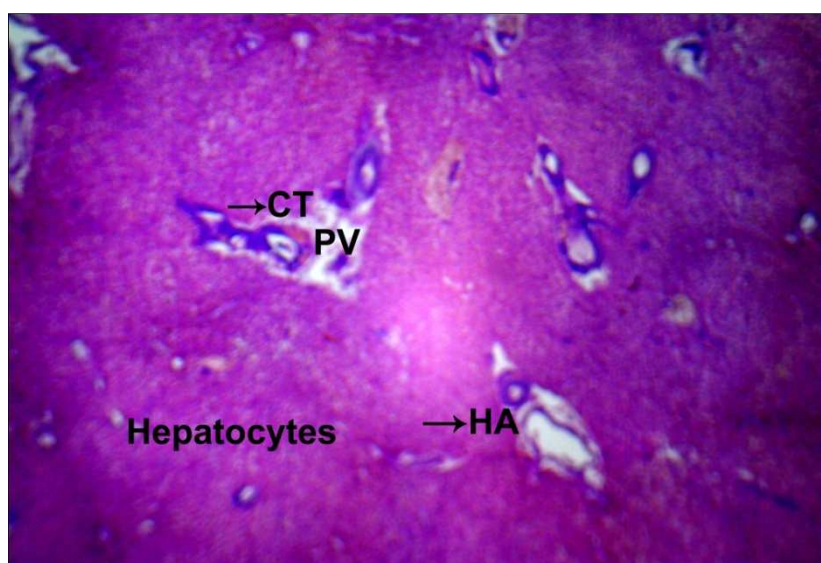
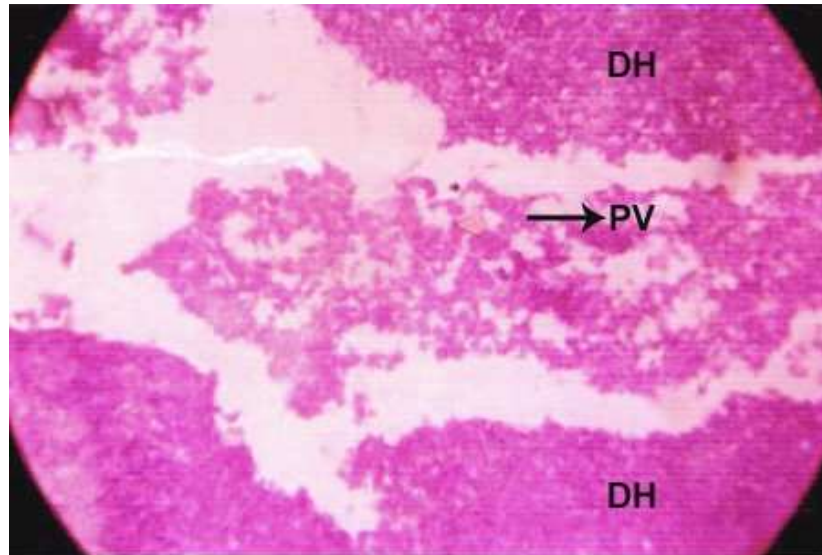


Fig.I Photomicrograph Of (GR 1 CF)liver *C.punctatus* Showing Numerous Hepatocytes With Normal Granular Cytoplasm , Central Vein & Portal Vein (HAEMATOXYLIN & EOSIN stain) 10X10

The liver of DEHP treated (GR2) showed severe degenerative and necrotic changes in most hepatocytes. Animals DEHP group showed severe degenerative and necrotic changes in most hepatocytes. Necrosis detectable in the form vacuolated cytoplasm with pyknotic, karyorrhetic or karyolytic nuclei. Some hepatocytes showed nuclei with chromatin margination and dark eosinophilic cytoplasm (Fig.2). Dilatation of blood sinusoids and central vein were detected. About three-fourth of hepatocytes (72.2%) in DEHP group showed severe affection .



.Fig.II Photomicrograph Of DEHP Treated Liver Of *C. punctatus* Showing Loss Hepatocytes, Vacuolation Of Cytolasm, Lack Of Central Vein & Portal Vein HAEMATOXYLIN & EOSIN Stain -10x

Electron microscopy of **CF(GR1)** shows normal cell organelles with endoplasmic reticulum, ribosomes, nucleus, nucleolus. In **DEHP treated (GR2)** alterations of total membrane integrity was observed condition shows necrosis with loss of ER, nucleus, mitochondria. However, Ashwagandha treated group(**ART GR**) showed signs of ameliorated condition with rejuvenation of nuclues, ribosomes, endoplsmic reticulum

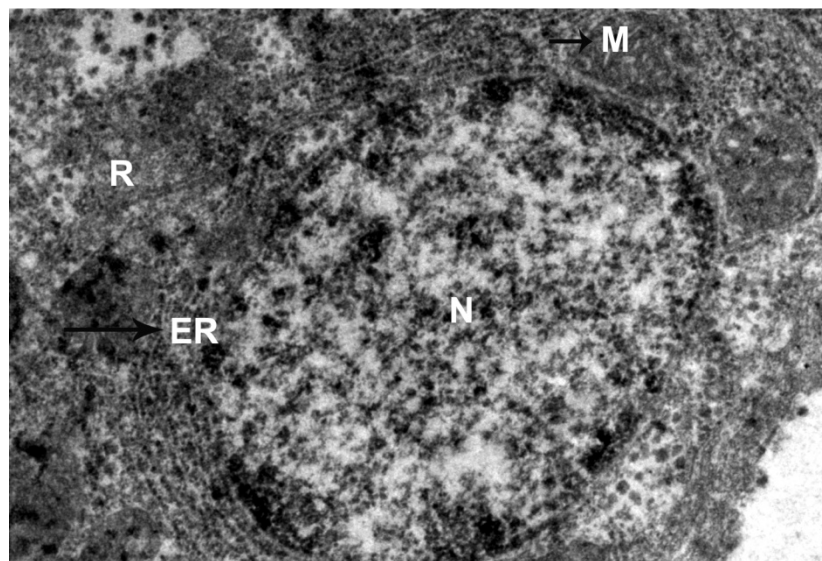


Fig .III 10,000x

An Electron Micrograph Of (CF) Control Liver of *C.punctatus* showing normal Rough Endoplasmic Reticulum, Nucleus, Nucleolus, Smooth Endoplasmic Reticulum, Chromatin Fibres

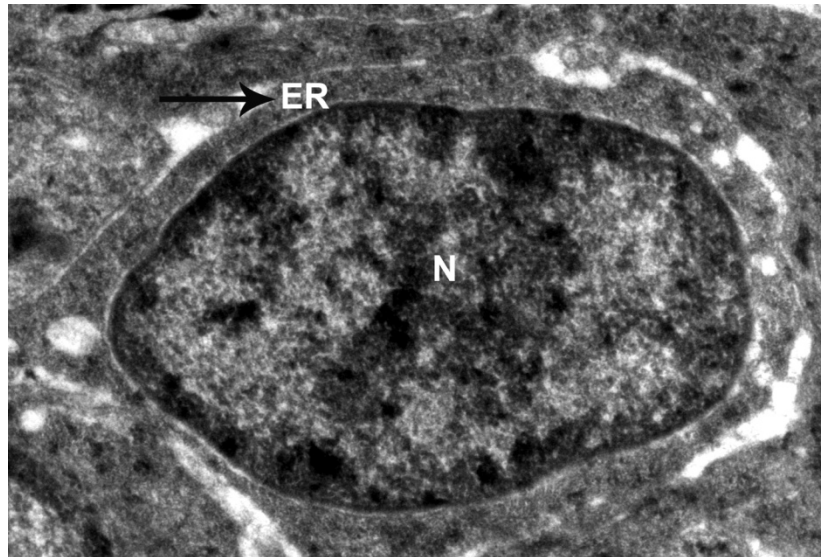


Fig.IV 10,000x

An Electron Micrograph Of (DT) DEHP Treated Liver In *C.punctatus* Showing Complete Dilation Of Nucleus , Endoplasmic Reticulum, Ribosomes, Swollen Mitochondria

Ashwagandha root treated group (ARTGR) of *C.punctatus* shows signs of amelioration showing regeneration of many cytoplasmic organelles which shows the amelioration. Ashwagandha is found to be a major ingredient of various adaptogenic and anti-stress tonics[11].



Fig.V Withania somnifera



Fig.VI Roots of Withania Somnifera

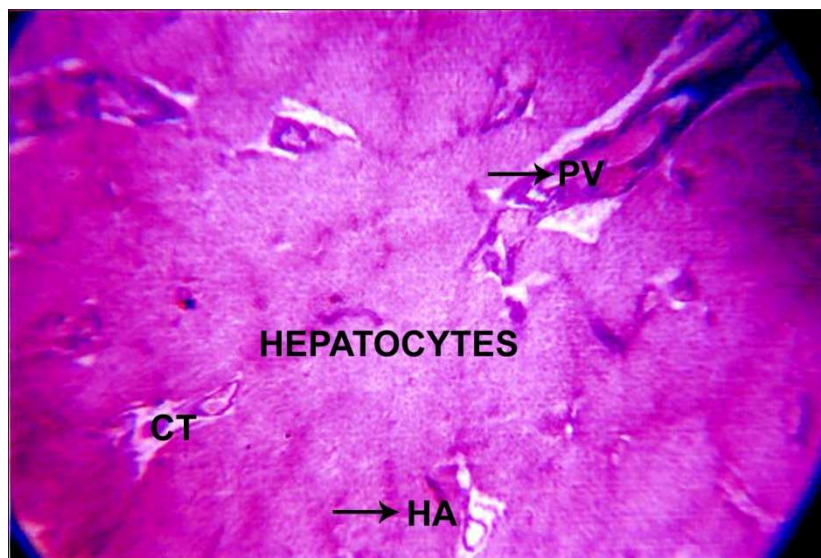


Fig.VII

Photomicrograph Of Liver Of Ashwagandha Root Treated In *C.punctatus* showing Rejuvenation Of Hepatocytes With Granular Cytoplasm,Central Vein & Portal Vein HAEMATOXYLIN & EOSIN STAIN- 10X

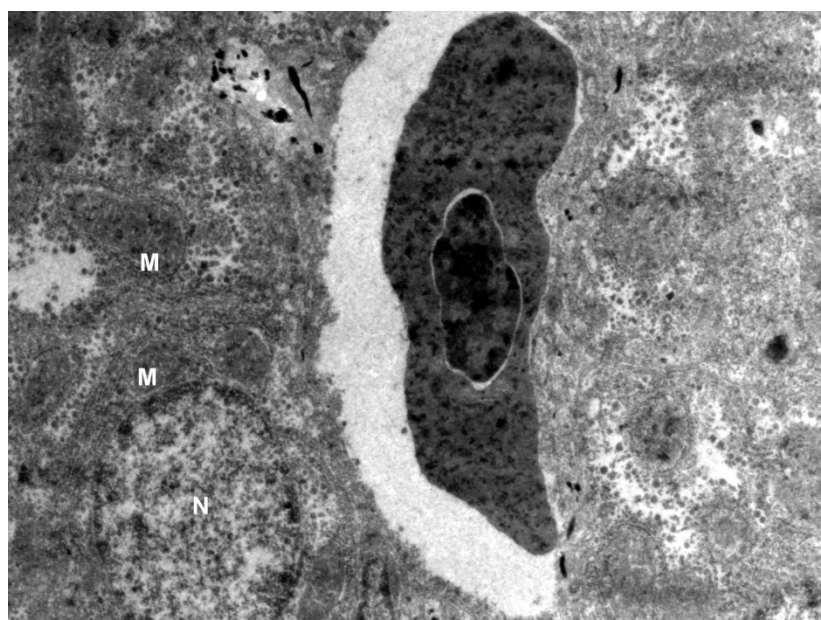


Fig.VIII 1600X

An Electron Micrograph Of Ashwagandha Root Treated Liver In *C.punctatus* Showing Regeneration Of The Cell Organells Namely Nucleolus And Nuclues And Also Mitochondria

DISSCUSSION

Studies indicated that oral exposure to DEHP resulted in toxic effects in human and rodent species. Oral studies in mice and rat have established that the main target of DEHP toxicity is the liver [12]. There have been no studies of specific techniques for reducing DEHP body burden [13]. The present study was undertaken to determine the postulated protective role of *Withania somnifera* against DEHP liver toxicity in *Channa punctatus*.

The group receiving DEHP showed DEHP hepatic cell hyperplasia; this is due to rapid cell division, which appears to be the initial physiological response to DEHP exposure [14, 15].

The group receiving DEHP also expressed marked fatty infiltration in hepatic cells, this was also shown by Price *et al.* [16] who claimed the presence of fatty infiltration in the hepatocytes and fat deposits in the periportal area of rats receiving oral DEHP. Studies done by David *et al* [12] indicated that the activities of the enzymes responsible for fatty acid catabolism (palmitoyl-CoA oxidase, enoyl-CoA hydratase, carnitine acyltransferase and aglycerophosphate dehydrogenase) were increased in rodents after exposure to DEHP by factors as great as 150%.

Also Bette [17] indicated that administration of DEHP for three days to rats resulted in significant increase in the phosphatidylcholine and phosphatidylethanolamine, leading to increase in total liver lipid content and total phospholipid.

Exposure to DEHP [18-19] or DBP[20] via ingestion has been shown to affect several hepatic enzyme activities in fetal, neonatal, suckling, and adult rats.

It is very clear with the results that *W. somnifera* extract contains many active constituents that have potential activity against many diseases. *W. somnifera* has therapeutic potential to be used as an anti-inflammatory agent, an antioxidant, an anti-aging agent, an anticancer agent, chemopreventive and immunomodulator, an adaptogen, an anti-anxiety and anti-depressant, an antiulcer agent, a cardioprotectant, a hypolipidemic and anti-atherogenic agent, an antihypertensive agent, a hypoglycemic agent, a hepatoprotectant, a treatment for hypothyroidism, an antimicrobial (antibacterial and antifungal) agent or as an adjunct to the above treatments.

The plant is chemically very complex and more than 80 compounds are known from it [21]. The constituents of *Withania somnifera* roots are the steroidal alkaloids and steroidal lactones. They belong to a class of constituents called the withanolides [22,23], with the main active chemical constituent Withaferin A, a phytosteroid [24].

CONCLUSION

Ashwagandha is found to be a major ingredient of various adaptogenic and anti-stress tonics [25]. The present attained findings may lead to the suggestion that factories should be prevented from pouring their effluents in rivers and tributaries. This is because such category is one of the most important aspects of pollution to the fresh water environments and drastic hazardous effects are proved by the result of the present study to be produced in internal organs of fishes living in such polluted waters. The objective has been explored and evaluated that ashwagandha root extract which may be consumed in future to trigger a new line of investigation against the DEHP toxicant and the use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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