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# Assessment of Toxic Effect of Dibutyl Phthalate (DBP) on Acid and Alkaline Phosphatase in Goldfish (*Carassius auratus*) With Ameliorative Efficacy of *Withania somnifera*

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**Abstract**

This study explores the metabolic responses of goldfish (*Carassius auratus*) to dibutyl phthalate (DBP) exposure, emphasizing the therapeutic potential of *Withania somnifera* (Ashwagandha). DBP, a prevalent environmental pollutant and endocrine disruptor, is commonly found in plastics and personal care products, leading to significant contamination of aquatic ecosystems. The objective of this research is to assess the impact of DBP on the metabolic health of goldfish and evaluate the efficacy of *Withania somnifera* in mitigating DBP-induced metabolic disturbances. Following DBP exposure, groups of goldfish were treated with *Withania somnifera* extracts. The therapeutic efficiency of *Withania somnifera*was evaluated by comparing metabolic parameters of the treated groups with those of the DBP-only exposed group and a control group. Treatment with *Withania somnifera*showed significant amelioration of these effects, with treated fish exhibiting enzyme activities closer to those of the control group. This study highlights the detrimental impact of DBP on the health of goldfish and underscores the potential of *Withania somnifera* as a therapeutic agent in counteracting these effects. These findings suggest promising applications of *Withania somnifera* in environmental toxicology and the preservation of aquatic organism health.

**Keywords :** Liver enzymes, Gold fish, Dibutyl Phthalate, *Withania somnifera*

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## Introduction

Human health relies on homeostasis, wherein hormones operate as chemical messengers that regulate numerous physiological processes. This regulation is delineated and structured at the molecular and biochemical levels. Recently, multiple articles have documented the negative effects of various xenobiotics, namely chemical substances from the environment that can directly influence hormonal homeostasis and signalling, such as pesticides, cosmetics, flavourings, and industrial chemicals (Jobling *et al.*,1995). This prompts an inquiry into the safety and degree of human exposure to compounds categorised as endocrine-disrupting chemicals (EDCs). The World Health Organisation (WHO) defines endocrine-disrupting chemicals (EDCs) as predominantly synthetic molecules present in diverse materials, including pesticides, metals, food additives, and personal care products. Endocrine-disrupting chemicals (EDCs) are suspected to affect reproductive function in both sexes, elevate breast cancer risk, induce aberrant growth patterns and neurodevelopmental delays in children, and modify immunological function (Huang *et al.*,2008). Numerous instances of low-dose effects of endocrine-disrupting chemicals (EDCs) exist, including parabens, phthalates, and bisphenol A (BPA). Phthalate esters are chemical compounds utilised as additions in various materials to enhance their flexibility. A variety of phthalate esters have been synthesised, posing a significant danger of human exposure to these compounds (Zeng *et al.*,2009). Dibutyl phthalate (DBP) is an ester of phthalic acid, extensively utilised as a plasticiser in sealants, paints, adhesives, cosmetics, children's toys, medical devices, nutritional supplements, and various packaging materials. Due to their physicochemical properties related to non-covalent bonding, these substances can gradually leach from products and permeate the environment (Wang *et al.* 2000; Li *et al.* 2016). The majority of sewage from industrial and home sources is treated prior to its release into aquatic bodies. In India, namely in Delhi, daily water usage reaches approximately 1,200 million litres, of which 960 million litres of untreated sewage containing 800 million kilogrammes of industrial garbage are discharged into the Yamuna River via 17 major drains. Different fish species may exhibit varying levels of susceptibility to DBP exposure, depending on their physiology, life history traits, and habitat preferences. DBP and other phthalates can accumulate in fish tissues over time. This bioaccumulation can lead to higher concentrations of DBP in predators that consume contaminated prey, resulting in biomagnification along the food chain (Sun *et al.*, 2015). Some in vitro studies have suggested that extracts or compounds from Ashwagandha may exhibit antiproliferative and cytotoxic effects on cancer cells. These effects could potentially slow down the growth of cancer cells or induce their death (apoptosis). Ashwagandha is believed to have immunomodulatory effects, meaning it can influence the immune system. Some research has explored the potential of Ashwagandha to enhance immune responses that may aid in the body's ability to recognize and destroy cancer cell, it also helps with chronic inflammation and oxidative stress are associated with cancer development and progression. Ashwagandha contains compounds with potential anti-inflammatory and antioxidant properties, which could have implications for cancer prevention or treatment. Therefore, in present study gold fish (*Carassius auratus*) has been taken as the experimental animal model for the study. The study aims to investigate the effects of DBP exposure and its various effects on enzymatic activity of the liver.

## Materials and Methods

Goldfish were acquired from the local fish market between September and April, when the room temperature fluctuates from 25 to 36°C and the water temperature ranges from 20 to 25°C. Following a thorough examination for injuries, they were immersed in a one percent potassium permanganate solution for several minutes to eliminate any cutaneous infections. Following a 15-day acclimatisation period, they were housed in enormous glass aquaria measuring 75 cm x 37.5 cm x 37.5 cm and were given boiled egg yolk and fish meal. To evaluate the impact of dibutyl phthalate (DBP), the fish were divided into five groups: one control group and four experimental groups, each including five fish. The fish were captured alive and severed at the peduncle to extract blood directly from the caudal vein for biochemical analysis. *Withania somnifera*was acquired from the market. Blood samples were taken in centrifuge tubes, and serum was isolated via centrifugation. The serum phosphatase will be assessed using King's technique (1959). The serum alkaline phosphatase will be quantified using the method established by Kind and King (1954). The data were analysed for correctness using the statistical program Ky plot.

## Results and Discussion

The data is represented in form of tables and graphs as below

Table-1: Toxic effect of DBP on Acid phosphatse enzyme of Gold Fish with amelioration by *Withania somnifera* for 15, 30, 45 and 60 days treatment

| **Experimental set** | **Experimental days** | | | |
|---|---|---|---|---|
| | **15 days** | **30 days** | **45 days** | **60 days** |
| **Control** | 45.50±1.20 | 45.50±1.20 | 45.50±1.20 | 45.50±1.20 |
| **DBP treated** | 55.80±1.70* | 68.50±1.95** | 82.20±1.50*** | 95.10±1.67*** |
| **DBP+*Withania somnifera* treatment** | 50.10±1.20* | 48.50±1.05** | 46.70±1.45*** | 45.90±1.37*** |

NS- Non-significant (p>0.05), *- Significant (p<0.05), **- Highly Significant (p<0.01), ***- Very Highly Significant (p<0.001)

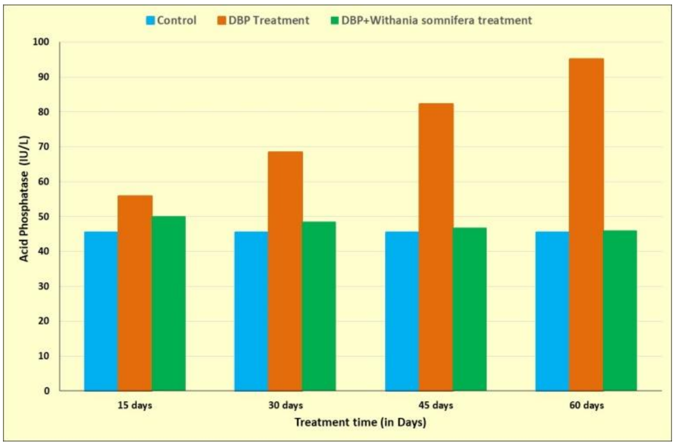


**Fig-1:** Toxic effect of DBP on Acid phosphatse enzyme of Gold Fish with amelioration by Withania somnifera for 15, 30, 45 and 60 day's treatent

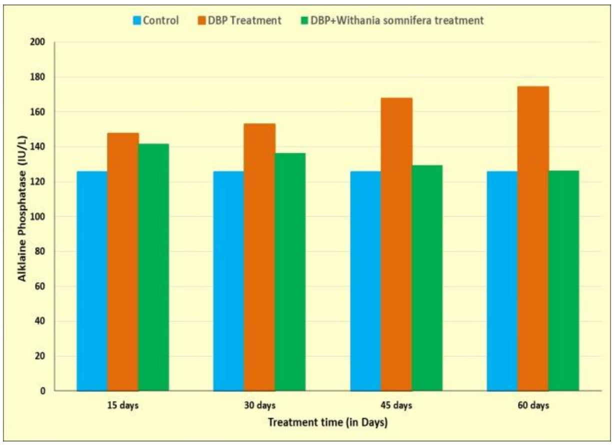


**Fig-2:** Toxic effect of DBP on Alkaline phosphatse enzyme of Gold Fish with amelioration by Withania somnifera for 15, 30, 45 and 60 days treatent

Table-2: Toxic effect of DBP on Alkaline phosphatse enzyme of Gold Fish with amelioration by *Withania somnifera*for 15, 30, 45 and 60 days treatment

| **Experimental set** | **Experimental days** | | | |
|---|---|---|---|---|
| | 15 days | 30 days | 45 days | 60 days |
| **Control** | 125.70±2.05 | 125.70±2.05 | 125.70±2.05 | 125.70±2.05 |
| **DBP treated** | 147.50±2.66** | 152.90±1.99*** | 167.75±2.55*** | 174.30±2.45*** |
| **DBP+*Withania somnifera* treatment** | 141.60±1.76$^{NS}$ | 136.10±1.05** | 129.37±1.95*** | 126.22±2.04*** |

NS- Non-significant (p>0.05), *- Significant (p<0.05), **- Highly Significant (p<0.01), ***- Very Highly Significant (p<0.001)

The effect of Di-n-butyl phthalate (DBP) on acid and alkaline phosphatase activities in goldfish can be significant, as DBP is a type of phthalate ester commonly used as a plasticizer, and it has been known to disrupt endocrine functions in aquatic organisms. Acid phosphatase is an enzyme that plays a role in the hydrolysis of phosphate esters at acidic pH levels (ATSDR, 1990). When goldfish are exposed to DBP, there could be an increase in ACP activity, which may indicate cellular stress or lysosomal membrane damage (Raisuddin and Mishra, 1998). This enzyme activity could reflect the body's response to detoxify and eliminate the harmful effects of DBP. Alkaline phosphatase is an enzyme that works optimally at alkaline pH and is involved in dephosphorylation processes (Devi 2023). Exposure to DBP may lead to altered ALP activity, which can disrupt normal metabolic functions such as the dephosphorylation of proteins and nucleotides. Changes in ALP activity could indicate liver damage or other systemic effects of DBP exposure (Pal and Sahu 2002). *Withania somnifera*, commonly known as Ashwagandha, is a medicinal herb with potent antioxidant and adaptogenic properties. Studies suggest that it can offer protective effects against various toxicities, including those induced by environmental pollutants like Di-n-butyl phthalate (DBP). DBP exposure may lead to an increase in acid phosphatase activity due to cellular stress or damage (Vijayan and Nair, 2023). *Withania somnifera* can potentially reduce this overactivity by stabilizing cellular membranes and reducing lysosomal damage, thereby minimizing the stress response. The herb's antioxidant properties can help neutralize free radicals generated by DBP, reducing oxidative stress and, consequently, the upregulation of ACP as a response to cellular damage (Gupta *et al*., 2001). DBP can disrupt ALP activity, leading to potential metabolic disturbances. *Withania somnifera* might help in normalizing these levels by protecting liver function and maintaining the integrity of cellular processes. ALP is often linked to liver function, and *Withania somnifera*'s hepatoprotective properties could mitigate the liver damage caused by DBP, thereby stabilizing ALP activity. *Withania somnifera* is rich in with anolides, which possess antioxidant and anti-inflammatory properties (Bhattacharya *et al.,* 2023). These compounds can mitigate the oxidative stress and inflammation induced by DBP exposure, thereby protecting enzyme activities. The herb may enhance the body's natural detoxification processes, helping to reduce the accumulation of DBP and its toxic effects on enzymes like ACP and ALP (Carlucci *et al.* 2024).). In conclusion, exposure to DBP may result in significant alterations in both acid and alkaline phosphatase activities in goldfish, potentially leading to various physiological and biochemical disturbances. These changes could serve as biomarkers for assessing the impact of DBP on aquatic organisms. *Withania somnifera* could offer a protective effect against DBP-induced toxicity in goldfish by stabilizing acid and alkaline phosphatase activities, reducing oxidative stress, and supporting overall cellular health (Ghosal and Bhattacharya 2023).

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