

Impact of Dibutyl Phthalate (Dbp) on Sgot and Sgpt in Goldfish (Carassius Auratus) with Therapeutic Efficacy Withania Somnifera

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Abstract

The increasing use of plasticizers such as dibutyl phthalate (DBP) in industrial and domestic products has raised significant concerns about their environmental toxicity, particularly in aquatic ecosystems. DBP, a widely used phthalate ester, is known for its endocrine-disrupting and hepatotoxic properties. The present study investigates the impact of DBP exposure on hepatic function in goldfish (*Carassius auratus*), as measured by serum levels of liver enzymes viz. serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Additionally, the study evaluates the therapeutic efficacy of *Withania somnifera*, commonly known as ashwagandha, a medicinal plant with known antioxidant and hepatoprotective properties, in mitigating DBP-induced hepatic damage. Goldfish were divided into three groups- control, DBP-exposed, DBP + *Withania somnifera* treated. Fish in the DBP-exposed group were subjected to a sub-lethal concentration of DBP followed by treatment with *Withania somnifera* extract in the therapeutic group. SGOT and SGPT levels were measured using standard biochemical assays through standard kit. Results demonstrated a significant increase in SGOT and SGPT levels in DBP-exposed fish, indicating hepatocellular injury. However, fish treated with *Withania somnifera* following DBP exposure showed a marked reduction in these enzyme levels, approaching normalcy compared to control. This suggests the hepatoprotective role of *Withania somnifera*, likely due to its antioxidative and anti-inflammatory bioactive compounds such as withanolides. In conclusion, DBP exerts a detrimental effect on liver function in *Carassius auratus*, as evidenced by elevated SGOT and SGPT. *Withania somnifera* demonstrates promising therapeutic potential in ameliorating DBP-induced hepatic toxicity, highlighting its relevance as a natural remedy in aquatic toxicology and fish health management. Further studies are recommended to elucidate the molecular pathways involved in its protective mechanism.

Keywords SGOT, SGPT, Gold fish, Dibutyl Phthalate, Therapeutic effect, *Withania somnifera*

INTRODUCTION

Environmental contamination by industrial pollutants has become a growing global concern, particularly due to its impact on aquatic ecosystems. Among various pollutants, phthalate esters such as dibutyl phthalate (DBP) have gained significant attention for their persistent and toxic nature. DBP is a commonly used plasticizer in the manufacture of flexible plastics, adhesives, paints, personal care products, and other consumer goods (Agus *et al.* 2015). Its widespread usage has led to its ubiquitous presence in the environment, especially in water bodies receiving industrial and municipal waste. Once released into the aquatic environment, DBP tends to accumulate in sediments and aquatic organisms, posing severe ecological and biological risks. Phthalates, including DBP, are well-documented as endocrine-disrupting chemicals that interfere with hormonal regulation and metabolic processes. DBP has been shown to exhibit hepatotoxic, nephrotoxic, reproductive, and developmental toxicity in various organisms (Sun *et al.* 2015). In aquatic organisms such as fish, DBP disrupts physiological homeostasis, affecting growth, reproduction, behavior, and organ function. One of the primary targets of DBP toxicity is the liver, a vital organ responsible for detoxification, metabolism, and homeostasis. Hepatotoxic effects of DBP are often reflected by changes in the levels of liver-specific enzymes such as serum glutamic oxaloacetic transaminase (SGOT, also known as AST) and serum glutamic pyruvic transaminase (SGPT, also known as ALT), which are reliable biomarkers for liver function and integrity (Li *et al.* 2016).

Increased levels of SGOT and SGPT in serum are indicative of liver damage, as these enzymes are normally contained within hepatocytes and are released into the bloodstream upon cellular injury or membrane disruption (Pal and Sahu 2002). Therefore, monitoring changes in these enzymes in response to DBP exposure provides valuable insight into the extent of hepatocellular damage and the physiological stress experienced by fish. The goldfish (*Carassius auratus*), a freshwater teleost, serves as an excellent model organism for toxicological studies due to its sensitivity to

environmental stressors, ease of maintenance, and well-characterized physiology. To counteract the toxic effects of environmental contaminants like DBP, there is increasing interest in the use of natural compounds and plant-based therapeutics. Medicinal plants offer a cost-effective and environmentally sustainable approach for mitigating chemical-induced toxicity in aquatic organisms. One such plant with notable pharmacological potential is *Withania somnifera*, commonly known as Ashwagandha (Ghosal and Bhattacharya 2023). It is a revered herb in traditional Indian medicine (Ayurveda) and has been extensively studied for its adaptogenic, antioxidant, anti-inflammatory, immunomodulatory, and hepatoprotective properties. The bioactive compounds in *Withania somnifera*, particularly withanolides, alkaloids, and flavonoids, are believed to play a critical role in its therapeutic efficacy (Chithaiya *et al.* 2024).

The hepatoprotective potential of *Withania somnifera* has been demonstrated in various *in vivo* and *in vitro* studies. Its antioxidant activity helps in scavenging free radicals and reducing oxidative stress, which is a major pathway through which DBP exerts its toxic effects (Xia *et al.* 2021). Additionally, *Withania somnifera* modulates the expression of liver enzymes, maintains cellular integrity, and enhances the overall physiological resilience of organisms under chemical stress. Despite extensive research on its therapeutic effects in mammals, relatively limited information is available on the application of *Withania somnifera* in aquatic toxicology, particularly in fish models. Given the significance of DBP as an environmental pollutant and the potential of *Withania somnifera* as a natural therapeutic agent, the present study aims to evaluate the impact of DBP on liver function in goldfish by measuring SGOT and SGPT levels and to assess the therapeutic efficacy of *Withania somnifera* in mitigating DBP-induced hepatotoxicity. By integrating toxicological assessment with phytotherapeutic intervention, this study seeks to contribute to a deeper understanding of fish physiology under chemical stress and explore the viability of herbal extracts in aquatic health management. The rationale behind focusing on SGOT and SGPT as primary biomarkers lies in their diagnostic significance. These enzymes, part of the transaminase family, are involved in amino acid metabolism and are localized in hepatic cells. Upon liver damage, such as that induced by exposure to toxicants like DBP, the permeability of liver cell membranes is altered, leading to the leakage of these enzymes into the bloodstream. Elevated serum levels of SGOT and SGPT are therefore considered early indicators of liver impairment. Understanding the pattern of changes in these enzymes can provide critical insights into the mechanism of toxicity and the protective effects of therapeutic agents. Moreover, goldfish as an experimental model offer several advantages in ecotoxicological research. Their widespread availability, ease of handling, and sensitivity to environmental pollutants make them suitable for evaluating chemical toxicity and the efficacy of detoxifying agents. Previous studies have used goldfish to investigate the toxicological effects of various pollutants, including heavy metals, pesticides, and pharmaceutical residues (Sharma *et al.* 2021). However, research focusing specifically on DBP and its interaction with herbal therapeutics in this species remains sparse.

The use of *Withania somnifera* in this study is due to its multifaceted pharmacological profile. Traditionally used to rejuvenate health and enhance vitality, Ashwagandha has shown promise in protecting against chemical-induced liver damage in rodent models. Its antioxidant defense system includes increasing levels of endogenous enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), which counteract oxidative stress. In addition, its anti-inflammatory and membrane-stabilizing properties are crucial in preventing cellular damage caused by toxicants like DBP (Malik *et al.* 2020). In this context, the current study hypothesizes that DBP exposure will result in elevated SGOT and SGPT levels, indicating liver damage in goldfish, and that treatment with *Withania somnifera* extract will alleviate these effects, thereby restoring liver enzyme levels toward normal. To test this hypothesis, goldfish were exposed to sub-lethal concentrations of DBP, followed by administration of *Withania somnifera* extract in a controlled laboratory setting. The choice of sub-lethal concentration ensures that physiological changes can be monitored without causing mortality, allowing for accurate assessment of therapeutic effects.

This research holds significance not only for aquatic toxicology but also for environmental risk assessment and the development of eco-friendly mitigation strategies. Understanding the interaction between environmental pollutants and natural therapeutics can aid in the development of integrated approaches to aquatic animal health management. Furthermore, this study may pave the way for future investigations into the use of herbal formulations in aquaculture, potentially reducing the dependency on synthetic drugs and chemicals. Through biochemical analysis and comparative evaluation of SGOT and SGPT levels, this study aims to establish a scientific basis for the use of herbal

remedies in protecting aquatic organisms from the harmful effects of environmental pollutants. The findings are expected to contribute to a growing body of knowledge on the interplay between xenobiotic stress and natural detoxification mechanisms, ultimately promoting healthier and more sustainable aquatic ecosystems.

MATERIALS AND METHODS

Gold fishes were purchased from the local fish market during September to April when the room temperature ranges from 25 to 36°C and water temperature from 20 to 25°C. After examining carefully for any injury they were kept in one percent solution of potassium permagnate for few minutes to get rid of any dermal infection. After acclimatization for 15 days they were reared in large glass aquaria measuring 75 cm X 37.5 X 37.5 cm and fed on boiled egg yolk and fish food. To assess the effect of dibutyl phthalate (DBP) the fishes were grouped in to three sets- one control and 2 experimental sets (DBP treated- 10mg/L and *Withania somnifera* supplementation- 800mg/kg b.wt.) each consisting five fishes. The fishes were taken live and cut at peduncle to collect the blood directly from caudal vein for biochemical estimations. The *Withania somnifera* was purchased from market. The blood samples were collected in the centrifuge tubes and serum was separated through centrifugation. The serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) has been estimated by King's method (1959). The serum alkaline phosphatase will be estimated by Reitman and Frankel (1957) method. The data were analyzed through statistical software Ky plot for accuracy.

RESULTS AND DISCUSSION

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

Table-1: Impact of DBP on serum glutamic oxaloacetic transaminase (SGOT) of Gold Fish and therapeutic effect of *Withania somnifera* for 15, 30, 45 and 60 days treatment

Experimental set	Experimental days			
	15 days	30 days	45 days	60 days
Control	19.50±1.15	19.50±1.15	19.50±1.15	19.50±1.15
DBP treated	25.85±1.10*	29.50±1.05**	36.10±1.20***	44.05±1.17***
DBP+ <i>Withania somnifera</i> treatment	22.05±1.01 ^{NS}	21.60±1.05**	21.50±1.35***	20.01±1.25***

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

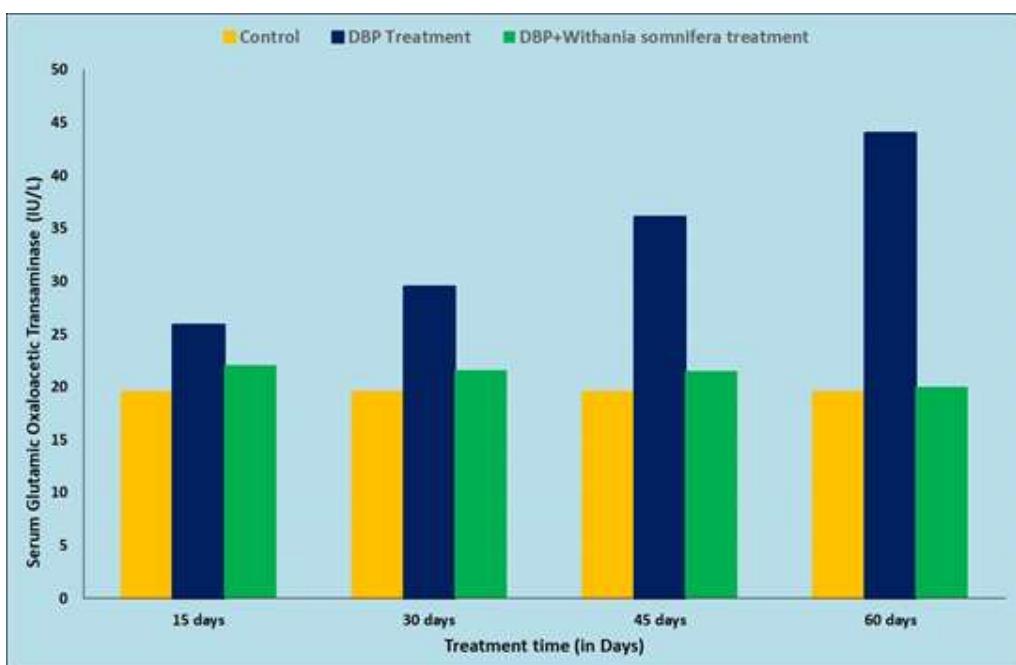


Fig-1: Impact of DBP on serum glutamic oxaloacetic transaminase (SGOT) of Gold Fish and therapeutic effect of *Withania somnifera* for 15, 30, 45 and 60 days treatment

Table-2: Impact of DBP on serum glutamic pyruvic transaminase (SGOT) of Gold Fish and therapeutic effect of *Withania somnifera* for 15, 30, 45 and 60 days treatment

Experimental set	Experimental days			
	15 days	30 days	45 days	60 days
Control	25.80±1.02	25.80±1.02	25.80±1.02	25.80±1.02
DBP treated	28.50±1.60 ^{NS}	35.10±1.10 ^{**}	39.50±1.50 ^{***}	48.50±1.40 ^{***}
DBP+ <i>Withania somnifera</i> treatment	28.00±1.05 ^{NS}	27.50±1.10 [*]	26.75±1.20 ^{***}	25.90±1.12 ^{***}

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

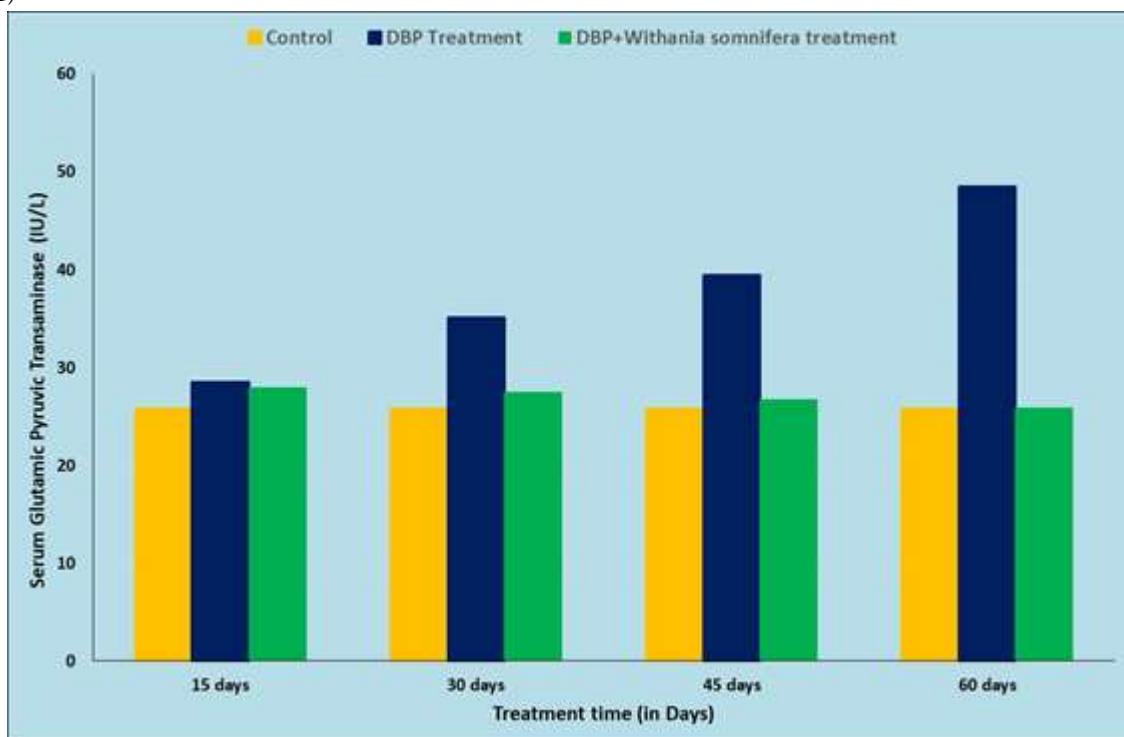


Fig-2: Impact of DBP on serum glutamic pyruvic transaminase (SGOT) of Gold Fish and therapeutic effect of *Withania somnifera* for 15, 30, 45 and 60 days treatment

The findings of the present study provide significant insights into the hepatotoxic effects of dibutyl phthalate (DBP) on *Carassius auratus* (goldfish) and the potential therapeutic efficacy of *Withania somnifera* in mitigating these effects. The observed alterations in the levels of hepatic enzymes viz. serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) serve as reliable biomarkers for evaluating liver damage in aquatic organisms exposed to environmental pollutants. The present findings align with existing literature on the hepatotoxic nature of phthalate esters and add new evidence supporting the hepatoprotective potential of *Withania somnifera* in fish models.

In the DBP exposed group, a significant elevation in SGOT and SGPT levels was observed when compared to the control group. This indicates considerable hepatic injury as a result of DBP exposure. The increase in these enzymes in serum is indicative of cellular damage or membrane leakage in hepatocytes, leading to the release of intracellular enzymes into the bloodstream (Sun *et al.* 2015). This hepatotoxic effect of DBP can be attributed to several factors, including oxidative stress, mitochondrial dysfunction, and interference with enzymatic activity at the cellular level. DBP and its metabolites are known to generate reactive oxygen species (ROS), which disrupt the antioxidant defense systems of cells and lead to lipid peroxidation, protein oxidation, and DNA damage (Agus *et al.* 2015). Such stress compromises cell membrane integrity and contributes to the observed elevation in transaminase levels. The findings are consistent with previous studies that report hepatotoxic responses in fish and other aquatic species exposed to

DBP and other phthalates (Pal and Sahu, 2002; Li *et al.* 2016). Similar elevations in liver enzyme levels have been documented by other researchers following phthalate exposure, highlighting the generalizable nature of DBP-induced liver toxicity in aquatic vertebrates. The sensitivity of goldfish to DBP, as observed in this study, further emphasizes their suitability as a model organism in aquatic toxicology research.

On the other hand, fish treated with *Withania somnifera* extract following DBP exposure exhibited a marked reduction in SGOT and SGPT levels. This reduction suggests that *Withania somnifera* plays a protective role in maintaining hepatic integrity and function, possibly by counteracting the oxidative stress and inflammatory responses induced by DBP (Bhattacharya *et al.* 2023). The hepatoprotective effects of *Withania somnifera* can be largely attributed to its rich phytochemical content, particularly withanolides, alkaloids, and flavonoids, which possess strong antioxidant and anti-inflammatory properties. *Withania somnifera* is known to enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work in tandem to neutralize ROS and prevent oxidative damage (Carlucci *et al.* 2024). By stabilizing cell membranes and reducing lipid peroxidation, *Withania somnifera* helps preserve hepatocyte structure and function, thereby limiting the leakage of liver enzymes into the bloodstream. Additionally, its immunomodulatory effects may reduce inflammation and promote tissue repair, further contributing to the normalization of liver enzyme levels (Malik *et al.* 2020).

These findings corroborate the results of earlier research on the hepatoprotective role of *Withania somnifera* in mammals (Vedi and Sabina 2016; Vijayan and Nair 2023). Several rodent studies have shown that *Withania somnifera* extract can significantly reduce SGOT and SGPT levels in cases of chemically induced liver injury, such as that caused by carbon tetrachloride, paracetamol, and ethanol. The present study extends this evidence to an aquatic model, suggesting that the therapeutic properties of *Withania somnifera* are not restricted to terrestrial animals but can also benefit fish under xenobiotic stress (Ojha and Arya, 2009).

The group that received *Withania somnifera* following DBP exposure did not show a complete restoration to baseline enzyme levels but demonstrated a significant improvement compared to the DBP-only group. This partial recovery suggests that while *Withania somnifera* is effective in mitigating liver damage, its efficacy may depend on the duration and severity of toxin exposure, as well as the dose and frequency of the herbal treatment (Devi, 2023). Future studies could explore optimized dosing regimens or combinational therapies to enhance the recovery of liver function following environmental toxicant exposure.

From an ecological perspective, the ability to use plant-based therapeutics such as *Withania somnifera* to counteract chemical toxicity offers promising implications for sustainable fish health management. With growing concern over the presence of phthalates and other EDCs in aquatic environments, there is a need to develop eco-friendly and non-invasive strategies to protect aquatic life (Ghosal and Bhattacharya 2023). The results of this study indicate that incorporating herbal supplements into fish diets or water systems may be a viable method to enhance resilience against environmental stressors, particularly in aquaculture operations.

In conclusion, the study confirms the hepatotoxic potential of dibutyl phthalate in goldfish, as indicated by elevated SGOT and SGPT levels, and demonstrates the therapeutic efficacy of *Withania somnifera* in ameliorating DBP-induced liver damage. The use of natural plant extracts like *Withania somnifera* offers a promising, sustainable approach for mitigating the impact of environmental pollutants on aquatic organisms. These findings lay the groundwork for further research into herbal therapeutics in aquaculture and underscore the importance of integrating traditional medicinal knowledge with modern toxicological assessments to address current environmental challenges.

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