Synergistic effect of Liquorice and Pyridoxial 5 Phosphate on Arsenic Induced Hepatotoxicity in Mice Model

Om Singh, Purnendra Gupta , Hariom Singh

Department of Pharmacy, Rameshwaram Institute of Technology and Management,

Lucknow, Uttar Pradesh 227202, India

Corresponding Author : singhom7275@gmail.com

ABSTRACT

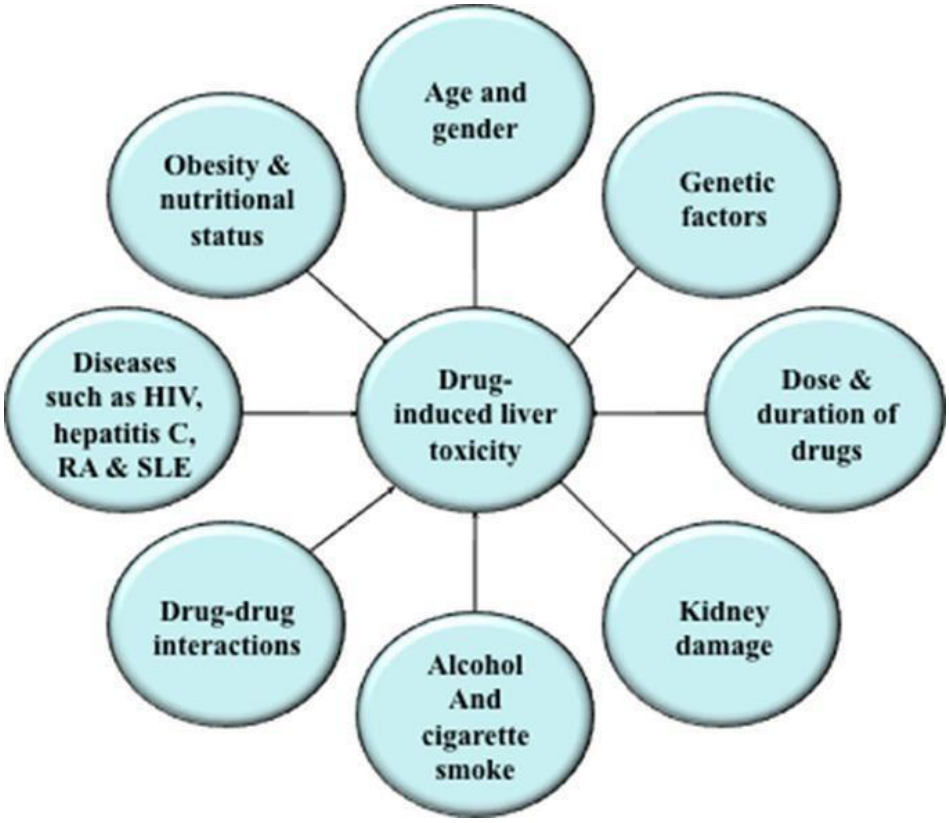
Arsenic contamination in the environment poses a significant threat to human health, particularly through hepatotoxicity. In this study, we investigated the potential synergistic protective effects of liquorice (*Glycyrrhiza glabra*) and pyridoxal 5-phosphate (PLP) against arsenic-induced hepatotoxicity in a mouse model. Mice were orally administered with arsenic, followed by co-administration of liquorice extract and PLP. Hepatic function markers, oxidative stress parameters, and histopathological changes were evaluated. Our findings demonstrate that combined treatment with liquorice and PLP significantly ameliorated arsenic-induced hepatotoxicity compared to individual treatments or arsenic alone. This protective effect was evidenced by decreased levels of liver enzymes, restoration of antioxidant defenses, and mitigation of histopathological alterations in the liver tissue. The synergistic action of liquorice and PLP suggests a promising strategy for mitigating arsenic-induced hepatotoxicity. Further research is warranted to elucidate the underlying molecular mechanisms and evaluate the translational potential of this combination therapy for human health.

Keywords: Arsenic, Hepatotoxicity, Liquorice, Pyridoxal 5-Phosphate, Oxidative Stress, Mouse Model

1.0 INTRODUCTION

In modern times, hepatic infections are one of the main problems posing a serious threat to people's welfare. It has repeatedly been demonstrated that liver damage and damage to the hepatic parenchyma affect the liver's ability to perform its many metabolic activities. Throughout the past few decades, liver disease has become more common and is mostly caused by prolonged contact with dangerous substances, medicines, including pollutants from the atm [1]. There have been efforts attempted to find effective a liver-protect drugs. However, there are currently no effective hepatoprotective therapies available. Hepatic diseases are treated in large part with plant-based drugs. Traditional medicine uses a variety of regenerative herbs and their products to treat hepatic problems in the absence of reliable hepatoprotective drugs in modern pharmaceuticals.

Figure 1.2 Various factors induced liver toxicity



Arsenic incited hepatotoxicity :-

In numerous components of the world, exposure to elevated amounts of inorganic arsenic poses a serious risk to public health, placing millions of people at risk for a wide range of systemic issues. Liver diseases including non-cirrhotic portal fibrosis, which can develop into portal hypertension, have been related to long-term exposure to arsenic. It is yet uncertain what mechanisms lead to liver damage from arsenic. Introduction to arsenic causes oxidative stressors to develop in the liver. Experimental studies demonstrate that the circulatory system's antioxidant defense mechanism starts to work after a brief stimulation and then breaks down with prolonged exposure. Hepatocellular fibrosis develops when the liver's antioxidant defence system is impaired as a result of persistent arsenic exposure. Prolonged oxidative stress in the liver can trigger pro- inflammatory cytokine-like TNF-α, which may contribute to enhanced deposits of collagen along with hepatic fibrosis.

Plant profile: *Glycyrrhiza glabra*

Glycyrrhiza glabra, frequently called licorice, is an annual herbaceous plant from Asia and southern Europe. It is distinguished by its sweet-tasting roots, which have been employed for generations in traditional medicine including confectionery. The name "Glycyrrhiza" words "glykys," meaning sweet, and "rhiza," which means root, emphasising the plant's most distinguishing trait. Licorice has a long history of production and use, extending back to ancient civilisations including the Egyptians, Greeks, and Romans, who valued it for its therapeutic benefits and flavouring.

Licorice plants normally have straight stems that can grow to be 1 to 1.5 metres (3 to 5 feet) tall and compound leaves with many leaflets. The little, pea-like blooms are grouped in clusters and range in colour from purplish blue to pastel violet. After fertilization, the blooms produce oblong-shaped legumes with numerous seeds.

Drug Profile: Pyridoxial 5-Phosphate :-

The active compound of vitamin B6, pyridoxal 5-phosphate (P5P), is essential for a number of bodily metabolic processes (Figure 1.6). Here's a drug profile outlining its uses, mechanisms of action, dosage, side effects, and precautions:

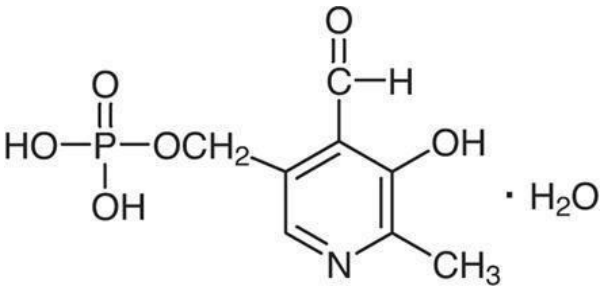


Figure 1.6 Pyridoxal 5-Phosphate (P5P)

Drug Class: Vitamin B6 Supplement

Uses:

1. **Treatment of Vitamin B6 Deficiency:** P5P is utilized in the management of vitamin B6 deficiency, which can lead to various neurological and metabolic disorders.
2. **Adjunctive Therapy:** It may be used as an adjunctive therapy in certain conditions like epilepsy, autism spectrum disorders, and premenstrual syndrome (PMS).

Mechanism of Action:

1. **Coenzyme:** P5P is a cofactor in many enzymatic activities related to heme synthesis, creation of neurotransmitters (including dopamine, serotonin, and gamma- aminobutyric acid), and the regulation of amino acid metabolism
2. **Neuroprotection:** P5P exhibits neuroprotective effects by modulating neurotransmitter levels and supporting myelin formation.

Dosage:

- **Oral Tablets:** Typically available in doses ranging from 25 mg to 100 mg.
- **Dosage:** The recommended dosage varies depending on the individual's age, medical condition, and severity of deficiency. It's usually prescribed by a healthcare professional.

Side Effects:

1. **Rare:** Adverse effects are rare when taken at recommended doses.
2. **Possible Side Effects:** May include nausea, vomiting, headache, and hypersensitivity reactions in some individuals.
3. **High Doses:** Long-term use of high doses may lead to peripheral neuropathy.

Collection and Authentication of selected plant :-The selected Liquorice was collected from a local supplier from Lucknow

Physicochemical parameters

- Determination of extractive value
- Determination of Ash value
    - Total ash
    - Acid insoluble ash value
    - Water soluble ash value
- Foaming index
- Swelling index

Preliminary Phytochemical test

Assessment of TPC and TFC

Pharmacological screening

Arsenic Induced Hepatotoxicity in Mice Model

- Effects of Liquorice and Pyridoxial 5-phosphate (PDP) on liver marker enzymes of Arsenic induced hepatotoxicity on Swiss albino mice the following parameters were estimated.

- SGOT
- SGPT
- ALP

➢ Serum parameters of Arsenic induced hepatotoxicity on Swiss albino mice

- Total protein
- Albumin
- Total cholesterol
- Estimation of triglycerides

➢ Histological changes of liver

Statistical Analysis

Compilation of Data

Reagents and Chemicals :-

LQ, which is more than 98% pure, was bought locally. Beijing SL Pharmaceutical Co., Ltd. (Beijing, China) provided the ATO. From SD Fine Pharmaceutical Ltd. in Mumbai, kits for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), CAT, GSH, MDA, along with SOD were acquired. Invitrogen provided the ELISA kits for mouse TNF-α and IL-6 (Carlsbad, CA, USA).

Plant Extract Preparation :-

Using a hot continuous infiltration approach in a maceration, the branching portions of *Glycyrrhiza glabra* being continuously divided using Aqueous (40-100 ^{0}C) about a period . Each of the concentrations were concentrated using a rotating evaporating before being freeze dried using a device called a lyophilizer to produce a fine powder

Phytochemical Tests [2, 3]

S. No.	Test	Aqueous xtract
a.	Alkaloids	P
b.	Carbohydrates and glycosides	P
c.	Phytosterols	N
d.	Coumarins	N
e.	Flavonoids	P

f.	Phenolic compounds and tannins	P
g.	Protein and Amino Acid	P
h.	Saponins	p
i.	Fixed oil and fats	N

P- Positive; N- Negative

Acquisition and the selection of *Glycyrrhiza glabra* :-

The ***Glycyrrhiza glabra*** (aerial parts) were procured from local supplier from Lucknow, India.

Extraction and determination of %age yield :-

According to the supercharged components were gradually separated with petroleum ether at different temperatures (40-600C) using maceration methods for 1 day. The concentrates were compressed using a rotary evaporator, then freeze dried in a lyophilizer to produce powder that is dry. Lock cap vials served to retain the extracted substances until future usage. Using extracts of polar solvents (Aqueous) *Glycyrrhiza glabra* yielded 9.2% w/w.

Table 6.2 Physicochemical analysis of *Glycyrrhiza glabra*

S. No.	Particulars	Percentage (% wt/wt)
1.	LOD	3.8
2.	Total ash	5.7
3.	Water soluble ash	3.6
4.	Acid insoluble ash	0.5
5.	Sulphated ash	2.1

Hepatoprotective activity of *Glycyrrhiza glabra* and Pyridoxial 5-phosphate by Arsenic Induced Hepatotoxicity

Experimental Animals :-31 female Swiss Albino mice (20+ 2 g) were procured from RITM College, Lucknow and maintained under conventional circumstances (23.0 ± 2.0 °C, 45-55% percent humidity, 12 hours of light and 12 hours of darkness), with unrestricted consumption of food and drink. Prior to testing, all mice were deprived overnight and kept in an environment for not less than 60 days. The Animal Experiments Ethics Committee atRITM College in Lucknow authorised and observed all experimental methods.

Treatment protocol :-31 female Swiss Albino mice were randomaly assigned to 6 treatment group including the control group, disease control group, standared group, test drug 1 (liquorice + Pyridoxial 5 phosphate) low dose 100mg\kg .test drug 2(liquorice +Pyridoxial 5 phosphate) high dose 200mg\kg . these study regulated 8 week each group contain 5 animal. And last is acute toxicity group here is 6 animals used ,3 animal used is control group and 3 animals used in test group. Twelve hours after the last treatment, the mice were weighed and put to sleep with sodium pentobarbital. After being gently removed, the liver was immediately rinsed in regular saltwater and its moist weight was measured. Each group's liver samples were fixed with either 4% para-formaldehyde or 4% glutaraldehyde and stored at -80°C until they were needed.

Blood Collection/Tissues Preparation :-

All research volunteers had to be murdered via cervical dislocation at the end of the 30-day trial. Blood was quickly drawn in 10% EDTA tubes for serum separation after the heart was punched. In order to extract blood cells, the liver was right away removed and submerged in a cold NaCl (0.15 M) buffer. Plasma was subsequently infused with the same combination, blotted on filter paper, quickly weighed, and homogenized using biochemical techniques. To analyze the metals As, Ca, Cu, and Zn, a portion of the liver was kept at -20°C.

Assay of Serum Hepatic Enzyme :-

Employing a fully computerized biochemical analyzer, serum derived from blood samples was subjected to analytical evaluations of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total protein, incorporating creatinine in plasma.

Estimation of biochemical parameters :-[4]

The retro-orbital plexus of the mice from where the blood samples checked after the hepatotoxicity induction in rats after day 21 of the adminstration with GSE. The biological parameters of SGOT, SGPT, and other tests were performed on the isolated serum. Alkaline Phosphatase [ALP] in all groups were measured.

Acute toxicity studies on different extracts of *Glycyrrhiza glabra :-*

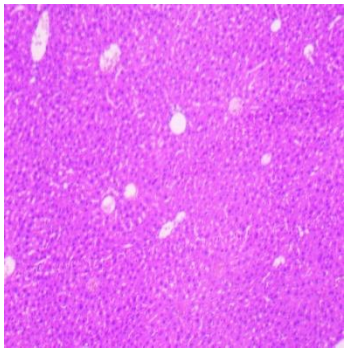
The acute toxicity of various concentrate of *Glycyrrhiza glabra* was conceded. The research

investigation was approved as stated in subsection 4.2.7 along with in accordance with the OECD-423 criteria for the safe dosage administering to animals. The results of the acute toxicity test indicated that the LD50 values of different *Glycyrrhiza glabra* concentrates were high, which seemed to indicate the acceptability of such preparations. A death rate of absolutely nothing across different concentrate of *Glycyrrhiza glabra* was showed at the doses of 2000mg/kg. additionally, to these the animals were noted continuously for four hours at first instance, and then at an interval of two hours for 24 hours to examine any change occurs (or) noted in the mice behaviour such as respiration, writhing, impulses, weakness in muscles, squeezing diarrhoea, CNS excitement increased anxiety, and dietary intake and mortality has been showed

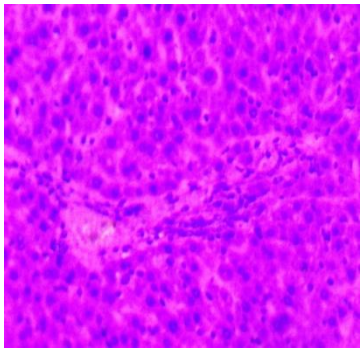
Experimental protocol- Mice were distributed into 6 groups of 5 mice all at random and equally, and group were define as-

- **Group 1 (Control Group):** Mice were administered with normal saline orally for 21 days.
- **Group 2 (Arsenic induced Group):** Mice were given Arsenic orally to induce Hepatotoxicity Disease up to 14 days..
- **Group 3 (Standard Group):** Mice were given Arsenic orally to hepatotoxicity Disease up to 14 days and then administered marketed drug silymarin .
- **Group 4 (test group Liquorice100mg/kg + pyridoxial 5 phosphate):** Mice were given Arsenic to induce Hepatotoxicity Disease up to 14 days and then administered aqueous extract of liquorice extract 100 mg/kg + pyridoxial 5 phosphate Orally (*p.o*) for 7 days.
- **Group 5 (test group Liquorice200mg/kg + pyridoxial 5 phosphate):** Mice were given Arsenic to induce Hepatotoxicity Disease up to 14 days and then administered aqueous extract of liquorice extract 200 mg/kg + pyridoxial 5 phosphate Orally (*p.o*) for 7 days
- **Group 6 (liquorice extract):** Administered liquorice aqueous extract (200 mg/kg) Orally (*p.o*) for 21 days.

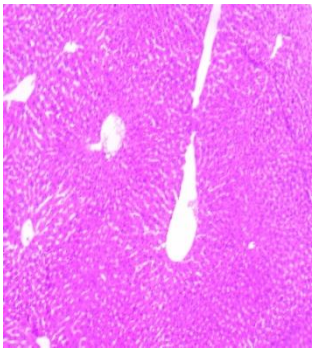
6.0 Histological changes of liver



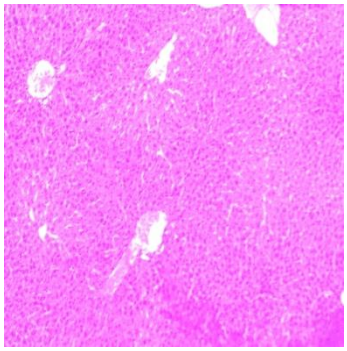
Control Group



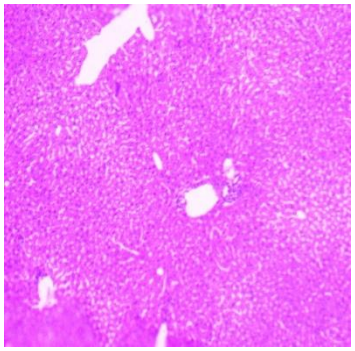
Toxin Group (Aesenic)



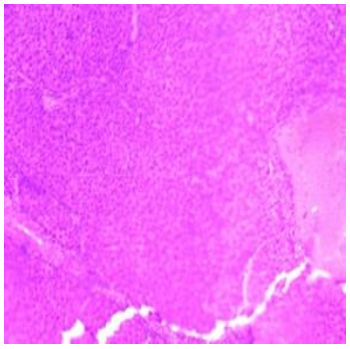
Toxin+ Standard



Toxin+Low Dose(100mg/kg



Toxin+ High Dose(200mg/kg



Test group

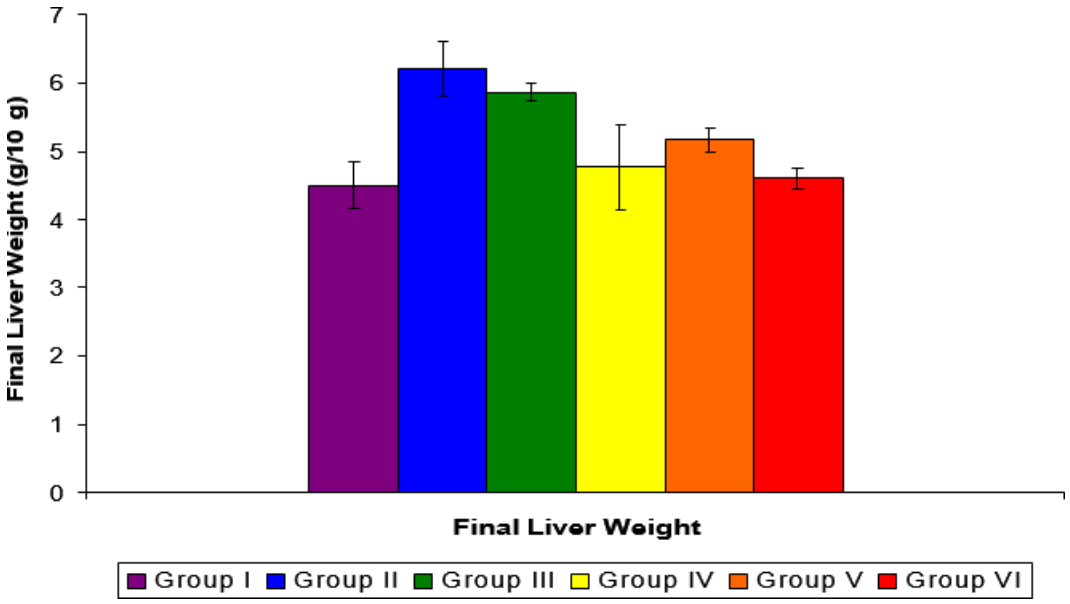
Results :- Effect of *Glycyrrhiza glabra* Aqueous extract (GGA) and Pyridoxial 5-phosphate (PLP) on average liver weight changes in normal and Arsenic Induced Hepatotoxicity in Mice Model**.**

Table 6.7 and Fig.6.1 appeared action of Aq. extract of *Glycyrrhiza glabra* and PLP mean variations in the liver's weight between healthy and mice given Arsenic to cause harm to the liver. The hepatotoxic control group of mice were exhibited increased weight of liver. The use of GGA was silymarin-treated connecting repaired the liver weight while delayed the liver's volume decreased.

- **Table 6.7 Influence of different kinds of *GGA* and Pyridoxial 5-phosphate (PLP) preparation on variations in median liver weight in animals**

Group	Weight of Liver in g/100g
GroupI	4.49±0.34
Group II	6.21 ± 0.40^{a}**
GroupIII	5.87 ± 0.12^{b}*
GroupIV	4.76 ± 0.62^{b}**

GroupV	5.17±0.18[b]*
Group VI	4.60±0.15[b]**

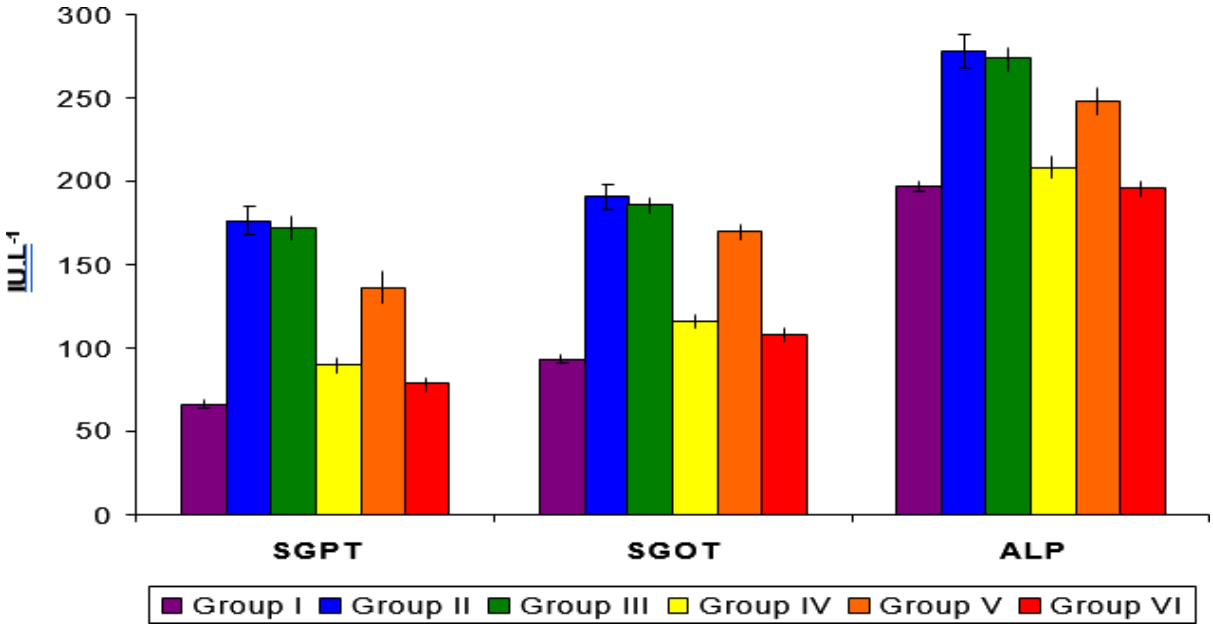


- **Fig 6.1 Effect of various extract of GGA and PLP on Hepatic enzymes and additional measurements in animals with liver damage brought on by Arsenic**

- **Table 6.8 Effect of various extract of GGA and PLP on the blood markers SGPT, SGOT, ALP, as well as GGT in animals with liver damage brought on by Arsenic**

Class	SGPT ($IU.L^{-1}$)	SGOT ($IU.L^{-1}$)	ALP ($IU.L^{-1}$)	GGT ($IU.L^{-1}$)
Group I (Control)	65.89±2.76	93.56±2.43	197.23±3.18	1.29±0.22
Group II (Disease control)	89.26±4.76[b]**	115.63±4.58[b]***	208.34±6.21[b]**	1.48±0.05[b]*
Group III (standard group)	172.12±6.52[b]**	185.48±4.34[b]**	273.65±6.87[b]**	1.89±0.12[b]**

Group IV (High) (GGA+PPD)	176.34±8.43[a]**	190.45±7.65[a]**	278.23±10.22[a]**	**1.96±0.14[a]****
Group V (Low) (GGA+PPD)	146.13±8.65[b]**	197.05±4.03[b]**	281.24±3.88[b]**	**1.91±0.18[b]****
Group VI (GGA)	**136.12±9.65[b]****	**169.65±4.23[b]****	**248.24±8.38[b]****	**1.64±0.12[b]****



- **Conclusion :-** The study conclusion that the synergistic effects of liquorice and pyridoxal 5-phosphate (PLP) offer promising therapeutic potential in mitigating arsenic-induced hepatotoxicity. Through comprehensive experimentation and analysis, this study has elucidated the enhanced hepatoprotective efficacy of the combination therapy compared to individual treatments or arsenic exposure alone .

- In both tests, extract dosages of 100 mg/kg have shown superior outcome than 200 mg/kg body weight.

Reference :-

1. Argaluza J, Domingo-Echaburu S, Orive G, Medrano J, Hernandez R, Lertxundi U. Environmental pollution with psychiatric drugs. World J Psychiatry. 2021 Oct 19;11(10):791-804. doi: 10.5498/wjp.v11.i10.791. PMID: 34733642; PMCID:

PMC8546762.

2. Khandelwal, K. R. “Practical Pharmacognosy,” Ninth Edition, Nirali prakashan, Delhi, 2002, pp. 149-153.

3. Kokate, C.K. (2014) Practical Pharmacognosy. 5th Edition, Vallabh Prakashan, Delhi, 25-30.

4. Ferdi NEH, Abla K, Chenchouni H. Biochemical Profile of an Adult Diabetic Population from Algeria in Relation with Anthropometric Parameters, Age and Gender. Iran J Public Health. 2018 Aug;47(8):1119-1127. PMID: 30186783; PMCID: PMC6123603