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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *WITHANIA COAGULANS* DUNAL FRUIT EXTRACT AGAINST DRUG-INDUCED HEPATOTOXICITY IN WISTAR RATS

Sugandha[1], Divya Singh[1]*, Aziz Ahmed[1]

[1]Jaipur College of Pharmacy, Jaipur 302033, Rajasthan, India

ABSTRACT

Liver diseases represent a significant global health burden due to increased exposure to drugs, environmental toxins, and metabolic disorders. The search for safe and effective hepatoprotective agents has led to growing interest in medicinal plants with antioxidant and cytoprotective properties. *Withania coagulans* Dunal is a well-known traditional medicinal plant reported to possess various pharmacological activities. The present study was designed to evaluate the hepatoprotective activity of *Withania coagulans* fruit extracts against drug-induced hepatotoxicity in experimental animals. The plant material was subjected to successive extraction using solvents of varying polarity, followed by preliminary phytochemical screening, which revealed the presence of bioactive constituents such as flavonoids, alkaloids, tannins, and saponins. Hepatotoxicity was induced using paracetamol and isoniazid in Wistar rats, and the protective effect of the extracts was assessed by evaluating biochemical markers including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and bilirubin levels. The results demonstrated that treatment with *Withania coagulans* extract significantly ($p < 0.05$) reduced elevated liver enzyme levels and restored biochemical parameters towards normal values when compared to the toxic control group. Histopathological studies further confirmed the protective effect by showing reduced hepatic necrosis, inflammation, and improved cellular architecture in treated groups. The hepatoprotective effect was found to be comparable to the standard drug silymarin. In conclusion, *Withania coagulans* exhibits significant hepatoprotective activity, which may be attributed to its antioxidant and membrane-stabilizing properties. The findings support its traditional use and suggest its potential as a natural alternative for the management of liver disorders.

Keywords: *Withania coagulans*, Liver, Hepatotoxicity, SGPT, SGOT, etc.

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INTRODUCTION

The liver is the largest internal organ of the human body and plays a central role in maintaining metabolic homeostasis. It accounts for approximately 2% of total body weight and performs a wide range of essential physiological functions, including metabolism of carbohydrates, lipids, and proteins, synthesis of plasma proteins, detoxification of xenobiotics, and bile production for digestion. Due to its strategic anatomical location between the gastrointestinal tract and systemic circulation, the liver is continuously exposed to various endogenous and exogenous toxic substances, making it highly susceptible to injury [1,2].

Liver diseases constitute a major global health burden, with conditions such as cirrhosis, fatty liver disease, fibrosis, and viral hepatitis contributing significantly to morbidity and mortality worldwide. Drug-induced liver injury (DILI) is one of the most common causes of acute liver failure and is often associated with commonly used drugs such as paracetamol, antitubercular agents, and alcohol consumption [3,4]. The pathogenesis of liver damage is primarily associated with oxidative stress, lipid peroxidation, and inflammatory responses, which ultimately lead to hepatocellular necrosis and dysfunction [5].

Despite advances in modern medicine, there is still a lack of highly effective and safe hepatoprotective drugs. Synthetic drugs used for liver disorders are often associated with adverse effects and limited efficacy. Therefore, there is an increasing interest in exploring natural products, particularly medicinal plants, as alternative therapeutic agents for liver diseases [6]. Herbal medicines have been widely used in traditional systems such as Ayurveda, Unani, and Traditional Chinese Medicine for the treatment of liver disorders due to their safety, accessibility, and therapeutic potential [7].

Medicinal plants are rich sources of bioactive phytoconstituents such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which possess antioxidant and hepatoprotective properties. These compounds help in scavenging free radicals, stabilizing cell membranes, and enhancing liver regeneration [8]. Several plant-based formulations, such as silymarin derived from *Silybum marianum*, have demonstrated significant hepatoprotective activity and are widely used as standard reference drugs in experimental studies [9].

Withania coagulans Dunal, commonly known as “Paneer Phool” or “Indian cheese maker,” is an important medicinal plant belonging to the family Solanaceae. It is widely distributed in India, Pakistan, and the Middle East and has been traditionally used for the treatment of various ailments including liver disorders, diabetes, inflammation, and digestive problems [10]. The plant is known to contain a variety of phytochemicals such as withanolides, alkaloids, flavonoids, tannins, and essential oils, which contribute to its diverse pharmacological activities [11].

Previous studies have reported that *Withania coagulans* exhibits significant antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective properties. The hepatoprotective effect is mainly attributed to its ability to reduce oxidative stress, inhibit lipid peroxidation, and improve the activity of endogenous antioxidant enzymes [12,13]. Moreover, the presence of steroidal lactones (withanolides) plays a crucial role in stabilizing hepatic cell membranes and promoting liver regeneration [14,15].

In view of its traditional usage and promising pharmacological profile, the present study aims to evaluate the hepatoprotective activity of *Withania coagulans* fruit extracts using drug-induced hepatotoxicity models in experimental animals. The study also seeks to validate its traditional claims and explore its potential as a natural alternative to synthetic hepatoprotective agents.

Collection and Authentication of Plant Material

The fruits of *Withania coagulans* were collected from Jaipur. These fruits were authenticated by Botanical Survey of India, Jodhpur with reference no BSI/AZC/2025/52. The collected fruits were washed, shade-dried, and coarsely powdered using a mechanical grinder for further experimental studies.

Preparation of Extracts

The dried powdered material of *Withania coagulans* fruits was subjected to successive extraction using solvents of increasing polarity such as petroleum ether, chloroform, ethanol, and methanol using a Soxhlet apparatus. The extraction process was continued until the solvent in the siphon tube became colorless. The extracts were concentrated under reduced pressure using a rotary evaporator and then dried in a desiccator. The percentage yield of each extract was calculated and stored in airtight containers for further use [16].

Phytochemical Screening

Preliminary phytochemical screening of various extracts was carried out to detect the presence of different classes of phytoconstituents such as alkaloids, flavonoids, tannins, saponins, steroids, and glycosides using standard qualitative methods [17].

Experimental Animals

Healthy adult Wistar albino rats (140–180 g) of either sex were procured and housed under standard laboratory conditions (25 ± 2°C, 55–65% humidity, 12 h light/dark cycle). Animals were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines [18].

Acute Toxicity Study

Acute oral toxicity study was performed according to OECD guideline 423. The extract was administered orally at different dose levels, and animals were observed for behavioral changes and mortality for 24 hours and further monitored for 14 days to determine the safe dose range [19].

Induction of hepatotoxicity by paracetamol

Paracetamol was prepared in 0.5% sodium carboxy methyl cellulose (CMC) solution and used for oral administration. Previous experiments for dose finding indicate paracetamol 2g/kg was selected as the toxicant dose in the present study [20]

Preparation of Silymarin

Silymarin was dissolved in normal saline and 100mg/kg per oral (p.o.) dose was selected as a standard [21].

Experimental Design

In the experiment, a total of 25 Wistar Rat were used. The Wistar Rat were divided into 5 groups,

comprising of 5 animals in each group

Group I: Normal control Wistar Rat received 1ml/100 g of 0.5% sodium carboxy methyl cellulose (CMC) using an intragastric tube for 7days.

Group II: Negative control Wistar Rat received paracetamol 2g/kg, p.o. for inducing hepatotoxicity on 6th day

Group III: Wistar Rat received silymarin 100 mg/kg, p.o. for 7 days and paracetamol 2 g/kg, p.o. on 6th day.

Group IV Wistar Rat received total alcoholic extract of *W. Coagulans* 100mg/kg once daily for 7 days and paracetamol 2 g/kg, p.o. on 6th day.

Group V Wistar Rat received methanolic extract of *W. Coagulans* 100mg/kg once daily for 7 days and paracetamol 2 g/kg, p.o. on 6th day.

Sample collection

At the end of the experiment on 7th day, Blood was collected by orbital puncture and allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 revolution per minute (rpm) at 30°C for 15 minutes. Wistar Rats were sacrificed by cervical dislocation for liver histopathology.

Induction of hepatotoxicity by isoniazid

Isoniazid (INH) solution was prepared in sterile distilled water. Wistar Rats were treated with INH at the dose of (100 mg/kg b. wt., i.p.) to the experimental animals for 10 days [22].

Experimental Design

In the experiment, a total of 25 Wistar Rat were used. The Wistar Rats were divided into 5 groups, comprising of 5 animals in each group.

Group I: Normal control Wistar Rat received 1ml/100 gm of 0.5% sodium CMC using an intragastric tube for 10 days.

Group II: Negative control Wistar Rat received isoniazid 100 mg/kg, i.p. for inducing hepatotoxicity

Group III: Wistar Rat received silymarin (100 mg/kg, p.o.) for 10 days and isoniazid100 mg/kg, i.p. for 10 days.

Group IV Wistar Rat received total alcoholic extract of *W. Coagulans* 100 mg/kg once daily for 10 days and isoniazid 100 mg/kg, i.p. for 10 days.

Group V Wistar Rat received methanolic extract of *W. Coagulans* 100 mg/kg once daily for 10 days and isoniazid 100 mg/kg, i.p. for 10 days.

Biochemical Analysis

Blood samples were collected and serum was separated for estimation of liver function biomarkers including: SGOT (AST), SGPT (ALT), Alkaline phosphatase (ALP), Total bilirubin These biochemical parameters serve as sensitive indicators of hepatocellular injury [23].

Histopathological Studies

Liver tissues were excised and fixed in 10% formalin. The tissues were processed, embedded in paraffin,

sectioned, and stained with hematoxylin and eosin (H&E) for microscopic examination of histological changes such as necrosis and inflammation [24].

Statistical Analysis

All data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant [25].

RESULTS AND DISCUSSION

Percentage Yield of Extracts

The successive extraction of *Withania coagulans* fruits with solvents of increasing polarity yielded different fractions. Among all extracts, the methanolic and ethanolic extracts showed higher percentage yield as compared to petroleum ether and chloroform extracts. This indicates that polar solvents are more effective in extracting bioactive phytoconstituents from the plant material. The variation in extractive values may be attributed to the differential solubility of phytochemicals in various solvents [26].

Table 5.1: Extractive value of extracts of *W. Coagulans*

S. No.	Extract name	Color/consistency	Percentage Yield
1	Total alcoholic	Dark brown/powder	47.43
2	Pet. ether	Yellow/sticky	01.81
3	Chloroform	Reddish brown/powder	04.48
4	Ethanolic	Dark reddish brown /powder	22.30
6	Methanolic	Reddish brown /powder	10.28
7	Water	Brown/powder	03.19

Phytochemical Screening

Preliminary phytochemical analysis revealed the presence of major bioactive constituents such as alkaloids, flavonoids, tannins, saponins, steroids, and glycosides in the extracts. The methanolic and ethanolic extracts showed a higher abundance of flavonoids and phenolic compounds, which are known for their antioxidant properties.

These phytoconstituents are reported to play a significant role in hepatoprotection by scavenging free radicals, inhibiting lipid peroxidation, and stabilizing cellular membranes. The presence of withanolides, a characteristic steroidal lactone of *Withania coagulans*, further supports its pharmacological potential [27,28].

Table 2. Phytochemical analysis of different extracts of *W. Coagulans*

Phytochemicals	Total Alcoholic	Pet. ether	Chloroform	Methanol	Water
Carbohydrates	++	+	-	+	+
Glycoside	+	-	-	++	-
Steroids	+	+	+	-	-

Saponins		++	-	-	+	+
Flavonoids		++	+	+	+++	+
Alkaloids	Dragendroff	+	+	-	+	+
	Wagner	+	+	-	+	+
Tannins and phenolic compoun	5% $FeCl_3$	++	-	-	++	++
	Lead Acetate	+	+	+	-	+
Protein and amino Acids	Biurete	+	-	-	-	+
	Sulpher contaning protein	+	-	-	-	++

Where '+' = Positive test, '-' = Negative test, '+' = Less color, '++' = Moderate, color, '+++' = Intense color

Acute Toxicity Study

The acute toxicity study conducted as per OECD guideline 423 revealed that the *Withania coagulans* extract was safe up to the tested dose levels. No mortality or significant behavioral changes were observed in experimental animals during the observation period. Based on these findings, suitable doses were selected for further hepatoprotective studies.

These results suggest that the extract possesses a wide safety margin, supporting its traditional use in herbal medicine [29].

Table 3: Acute toxicity study of petroleum ether extracts of *W. coagulans*

S. No.	Dose (mg/kg b.wt)	Numbers of animals	Observation
1	5	3	All animals survived
2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived
6	1000	3	All animals survived
7	2000	3	All animals survived

Table 4: Acute toxicity study of ethanol extracts of *W. coagulans*

S. No.	Dose (mg / kg b. wt)	Numbers of animals	Observation
1	5	3	All animals survived
2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived

6	1000	3	All animals survived
8	2000	3	All animals survived

Mortality was not observed in any groups of rats up to the dose level of 5000 mg/kg body weight. As per OECD guideline, these extracts come under the category -5 Hence, these are safe. The LD_{50} as per OECD guideline, this extract under category was found to be 2000 mg/kg. The LD_{50} of these extract should be effective 1/4 to 1/20. Therefore, dose of petroleum ether, ethanolic extract, methonolic extract of *W.coagulans* for hepatoprotective was found to be 100 mg/kg b. wt.

Effect on Biochemical Parameters

Administration of paracetamol and isoniazid resulted in a significant ($p < 0.05$) increase in serum levels of SGOT (AST), SGPT (ALT), ALP, and bilirubin in the toxic control group, indicating severe hepatic damage. Treatment with *Withania coagulans* extract at different doses significantly ($p < 0.05$) reduced the elevated levels of these enzymes compared to the toxic control group. The results were comparable to the standard drug silymarin.

The normalization of these biochemical markers indicates stabilization of hepatocyte membranes and regeneration of liver cells [30,31].

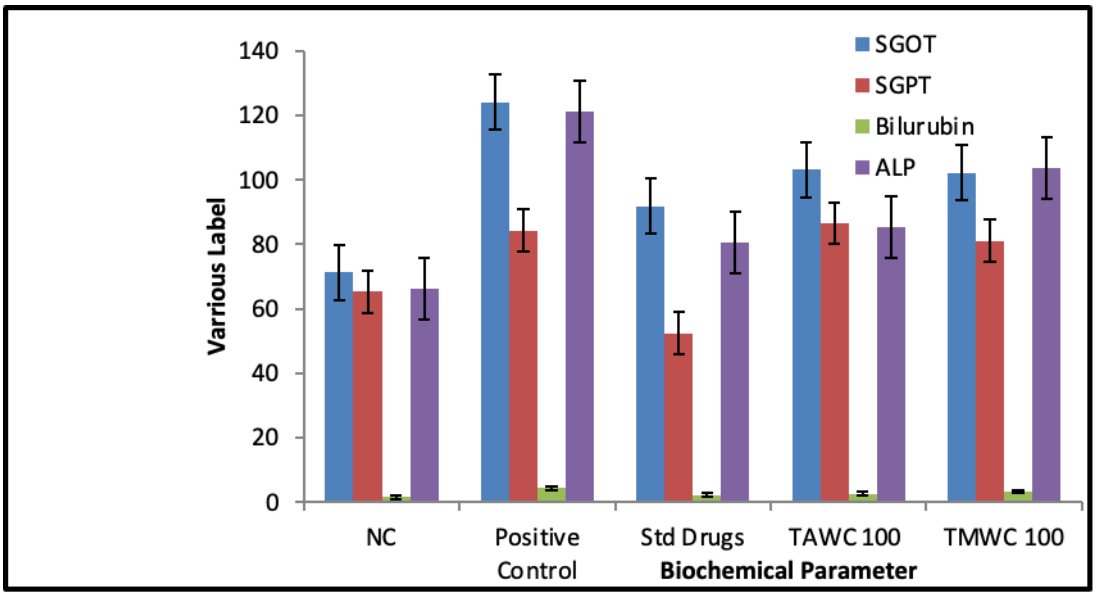


Figure 1. Effect of *Withania coagulans* extract on Biochemical Parameters

On the basis of biochemical parameters analysis for paracetamol induced hepatotoxicity, control group animals showed normal level of all enzymes SGOT (71.23 ± 0.48 u/l), SGPT (62.73 ± 2.94 u/l), total bilirubin (1.36 ± 0.17 mg/dl), ALP (64.23 ± 1.46 u/l). After treatments with paracetamol on negative control group, enzymes level were found to be elevated SGOT (126.07 ± 3.78 u/l), SGPT (87.28 ±2.76 u/l), total bilirubin (4.19 ± 0.28 mg/dl), ALP (124.18 ± 2.31 u/l) compared to normal group treated animals.Methanolic extract of *W. Coagulans* at the dose of 100 mg/kg b. wt. produce more significant hepatoprotective activity as compare to total alcoholic extract of *W. Coagulans* at the dose of 100 mg/kg b. wt. dose.

Histopathological Observations

Histopathological examination of liver tissues supported the biochemical findings:

Normal control group: Showed normal hepatic architecture with intact hepatocytes

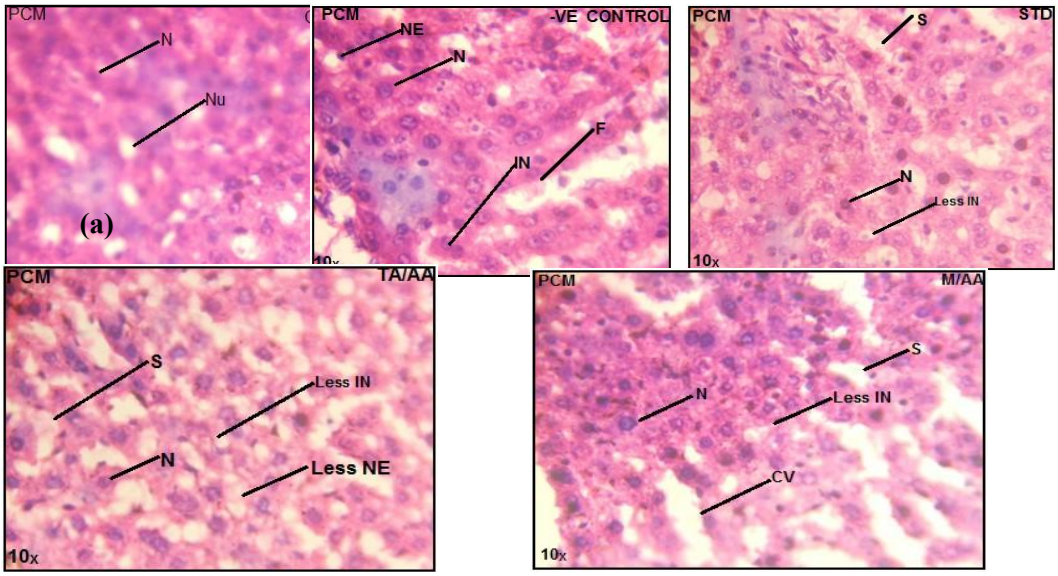
Toxic control group: Showed severe necrosis, fatty degeneration, inflammatory cell infiltration, and loss of cellular integrity

Standard (Silymarin) group: Showed near-normal architecture with minimal damage

Test groups: Demonstrated dose-dependent protection with reduced necrosis, decreased inflammation, and improved cellular structure

The protective effect observed in treated groups confirms the hepatoprotective potential of *Withania coagulans* extract [32].

Histopathology of paracetamol induced Hepatotoxicity in Wistar Rat liver



* N or H- Hepatocytes, Nu- Nucleus, * NE- Necrosis, N or H- hepatocytes, F- Fibrosis, IN- Inflammatory cell, IN- Inflammatory cell, N or H- hepatocytes, S- sinusoids, NE- Necrosis

Figure 2 (a) Histological section of group I (control) animals in paracetamol induced hepatotoxicity showed normal hepatocytes with well-preserved cytoplasm, nucleus and central vein. There was no sign of inflammation, fatty change or necrosis in these animals Histological section of group II (negative control paracetamol treated) animals in paracetamol induced hepatotoxicity showed hepatic injury induced by paracetamol showed massive fatty changes, gross necrosis, and disturbed sinusoids with a prominent infiltrate of neutrophil polymorphs (c) Histological section of group III (silymarin) animals in paracetamol induced hepatotoxicity showed almost normal liver lobule with no sign of necrosis in the centrizonal area. Only a few inflammatory cells were observed in the centrizonal area. (d) Histological section of group IV (Total alcoholic extract of *W. Coagulans* 100 mg/kg) animals in paracetamol induced hepatotoxicity showed greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein. (e) Histological section of group V (methanolic extract *W. Coagulans* 100 mg/kg) animals in paracetamol induced hepatotoxicity showed less reduction of necrosed area and inflammatory infiltrates in the centrizonal area.

Histopathological examination of liver sections obtained from Wistar Rat of different treatment

substantiated the observation of enzyme study. In paracetamol treated group centrilobular necrosis was prominent. The hepatic cells were depleted of cytoplasm and vacuolated hepatic cells were observed. Cell boundries were indistinct. In total alcoholic extract and methanolic extract of *W. Coagulans* groups necrosis were portal. Lobular vein was prominent with blood cells. Cell nucleus and nucleoli were prominent. Cytoplasm was hyalinised. The cell walls were shrunken and vacuolization was less. The total alcoholic extract and methanolic extract of *W. Coagulans* were active but methanolic extract has more potential then total alcoholic extract of *W. Coagulans*.

Conclusion

The present study demonstrated that *Withania coagulans* Dunal fruit extract possesses significant hepatoprotective activity against drug-induced hepatotoxicity in experimental animals. The extract showed the presence of important phytoconstituents such as flavonoids, alkaloids, tannins, and withanolides, which are known to contribute to antioxidant and cytoprotective effects. Administration of hepatotoxic agents like paracetamol and isoniazid resulted in marked elevation of liver biomarkers, including SGOT, SGPT, ALP, and bilirubin, indicating severe hepatic injury. However, treatment with *Withania coagulans* extract significantly restored these biochemical parameters toward normal levels and improved liver function. Histopathological observations further confirmed the protective effect by demonstrating reduced necrosis, inflammation, and preservation of normal hepatic architecture in treated groups. The hepatoprotective effect of the extract was found to be comparable to the standard drug silymarin, suggesting its potential as a natural therapeutic agent. The observed activity may be attributed to its antioxidant properties, inhibition of lipid peroxidation, and stabilization of hepatocellular membranes. In conclusion, the findings of this study scientifically validate the traditional use of *Withania coagulans* in the treatment of liver disorders and highlight its potential as a safe and effective alternative to synthetic hepatoprotective drugs. Further studies involving isolation of active constituents and clinical evaluation are recommended to establish its therapeutic applicability.

REFERENCES

1. Mitra, V., & Metcalf, J. (2009). Metabolic functions of the liver. *Anaesthesia and Intensive Care Medicine, 10*(7), 334–335.
2. Vaja, R., & Ghuman, N. (2017). Drugs and the liver. *Anaesthesia and Intensive Care Medicine, 19*(1), 30–34.
3. Chiang, J. Y. L. (2014). Liver physiology: Metabolism and detoxification. In L. M. McManus & R. N. Mitchell (Eds.), *Pathobiology of human disease* (pp. 1770–1782). Elsevier.
4. Reinke, H., & Asher, G. (2016). Circadian clock control of liver metabolic functions. *Gastroenterology, 150*(3), 574–580.
5. Allameh, A., Niayesh-Mehr, R., Aliarab, A., Sebastiani, G., & Pantopoulos, K. (2023). Oxidative stress in liver pathophysiology and disease. *Antioxidants*, *12*(9), 1653.
6. Del Prete, A., Scalera, A., Iadevaia, M. D., Miranda, A., Zulli, C., Gaeta, L., ... & Loguercio, C. (2012). Herbal products: benefits, limits, and applications in chronic liver disease. *Evidence-based*

Complementary and Alternative Medicine, *2012*(1), 837939.

7. Singh, D., Cho, W. C., & Upadhyay, G. (2016). Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. *Frontiers in physiology*, *6*, 363.
8. Hong, M., Li, S., Tan, H. Y., Wang, N., Tsao, S. W., & Feng, Y. (2015). Current status of herbal medicines in chronic liver disease therapy: the biological effects, molecular targets and future prospects. *International journal of molecular sciences*, *16*(12), 28705-28745.
9. Singla, R. K., Singh, D., Verma, R., Kaushik, D., Echeverría, J., Garg, V., ... & Shen, B. (2024). Fermented formulation of silybum marianum seeds: Optimization, heavy metal analysis, and hepatoprotective assessment. *Phytomedicine*, *124*, 155286.
10. Ashtekar, A. (2026). Withania coagulans (Stocks) Dunal. In *Medicinal and Aromatic Plants of India, Vol. 4* (pp. 449-463). Cham: Springer Nature Switzerland.
11. Awuchi, C. G. (2020). The biochemistry, toxicology, and Uses of the acologically active phytochemicals: Alkaloids, terpenes, polyphenols, and glycosides. *Merit Research Journals*, *5*(1), 6-21.
12. Yasmin, A., Jindal, S., Monga, V., Gupta, G. D., & Goyal, K. (2025). Withania coagulans: a comprehensive exploration of its botanical, phytochemical, and pharmacological properties. *Medicinal Chemistry Research*, *34*(8), 1663-1687.
13. Kumar Bharti, S., Krishnan, S., Kumar Sharma, N., Kumar, A., Prakash, O., Kumar Gupta, A., & Kumar, A. (2015). In vivo and in silico investigation of antidiabetic activity of fruit of Withania coagulans Dunal. *Current hypertension reviews*, *11*(2), 143-158.
14. Misico, R. I., Nicotra, V. E., Oberti, J. C., Barboza, G., Gil, R. R., & Burton, G. (2011). Withanolides and related steroids. *Progress in the Chemistry of Organic Natural Products Vol. 94*, 127-229.
15. Kumar, P., Banik, S. P., Goel, A., Chakraborty, S., Bagchi, M., & Bagchi, D. (2024). Revisiting the multifaceted therapeutic potential of Withaferin A (WA), a novel steroidal lactone, W-ferinAmax ashwagandha, from Withania Somnifera (L) Dunal. *Journal of the american nutrition association*, *43*(2), 115-130.
16. Kumar, P., Banik, S. P., Goel, A., Chakraborty, S., Bagchi, M., & Bagchi, D. (2024). Revisiting the multifaceted therapeutic potential of Withaferin A (WA), a novel steroidal lactone, W-ferinAmax ashwagandha, from Withania Somnifera (L) Dunal. *Journal of the american nutrition association*, *43*(2), 115-130.
17. Yadav, M., Chatterji, S., Gupta, S. K., & Watal, G. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int J Pharm Pharm Sci*, *6*(5), 539-42.
18. Patel, S., Patel, S., Kotadiya, A., Patel, S., Shrimali, B., Joshi, N., & Jain, M. (2024). Age-related changes in hematological and biochemical profiles of Wistar rats. *Laboratory Animal Research*, *40*(1), 7.
19. Jonsson, M., Jestoi, M., Nathanail, A. V., Kokkonen, U. M., Anttila, M., Koivisto, P., &

Peltonen, K. (2013). Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. *Food and chemical toxicology*, *53*, 27-32.

20. Kumar, R., & Dubey, A. (2020). Phytochemical investication and heptoprotective evalution acacia rubica extract isonized and paracetamol indused animal toxicity. *Turkish Journal of Physiotherapy and Rehabilitation*, *32*(3), 65-69.

21. Attia, H. G., Mohamed, B. M., Alasmari, S. M., AbdelRahman, S. S., Aleraky, M., Alqahtani, A., ... & Fayed, H. M. (2025). Effect of effervescent formulation of silymarin against experimental hepatotoxicity in rats: involvement of NRF2/HO-1, PI3K/AKT and TLR4/NFκB pathways. *Journal of Applied Veterinary Sciences*, *10*(2), 18-27.

22. Metushi, I., Uetrecht, J., & Phillips, E. (2016). Mechanism of isoniazid-induced hepatotoxicity: then and now. *British journal of clinical pharmacology*, *81*(6), 1030-1036.

23. Hasan, A. H., Abdulzahra, M. A., Hashim, A. M., & Al-Mawlah, Y. H. (2024). Biochemical markers of liver function test (ALT, AST, ALP) in thyroid dysfunction (hyperthyroidism and hypothyroidism). *Journal of Biotech Research*, *16*, 167-174.

24. Aydin, G., Gökçimen, A., Öncü, M., Çicek, E., Karahan, N., & Gökalp, O. (2003). Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. *Turkish journal of veterinary & animal sciences*, *27*(5), 1131-1140.

***Corresponding Author:** Divya Singh, Jaipur College of Pharmacy, Jaipur, Rajasthan, India