Effects of Ashwagandha Root Extract on Hepatic Tissue in a CFA-Induced Rheumatoid Arthritis Model

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Abstract

No previous studies investigated the hepatoprotective potential of *Withania somnifera* (*Ashwagandha*) ethanolic root extract as compared to supplementation of dexamethasone and ibuprofen, alone or in combinations with crude root powder in Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis (RA) rats. Materials: Thirty-six adult male albino rats were randomly assigned to six different groups. Rheumatoid arthritis model was induced by intraplantar administration of 200 µL CFA, and 14-day oral treatment regimens. Liver tissues were excised and fixed with formalin . CFA-induced animals demonstrated extensive hepatic pathology, including lymphocytic infiltration, hepatocellular degeneration and necrosis, sinusoidal dilation, fibroblast proliferation, and vascular congestion — collectively consistent with a systemic inflammatory hepatotoxic response. Oral administration of 300 mg/kg BW of the ethanolic extract resulted in near-total histological restoration of hepatic architecture, apparently the hepatoprotective effects of dexamethasone, ibuprofen and crude powder formulations. Both the traditional pharmacological agents caused chronic hepatic lesions including fatty degeneration, collagen deposition, and necroptotic changes signifying their intrinsic hepatotoxicity. The hepatoprotective effect of the ethanolic extract may be attributed, at least in part, to bioactive constituents such as withanolides, which have been reported to possess anti-inflammatory and antioxidant properties. powerful antioxidant activity.

Keywords: *Withania somnifera* (*Ashwagandha*), Rheumatoid arthritis, Complete Freund's Adjuvant, dexamethasone, ibuprofen.

Introduction

Rheumatoid arthritis (RA) is a long-standing autoimmune inflammatory disease mainly manifested by synovial membrane dysfunction which clinically presents as pain, swelling and stiffness of joints. Such pathological changes increasingly lead to functional limitations and reduced mobility, which finally manifest as irreversible physical disability and morphological damage of the affected joints[1]. Therefore, effective early diagnosis and early treatment of the disease are of paramount importance in limiting disease progression and avoiding long-term sequelae. The current study intended to compare the efficacy of standard pharmacological agents that are both steroidal and non-steroidal anti-inflammatory drugs, with Withania somnifera (*Ashwagandha*) as a possible therapeutic agent.

Withania somnifera (WS) is widely used in traditional medicine for musculoskeletal

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and joint function as well as for its antiinflammatory properties. Due to the well-characterized pharmacological properties possessed by this plant, it is commercially available in different supplementary forms including capsules, tablets and powder, and is relatively safe for short term application in general population. [2] .

The withanolides are considered to be among the most bioactive constituents of Withania somnifera — a class of naturally occurring, predominantly plant-derived steroidal lactones. These compounds have been reported to be the active principle of the pharmacodynamic action of the plant in relation to the anti-inflammatory and immunomodulatory activity in the course of arthritic diseases. Recent studies suggest that withanolides strongly inhibit inflammatory signal transduction pathways by inhibiting the production of proinflammatory cytokines (e.g., Tumor Necrosis Factor α (TNF-α) and Interleukin-6 (IL-6)) which are key pathogenic mediators of the initiation and persistence of RA. Moreover, withanolides act as strong scavengers of radicals, and reduce free radical-induced oxidative injury in joint tissues, which can preserve cartilage, and decrease pain and inflammatory edema.. In light of these findings, the therapeutic application of *Ashwagandha* and its withanolide derivatives is increasingly regarded as a promising adjunctive treatment modality for patients with Rheumatoid Arthritis, particularly those who experience adverse effects associated with conventional pharmacotherapy, including corticosteroids [3]

Material and Methods

Source and Preparation of Plant Extract

Roots of W. somnifera were purchased from local grain markets in Kirkuk and Baghdad cities, Iraq. The roots were cleansed, dried under shade and powdered to fine powder. The powder material was eluted with 70% ethanol. The filtered extract was concentrated under reduced pressure, evaporated to dryness and kept at 4 °C till further use.

Phytochemical Screening

Preliminary phytochemical screening of the ethanolic root extract was done to identify the primary bioactive agents (flavonoids, phenols, alkaloids, tannins, saponins, glycosides, steroids and terpenoids) following standard procedures [4] .

Experimental Animals

The animals Albino male rats (300–350 g weight and 3–4 months old) were purchased from the Laboratory Animal House, Faculty of Veterinary Medicine, Tikrit University. The animals were acclimatized for 1 month and were kept under controlled environment (25 °C, 12 h light/dark cycle) with ad libitum standard pellet diet and water.

Experimental Design

Thirty-six rats were randomly assigned into six groups (n = 6):

- Group I non-induced) normal saline).
- Group II: CFA-induced RA + normal saline [5] .
- Group III: CFA-induced RA + ethanolic root extract of Withania somnifera (300 mg/kg, orally for 14 days) [6] .
- Group IV: CFA induced RA + Withania somnifera root powder (10% mg/kg, orally) [7] .

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- Group V: CFA-induced RA + dexamethasone (0.1 mg/kg, orally for 14 days) [8] .
- Group VI: CFA-induced RA + ibuprofen (40 mg/kg, orally for 14 days) [9] .
- Treatment was started on day 8 and ended at the end of day 21.

Induction of Rheumatoid Arthritis

Arthritis was induced by injecting 200 µL of Complete Freund's Adjuvant (CFA; Sigma, USA) into the plantar surface of the right hind paw with a 25-gauge needle on day [10] . Redness, swelling, and increased sensitivity (hyperalgesia), signs of inflammation, appeared within hours and reached the peak within 12–24 h.

Histological study

All animals were sacrificed following the administration of a lethal dose of ketamine-xylazine anesthetic combination. The organs (liver and kidneys) were immediately harvested and fixed in 10% neutral buffered formalin. Tissue processing was performed in accordance with the protocol described by [11]

Results and Discussion

Control Group

The histopathological findings of the present study demonstrated that the hepatic tissue of the healthy control group exhibited a fully preserved normal architecture. The central vein (CV) appeared with its typical morphology, surrounded by well-organized hepatocytes (HC) arranged in regular radial cords. The sinusoids (S) were of normal caliber and configuration, and Kupffer cells (KC) were identified along the sinusoidal lining, fulfilling their established immunosurveillance role within the hepatic parenchyma (Figure 1).

CFA-Injected Group

Marked histopathological alterations were observed in the group subjected to Complete Freund's Adjuvant (CFA) injection. The hepatic sections revealed a constellation of advanced pathological changes, including: central vein wall thickening (CVWT), prominent lymphocytic infiltration (LI), presence of fibroblasts (FB), vascular congestion, hepatocellular degeneration (D), hepatocellular necrosis (N), hemolysis (He), and sinusoidal dilation (S) (Figures 2 and 3).

Alcoholic Extract of *Ashwagandha* Root (300 mg/kg BW)

Histopathological examination of liver sections from the group administered the alcoholic extract of *Ashwagandha* (Withania somnifera) root powder at a dose of 300 mg/kg body weight — in which rheumatoid arthritis had been induced via CFA — revealed a substantial improvement compared with all other CFA-treated groups, including: the group supplemented with 10% *Ashwagandha* root powder in standard diet, the Dexamethasone-treated group (0.1 mg/kg BW), and the Ibuprofen-treated group (40 mg/kg BW). Specifically, lymphocytic infiltration and hepatocellular degeneration and necrosis were reduced to trace levels, with no detectable fibroblast formation, central or hepatic vein wall thickening, hemolysis, vascular congestion, or sinusoidal dilation (Figures 4 and 5).

10% *Ashwagandha* Root Powder Diet, Dexamethasone (0.1 mg/kg BW), and Ibuprofen (40 mg/kg BW) Groups

In contrast, the groups fed a standard diet supplemented with 10% *Ashwagandha*

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root powder, as well as those treated with Dexamethasone (0.1 mg/kg BW) or Ibuprofen (40 mg/kg BW) — all of which were subjected to CFA-induced arthritis — continued to exhibit numerous histopathological alterations. These included: central vein wall thickening (CVWT), lymphocytic infiltration (LI), fibroblast presence (FB), hepatocellular degeneration (D), hemolysis (He), necrosis (N), nuclear degeneration (DN), hyaline necrosis (HN), blood vessel wall damage (DV), and hemorrhage (H) (Figures 6,7,8,9,10).

Control Group : The histopathological findings of the present study confirmed that the hepatic tissue of the healthy control group maintained its complete normal architecture. The central vein was readily highlighted in its normal pattern, surrounded by hepatocytes lining up in regular radial cords. Kupffer cells accountable for hepatic immune surveillanc Distinct sinusoids had been simply identifiable. This normal histology provides a morphometric baseline for comparing the pathological change that follows with the intervening experimental groups. These findings are in line with those of [10] which suggested lack of any evidence for inflammation in hepatic tissue from control animals which received seminal normal saline, and agree with many studies showing no morphological changes in liver in control groups of rats.

Hepatic Pathological Changes: Histopathological alterations in CFA-injected group comprise the major clinical findings of current study. Histologically, there was a wide range of effective pathological changes. Central vein wall thickening was prominent with marked lymphocytic infiltration interpreted as a systemic immune response induced by CFA administration. [12] . confirmed that CFA induces a polyarticular systemic inflammatory state reflected across multiple hematological and inflammatory parameters, which supports the interpretation that the hepatic effects observed in the current study represent an extension of systemic inflammatory response rather than a localized phenomenon. At the cellular level, fatty degeneration was prominently observed in hepatocytes as an early indicator of hepatocellular metabolic stress. In the advanced stages documented in Figures 3–4, this degenerative process was accompanied by collagen fiber deposition, indicative of the onset of hepatic fibrosis. In this context, [13] established a mechanistic link between inflammatory effects and the progression of non-alcoholic steatohepatitis (NASH), elucidating the cell death pathways associated with this process. Furthermore, hepatocellular necrosis concomitant with karyolysis was observed, indicating the transition from reversible to irreversible cellular injury. Previous research has recognized central vein dilation and hepatocellular necrosis as key contributors to severe liver injury resulting from systemic inflammation. These findings collectively indicate that CFA has systemic hepatotoxic effects with several levels of integration, which reflect end processes of steatohepatitis in humans.

Extract of *Ashwagandha* And Hepatoprotective Effects: Histopathological images of the liver sections obtained from the extract of *Ashwagandha* alcohol group demonstrated a significant improvement of hepatic architecture. The central vein returned to its normal morphology, the arrangement of hepatocytes and sinusoids with Kupffer cells approached the control group level, and only minor lesions were present. This hepatoprotective impact is associated with the antioxidant and anti-inflammatory effects of withanolides—principal

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bioactive constituents of *Ashwagandha* roots. [14] found that 14 days daily administration of Withania somnifera extract to the rats markedly ameliorated hepatic injury and liver function parameters. This protection was further explained at the molecular level by [15], where protein expression studies showed *Ashwagandha* inhibition of cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β), the same pathways activated by CFA to induce arthritis in the present experiment

The same dose by alcohol extract treated group showed more pronounced histopathological restoration in contrast to 10% *Ashwagandha* root powder fed diet. Residual inflammatory changes (fatty degeneration, necrosis, nuclear degeneration, nuclear condensation, also persistent lymphocytic infiltration) remained there. This difference between powder and extract formulations indicates that the bioactive constituents of the alcoholic extract are concentrated to a greater proportion and perhaps exist in a more bioavailable form than in a crude powder that is mixed into the diet, providing a rationale for the type of the dietary matrix composition that would affect the absorption of withanolides. Recently, a subacute toxicity study of *Ashwagandha* root extract in Wistar rats showed that doses and preparation methods can modulate hepatic responses, with mild hepatic cellular inflammatory infiltration found in certain exposure groups, which corroborates the interpretation of the formulation-related variation noted in our study. [14] .

Discussion and Results Dexamethasone Group, *Potential Hepatic Effects of Dexamethasone Histopathological Findings in the Dexamethasone Group*.Pharmacological Paradox:Analysis of hepatic at least two different pattern in Dexamethasone group and found pharmacological paradox Histological sections revealed sustained fat degeneration, necrosis, and nuclear degeneration, changes that were accompanied by an increase in thickness of the wall of the central and hepatic veins, collagen deposition, and vascular congestion, despite having been well-established as an anti-inflammatory reference drug. The pattern is consistent with the cumulative documented ability of corticosteroids to cause hepatic fatty degeneration as a recognized metabolic side effect, despite their anti-inflammatory action. [13] shows that the dexamethasone induces apoptosis and necroptosis in liver tissue and explains the histopathological changes.mechanism that can explain changes in histopathology. Other studies have shown that moderate dexamethasone doses induce central vein dilation and hepatocellular necrosis in a dose-dependent manner 56, and this evidence is consistent with this study's findings showing that the anti-inflammatory gain with dexamethasone was accompanied by considerable hepatic drawback. [16] .

Ibuprofen Group; NSAID-Related Hepatotoxicity:Similar histological findings, but overall a pattern that was broader than that seen in the Dexamethasone group, of central vein wall thickening, fatty degeneration, necrosis, degenerating nuclei, and lymphocytic infiltration were seen in the Ibuprofen group. These results are consistent with the pharmacological mechanism of NSAIDs and their recognized hepatotoxicity. Studies have shown that Ibuprofen produces hepatic histopathological lesions of severity similar to those produced by high-dose acetaminophen and these are associated with the induction of cholestatic hepatitis. Simultaneously, other studies have validated the incurred systemic

oxidative stress by NSAIDs forced cellular necrosis and fibrosis alongside the concurrent hepatic depletion of glutathione levels. In fact, in some respects the degree of histopathological changes observed in the Ibuprofen group was more pronounced than in the Dexamethasone group, which could be explained by a dual mechanism of cumulative injury, a direct hepatotoxic effect of the drug on top of CFA-induced systemic inflammatory damage [17]

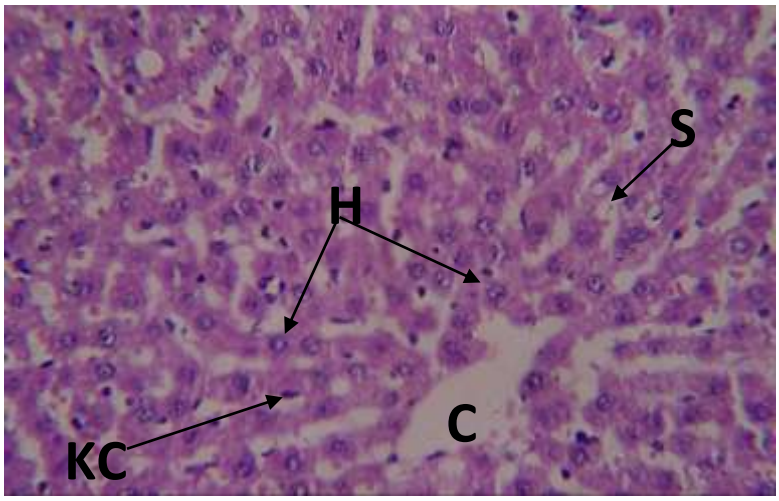

Fig.1. A liver section of the control group demonstrating the normal morphology of the central vein (CV) and hepatocytes (HC), with sinusoids (S) visible between the cells and containing Kupffer cells (KC). H&E ×400.

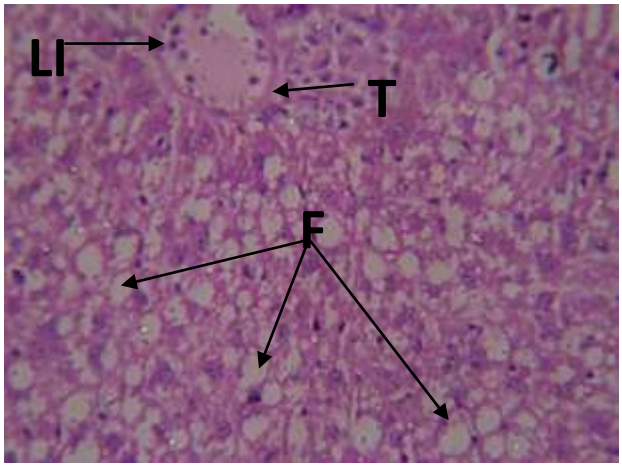

Fig.2. A liver section of the CFA group demonstrating central vein wall thickening (TW) with lymphocytic infiltration (LI), along with the presence of fibers and fatty degeneration (FD) of hepatocytes, indicative of suggestive of inflammatory and degenerative hepatic changes H&E ×400.

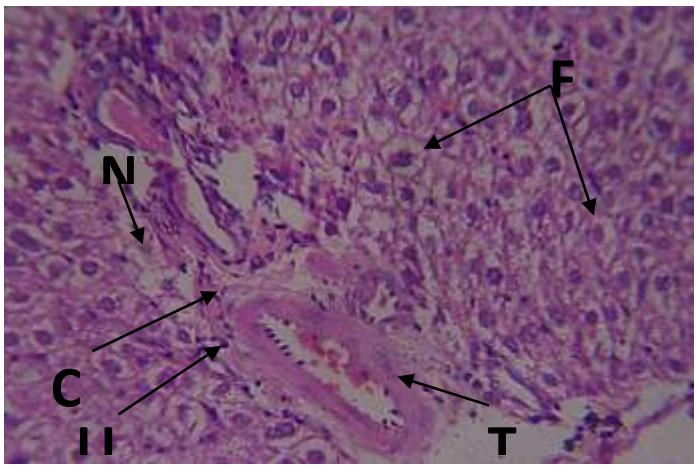

Fig.3.A liver section of the CFA group demonstrating central vein wall thickening (TW) resulting from collagen protein deposition and the presence of collagen fibers (CF), accompanied by lymphocytic infiltration (LI), necrosis (N), and fatty degeneration (FD) of hepatocytes — indicative of advanced-stage

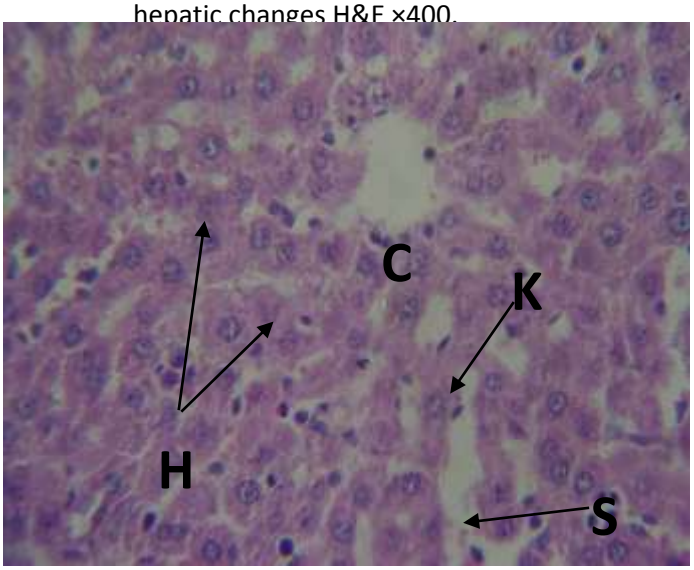

Fig.4. A liver section of the CFA group administered the alcoholic extract of Ashwagandha (*Withania somnifera*) root powder (300 mg/kg BW), demonstrating the normal morphology of the central vein (CV) and hepatocytes (HC), with sinusoids (S) visible between the cells and containing Kupffer cells (KC). H&E ×400.

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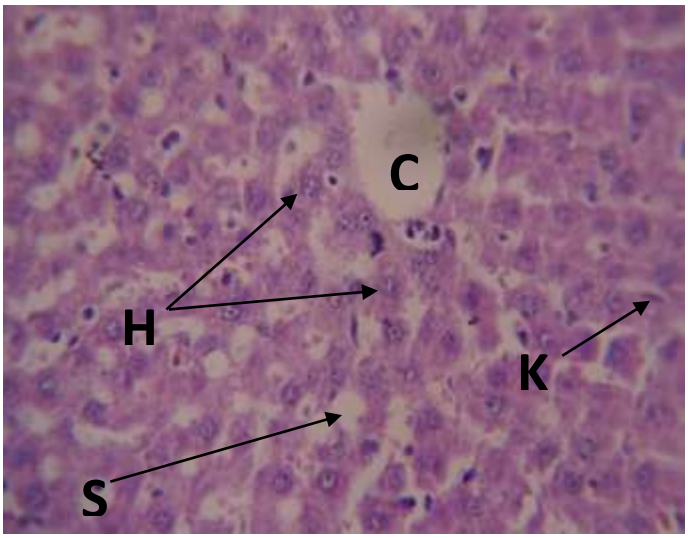

Fig.5. A liver section of the CFA group administered the alcoholic extract of Ashwagandha (*Withania somnifera*) root powder (300 mg/kg BW), demonstrating the restored normal morphology of the central vein (CV) and hepatocytes (HC), with sinusoids (S) visible between the cells and containing Kupffer cells (KC). H&E ×400.

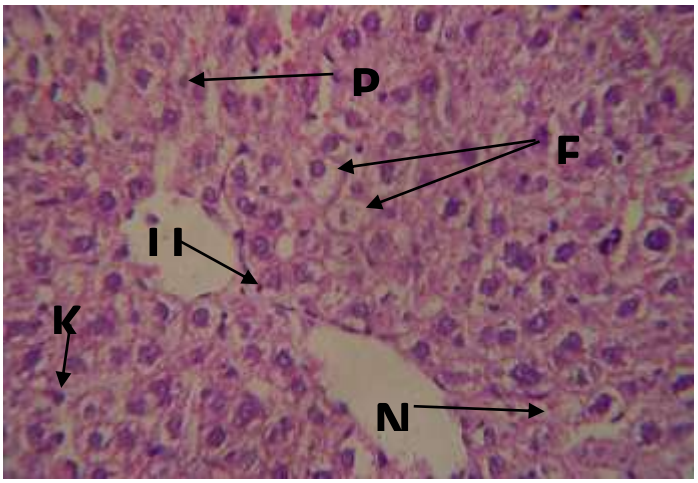

Fig.6. A liver section of the CFA group fed a standard diet supplemented with 10% Ashwagandha root powder, demonstrating fatty degeneration (FD) and necrosis (N) of hepatocytes, with karyolysis (KL) and pyknosis (PN) of some nuclei, along with lymphocytic infiltration (LI). H&E ×400.

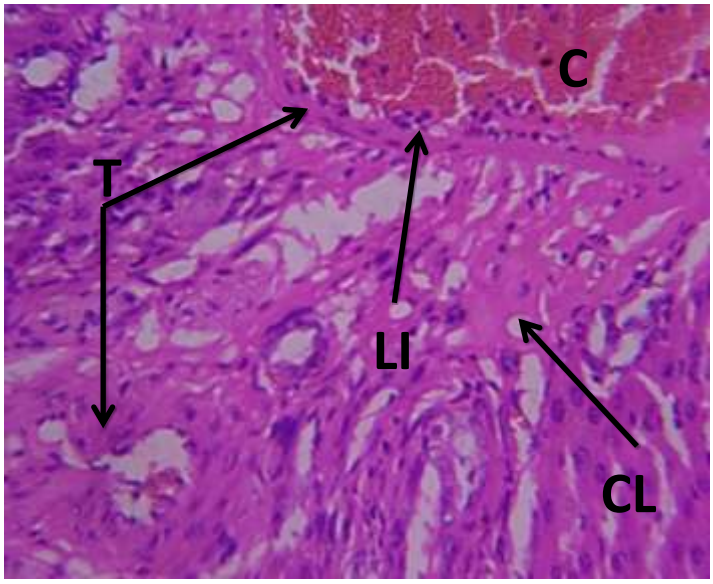

Fig.7. A liver section of the CFA group treated with Dexamethasone (0.1 mg/kg BW), demonstrating central and hepatic vein wall thickening (TW), collagen deposition (CLG), lymphocytic infiltration (LI), and vascular congestion (CON). H&E ×400.

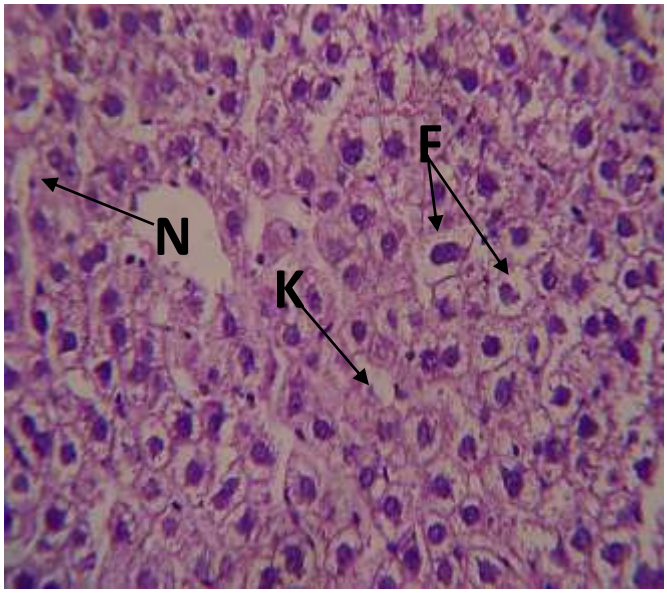

Fig.8. A liver section of the CFA group treated with Dexamethasone (0.1 mg/kg BW), demonstrating fatty degeneration (FD) and necrosis (N) of hepatocytes, with karyolysis (KL) of some nuclei. H&E ×400.

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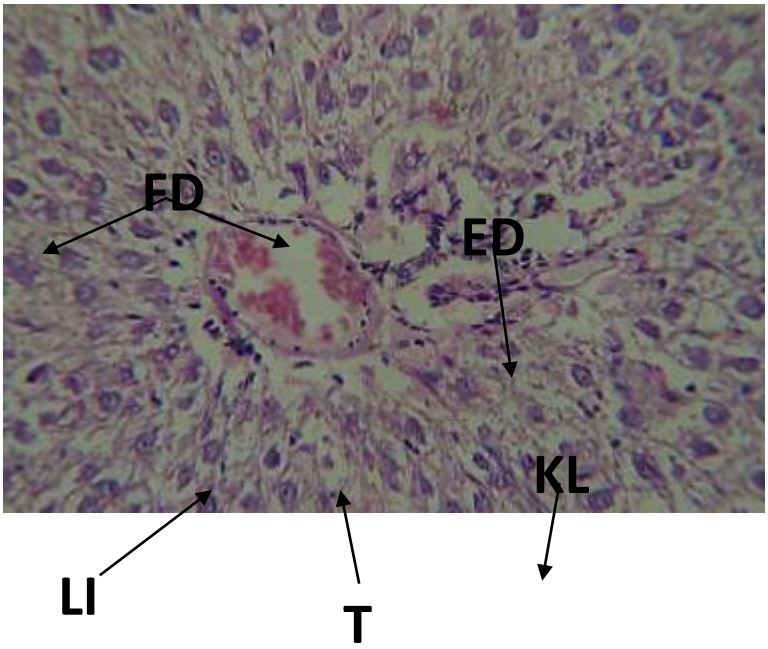

Fig.9. A liver section of the CFA group treated with Ibuprofen (40 mg/kg BW), demonstrating central vein wall thickening (TW), fatty degeneration (FD), hepatocellular necrosis (N),

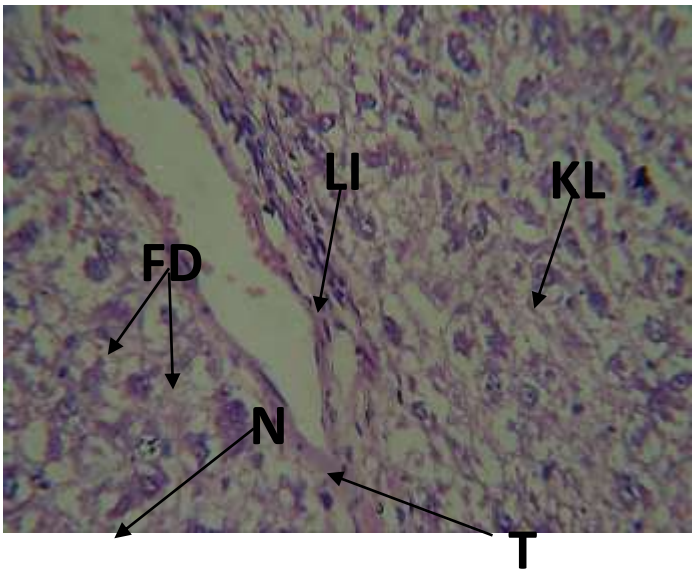

Fig.10. A liver section of the CFA group treated with Ibuprofen (40 mg/kg BW), demonstrating central vein wall thickening (TW), fatty degeneration (FD) of hepatocytes, karyolysis (KL) of some nuclei, and lymphocytic infiltration (LI). H&E ×400.

Conclusion

CFA-induced rheumatoid arthritis elicits substantial systemic hepatotoxicity in rats, manifesting as a multi-tiered histopathological syndrome encompassing hepatocellular necrosis, lymphocytic infiltration, fibrogenesis, and sinusoidal disruption — collectively indicative of a steatohepatitis suggestive of inflammatory and degenerative hepatic changes -like progression driven by systemic immune dysregulation. In this pathological background, ethanolic extract /W. somnifera root 300 mg/kg BW exhibited superior hepatoprotective efficacy over all comparator groups, restoring normal hepatic cytoarchitecture while demonstrating its therapeutic potential as a hepatoprotective adjuvant against CFA-induced inflammatory disease. In comparison, supplementation with crude root powder (10% of the diet) offered markedly less protection to the liver that the ethanolic extract, an effect likely due to low bioavailability of withanolides within non-purified preparations and their labile nature in the GI matrix — providing further evidence of the significant influence of extraction methodology on therapeutic efficacy. With regard to conventional pharmacological comparators, dexamethasone, whilst having an anti-inflammatory reference-standard designation, caused paradoxical hepatic injury characterised by corticosteroid-induced steatosis, apoptotic and necroptotic signalling, and collagen

accumulation, establishing that systemic anti-inflammatory benefit is greatly negated by large hepatic metabolic toxicity. Likewise, ibuprofen elicited hepatotoxic lesions of similar or greater severity as dexamethasone, consistent with NSAID-mediated cholestatic hepatitis and oxidative stress-mediated hepatocellular necrosis, exacerbated by the concomitant inflammatory burden of CFA-induced arthritis an observation which emphasizes the cumulative hepatotoxic risk attributable to traditional anti-inflammatory pharmacotherapy. These withanolide constituents inhibit COX-2, TNF-α and IL-1β, which are endogenous regulators that mediate both the pathogenesis of RA and the CFA-induced hepatopathy, thus providing a mechanistic basis for the hepatoprotective superiority of W. somnifera; this mechanistic rationale indicates that *Ashwagandha* can serve as an ideal dual-target therapeutic for both articular and hepatic components of inflammatory disease. Cumulatively, these results indicate that standardized ethanolic W. somnifera extracts demonstrate important potential for future, detailed clinical assessment as hepatoprotective adjunct therapy for RA, in patients at increased risk for drug-induced hepatotoxicity, particularly with chronic corticosteroid or NSAID use.

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