Comparative clinicopathological studies on the anti-inflammatory effect of ashwagandha and diclofenac in rats

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Abstract

Arthritis is a painful inflammatory illness, characterized by joint destruction and disability. Non-steroidal anti-inflammatory drugs (NSAIDs) are applied extinsively as pain relievers, however, their prolonged administration can result in undesirable side impacts on various body organs. Ashwagandha (*Withania somnifera*) has drew researchers' consideration by virtue of its several medical uses. The current study investigated the anti-inflammatory action of ashwagandha (Ash) versus that of diclofenac sodium (Dic) via the evaluation of paw thickness, hematological, biochemical, inflammatory parameters and histopathological alteration in rats. Rats were distributed into 6 groups and kept for 21 days: control group; received normal saline orally, Ash group; orally treated with Ash (200 mg/kg body weight), Dic group; orally treated with Dic (2 mg/kg b.wt), Arthritic group (Arth); rats were subjected to 0.1 ml sub-plantar inoculation of formalin (2.5%) at right hind paw on day 1 and received normal saline orally, Arth+Ash group; arthritis was induced as explained in Arth group then treated with Ash (at the same dose as Ash group) orally, Arth+Dic group; arthritis was induced as explained in Arth group then treated with Dic (at the same dose as Dic group) orally. Paw thickness was significantly increased in Arth rats than the control ones at various estimated times along the experiment, however, significant decline was recorded in Arth+Ash and Arth+Dic groups. Upon completion of the research (21 days), blood and tissue specimens were harvested. Hematological outcomes announced normocytic normochromic anemia in Arth rats, and this effect was alleviated upon administration of Ash to Arth rats. While, Dic had negative drawbacks on erythrogram. Serum ALT, urea, creatinine, IL-6, CRP, COX2 and hepatic TOC exhibited substantial increment along with substantial decrement in serum IL-10 and hepatic TAC in Arth group. Meanwhile, Arth+Ash and Arth+Dic groups showed

significant improvement in these parameters in comparison to Arth group. In addition, our histopathological assessment illustrated the adverse sequel of Dic on miscellaneous rat's body tissues and that Ash displayed invulnerable action. Lastly, oral administration of *W. sominefra* extract to rats with arthritis induced by formalin, remarkably decreased paw thickness and alleviated unfavorable modifications in all measured variables in a way better than diclofenac. Then, *W. sominefra* can be used to treat rheumatoid arthritis without causing any harm because it owns anti-arthritic and anti-inflammatory impacts.

Key words: ashwagandha, arthritis, diclofenac, erythrocytes, oxidant, inflammation, rats.

Introduction

Inflammation is a model defensive attitude to tissue harm provoked through trauma, pathogens and irritants ***(Medzhitov, 2008)***. Rheumatoid arthritis (RA) is classified as an inflammatory disorder that creates synovial joints' inflammation. It is linked to the growth of synovial cells and the infiltration of stimulated inflammatory cells, like macrophages, plasma cells, and memory T cells, which eventually destroys the cartilage and bones ***(Hitchon and El-Gabalawy, 2004)***.

The predominant prescriptive medications for treating rheumatic arthritis are non-steroidal anti-inflammatory medicines (NSAIDs) ***(Khaled et al., 2010)***. Diclofenac, a by-product of phenyl acetic acid, is one of NSAIDs that is recommended for treatment in rheumatic arthritis and various non-rheumatic illnesses that cause pain and inflammation ***(Todd and Sorkin, 1988)***. Diclofenac is a cyclooxygenase (COX) inhibitor ***(Imamura et al., 2013)***, this enzyme manages generating inflammatory mediators. The broad usage of these medications is confined due to their unwanted secondary effects and toxicities ***(Svensson et al., 2020; Wang et al., 2018)***. As a result, efforts are continually conducted to find medicinal products that are safer and less likely to cause negative consequences.

The demand for herbal remedies has become high, and the use of them has grown over time. Ashwagandha (*Withania somniferous*) is a particular herbal of conventional Indian medication and is appropriated from South-East Asia up to the Mediterranean district ***(Paul et al., 2021)***. Numerous pharmacological benefits of ashwagandha have been reported involving anti-stress, neuroprotective, anticancer, diuretic, anti-inflammatory, anti-arthritic, immunomodulatory, antioxidant, hemopoietic and anti-ulcerogenic properties ***(Parihar, 2022)***.

In the current investigation, we aimed to check the potential effect of Ashwagandha on relieving pain of induced arthritis in comparison with synthetic anti-inflammatory drug through detecting paw thickness, hematological picture, some serum biochemical, tissue oxidant status, inflammatory markers and histopathological assessments.

Material and Methods

Experimental rats

Sixty male Albino rats, 2–3 months old, 120–130 g in weight, were acquired from Pharmacy Faculty, Suez Canal University, and transported to Faculty of Veterinary Medicine Lab Animal House, Suez Canal University, where they spent a week for acclimatization. Rats were accommodated in polystyrene cages (thoroughly cleaned and disinfected), with base covered by sawdust (that was changed continuously) to prevent contact with rough surface. Prior to the testing, they had frequent handling to acclimatize them to the handling of the researcher. Rats were retained at room condition of 25°C on a 12-h light-dark session with unlimited reach to water and food.

Ethical approval

The study design was authorized by Faculty of Veterinary Medicine's scientific research morality board, Suez Canal University before any work was done with an Approval No. (2022035).

Ashwagandha (*Withania somnifera*)

Ashwagandha Standardized Extract Veg Capsules 450 mg (*Withania somnifera*) were obtained from NOW Foods. Ashwagandha dissolved in carboxy methyl cellulose sodium salt 1% (CMC) obtained from ARMCHEM., Bahadrugarh, Haryana, India. One capsule was dissolved in 11.25 ml of CMC solution.

Medication and Formalin

Diclofenac Sodium (Voltaren 50 mg) (Novartis Pharma AG., Basle, Switzerland) was applied in the study. Formalin 33% obtained from TOPCHEM company, Egypt, was prepared at a concentration of 2.5% to be used.

Experimental protocol

Randomly, sixty male albino rats were separated forming six groups, with ten rats each one. Treatment was as follow: Control group (C); received normal saline orally during 21 days, Ashwagandha group (Ash); orally received Ashwagandha 200mg/kg body weight along 21 days ***(Khaled et al., 2022)***, Diclofenac group (Dic); orally receiving Diclofenac sodium 2 mg/kg bwt through 21 days ***(Yasmeen et al., 2007)***, Arthritic group (Arth); injected with 0.1 ml formalin (2.5%) at sub-plantar area of right hind paw of rats on day 1 ***(Arzi et al., 2015)*** and received normal saline orally over 21 days, Arthritic+Ashwagandha group (Arth+Ash); injected with 0.1 ml formalin (2.5%) at sub-plantar area of right hind paw of rats on day 1, then treated with Ash (at the same

dose as Ash group) orally for 21 days, Arthritic+Diclofenac group (Arth+Dic); injected with 0.1 ml formalin (2.5%) at sub-plantar area of right hind paw of rats on day 1, then treated with Dic (at the same dose as Dic group) orally for 21 days.

Induction of arthritis

At the beginning of the experiment (1st day), Arth, Arth+Ash and Arth+Dic rats were subjected to sub-plantar inoculation of 0.1 ml formalin (2.5%) into their right hind paws to induce arthritis ***(Arzi et al., 2015)*** using insulin syringe. After half an hour, signs of inflammation appeared including; redness, swelling, and increased sensitivity to undesirable stimulants, limited only to the inoculated paw accompanied by abnormal movement due to pain. After appearance of the signs, different treatments were carried out for each group and lasted for 21 days.

Measurement of paw thickness

Thickness of rats' right hind paws of arthritis and treated groups were assessed every 3 days by caliber (micrometer screw gauge) to quantify the changes due to the inflammatory response toward formalin and the results were statistically analyzed.

Blood samples

At the completion of the experiment (21st day), 90 mg/kg ketamine and 10 mg/kg xylazine were administered intraperitoneal to five rats from every group ***(Akpolat et al., 2009)***, then blood samples were extracted into 2 tubes; the first tube contained potassium EDTA, was utilized for hematological analysis. The second sample was taken and placed in sterile, plain centrifuge tubes. It was then allowed to coagulate at ambient temperature then it was centrifuged to separate the sera. The clear sera were utilized for immunological and biochemical analyses after being thoroughly separated.

Tissue sampling

Following sacrifaction, the right hind paw and internal organs were grossly examined. A part of the liver from each rat was separated, washed with buffer and kept at -80 °C for further preparation of tissue homogenate to determine oxidant antioxidant status. Hind paw joint, other part of the liver, kidney and stomach were detached and preserved in 10% formalin for histological analysis. Furthermore, paw was decalcified by 10% formic acid and dehydrated by alcohol. After samples were cleansed in xylene, each sample was inserted in paraffin wax and incised into segments, then stained with H&E and finally examined as explained by ***Chinnasamy et al. (2019)***.

Hematological studies

Red blood cells count (RBC), hemoglobin (Hb) content, packed cell volume (PCV), erythrocytic indices, total leukocytes count (T.L.C) and differential leukocytes count (D.L.C) were carried out according to ***Feldman et al. (2000)***.

Biochemical, oxidative and inflammatory indices assay

Kits of different analytes were purchased from reputable companies. Serum total proteins (TP), albumin, alanine aminotransferase (ALT), urea and creatinine (Clinichem Co., Budapest, Hungary). Hepatic total oxidant capacity (TOC) and total antioxidant capacity (TAC) (Labor Diagnostika Nord GmbH & Co KG, Nordhorn, Germany). ELISA kits for serum cyclooxygenase 2 (COX-2), interleukin 6 (IL6) and interleukin 10 (IL10) (CUSABIO Technology LLC, Houston, USA). ELISA kit of C-reactive protein (CRP) (KAMIYA Biomedical Company, Seattle, USA).

Statistical evaluation

For each group of five animals, data was displayed like mean and its standard error. Analysis was implemented using SPSS (IBM 20.0 version software) ***(Levesque, 2005)***, 1-way analysis of variance and Duncan's multiple range test were performed to contrast groups. Significant difference was recognized at *P* values below 0.05.

Results

Clinical signs and paw thickness

As shown in Fig. (1), after half an hour of right hind paw injection with 0.1 ml of formalin 2.5%, redness and swelling associated with abnormal movement due to pain were noticed in arthritic rats opposed to the control ones (Fig. 2). Regarding paw thickness, a significant ($P\leq0.05$) rise was pronounced in Arth group in comparison with C group. As for, Arth+Ash and Arth+Dic groups, a substantial ($P\leq0.05$) improvement in paw thickness was recorded in contrast to Arth group. On 12^{th}, 15^{th}, and 21^{st} days, significant ($P\leq0.05$) decreases were noticed in paw thickness of Arth+Ash rats versus Arth+Dic ones (Table, 1).

Hematological results

As shown in Table (2), at 21^{st} day of the study, erythrogram findings revealed insignificant variation between control and Ash groups in all measured parameters. While, Dic treated rats revealed significant ($P\leq0.05$) decline in Hb content and PCV value in comparison with control rats. Also, there were significant ($P\leq0.05$) decreases in Hb, PCV and RBCs of Arth rats in contrast to the control ones. On the other hand, Arth+Ash rats exhibited significant ($P\leq0.05$) increment of Hb, PCV and RBCs when related to Arth group. While, Arth+Dic revealed insignificant alteration from the Arth one. Erythrocytic indices (MCV, MCH and MCHC) showed insignificant changes in all groups of the study. Concerning leukogram results (Table, 3), insignificant variation was recorded among control, Ash and Dic groups. Whereas, remarkable ($P\leq0.05$) leukocytosis, neutrophilia and lymphocytosis was noted in Arth group in contrast to control group. Significant ($P\leq0.05$) decline in T.L.C and lymphocytes count was noticed in arthritic treated groups in comparison with arthritic non-treated group. This reduction was

more pronounced in Arth+Dic group than Arth+Ash one.

Biochemical results

The current study revealed insignificant changes in all tested serum biochemical parameters between control and Ash groups. While, there were substantial (*P≤0.05*) hypoproteinemia and hypoalbuminemia with considerable (*P≤0.05*) increment in ALT activity, urea and creatinine levels in Dic group and Arth group in contrast to the control one. Whereas for, treated arthritic groups against the arthritic one, substantial (*P≤0.05*) improvements in all serum tested parameters were recorded (Table, 4).

Hepatic tissue total oxidants and total antioxidants capacities results

As shown in Table (5), TAC was remarkably (*P≤0.05*) raised in hepatic tissue of Ash group in contrast to the control. However, hepatic TAC was considerably (*P≤0.05*) diminished in Arth group to the control one. Whereas, it was significantly (*P≤0.05*) promoted in arthritic treated groups versus arthritic non treated one. As for, TOC in hepatic tissue, a significant (*P≤0.05*) increment was detected in Arth rats versus the control rats. But arthritic treated groups displayed significant (*P≤0.05*) decrement in TOC against arthritic non treated group.

Serum inflammatory markers results

The present study exhibited insignificant changes in values of serum IL6, IL10, CRP and COX-2 of Ash and Dic groups in comparison with the control. There were significant (*P≤0.05*) rises in IL6, CRP and COX-2 serum concentrations with noticable (*P≤0.05*) drop in IL10 value of Arth group versus the control one. While, Arth+Ash and Arth+Dic groups revealed significant (*P≤0.05*) diminish in IL6, CRP and COX-2 levels with significant (*P≤0.05*) rise in IL10 level against Arth one (Table, 6).

Histopathological results

Hind paw: On 21st day of the experimental period, histopathological examination for H&E-stained sections of paw joint obtained from groups (C, Ash and Dic) revealed a normal smooth articular surface and normal synovial membrane (Fig. 3a, b & c). Joints obtained from Arth group showed severe hyperplasia of the synovial membrane with mild congestion, edema, leukocytic infiltrations, and collagen deposition (Fig. 3d). The Arth+Ash group exhibited synovial membrane hyperplasia in addition to mild edema as well as the joint space was maintained (Fig. 3e). The Arth+Dic group showed thickened synovium, mild leukocyte infiltration, and edema with moderated collagen deposition (Fig. 3f).

Liver: Hepatic tissue of C and Ash groups showed normal central vein and hepatic cells (Fig, 4a &b). While, the Dic group revealed dilatation of central veins along with activation of Von Kupffer cells (Fig. 4c). The Arth group showed mild

congestion of blood vessels (Fig. 4d), as well as Arth+Ash group showed mild congestion of central veins (Fig. 4e). The Arth+Dic group rats showed mild congestion of blood vessels with activation of Von Kupffer cells (Fig. 4f).

kidney: The renal tissue of C and Ash groups had normal glomeruli and renal tubules (Fig. 5a &b). Whereas the Dic group and Arth group displayed mild to moderate congestion of blood vessels (Fig. 5c &d). Arth+Ash group presented a normal histological picture of both glomeruli and renal tubules (Fig. 5e). The Arth+Dic group rats showed mild congestion of blood vessels (Fig. 5f).

Stomach: Histopathological examination of the stomach of C and Ash groups showed normal gastric epithelium and gastric submucosa (Fig. 6a &b). In contrast, the Dic group revealed moderate congestion of submucosal blood vessels along with focal desquamation of gastric epithelial cells (Fig. 6c). Arth group showed normal mucosa and submucosa (Fig. 6d). The Arth+Ash group showed a normal histological picture of the stomach with intact gastric epithelium (Fig. 6e). Arth+Dic group exhibited moderate congestion of submucosal blood vessels along with mild desquamation of gastric epithelium (Fig. 6f).

Table (1): *Effect of Arthritis, Ashwagandha and Diclofenac on the paw thickness (mm) of rats over 21 days*

Group / Time	Control	Ash	Dic	Arth	Arth+Ash	Arth+Dic
The day before inject. (0 day)	1.97±0.06^{b}	1.98±0.02^{ab}	2.06±0.03^{ab}	2.05±0.03^{ab}	2.10±0.05^{a}	2.03±0.02^{ab}
1st day of inject.	1.97±0.05^{c}	1.98±0.02^{c}	2.07±0.03^{c}	7.92±0.66^{a}	6.81±0.47^{b}	5.83±0.17^{b}
3rd day of inject.	1.95±0.05^{c}	2.03±0.02^{c}	2.06±0.03^{c}	7.92±0.64^{a}	6.25±0.29^{b}	6.43±0.19^{b}
6th day of inject.	2.00±0.07^{c}	2.03±0.02^{c}	2.12±0.03^{c}	7.75±0.48^{a}	5.95±0.29^{b}	6.32±0.31^{b}
9th day of inject.	2.03±0.06^{c}	2.12±0.03^{c}	2.17±0.02^{c}	7.80±0.47^{a}	5.98±0.25^{b}	6.25±0.31^{b}
12th day of inject.	2.12±0.05^{d}	2.13±0.03^{d}	2.17±0.02^{d}	7.25±0.36^{a}	5.75±0.17^{c}	6.57±0.19^{b}
15th day of inject.	2.13±0.04^{d}	2.13±0.04^{d}	2.20±0.03^{d}	7.33±0.36^{a}	5.83±0.17^{c}	6.48±0.22^{b}
18th day of inject.	2.20±0.05^{c}	2.17±0.02^{c}	2.22±0.03^{c}	7.50±0.45^{a}	5.83±0.17^{b}	6.42±0.27^{b}
21st day of inject.	2.22±0.04^{d}	2.17±0.03^{d}	2.27±0.02^{d}	7.50±0.45^{a}	5.75±0.17^{c}	6.42±0.27^{b}

Values are presented as means ± SE; a-d unlike superscripts in the row itself are regarded as significant when ($P \leq 0.05$)

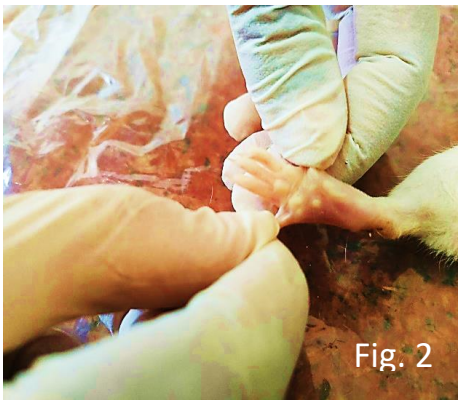

Fig. (1): Signs of inflammation in paw of rat in Arth group after half an hour of formalin injection.

Fig. (2): Normal paw of rat in control group

Table (2): *Effect of Arthritis, Ashwagandha and Diclofenac on erythrogram of rats on 21st day of the experimental period*

Parameters / Groups	Hb (g/dl)	RBCs ($\times10^6$/µl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control	14.43±0.17^a	8.14±0.59^a	46.37±1.10^a	57.43±3.29^a	17.95±1.52^a	31.18±1.05^a
Ash	13.90±0.42^a	8.12±0.22^a	45.70±1.30^a	56.43±2.76^a	17.12±0.11^a	30.50±1.64^a
Dic	12.07±0.43^b	7.28±0.31^{ab}	40.87±1.51^b	56.37±3.45^a	16.61±0.70^a	29.68±2.07^a
Arth	10.32±0.49^c	5.23±0.41^c	35.30±1.12^c	68.17±5.11^a	19.92±1.56^a	29.21±0.47^a
Arth+Ash	11.71±0.18^b	6.47±0.33^b	40.30±1.29^b	62.51±2.46^a	18.18±0.83^a	29.08±0.54^a
Arth+Dic	9.87±0.74^c	5.17±0.29^c	34.83±1.00^c	67.68±3.23^a	19.09±1.05^a	28.40±2.43^a

Values are presented as means ± SE; a-c unlike superscripts in the column itself are regarded as significant when ($P\leq0.05$)

Table (3): Impact of Arthritis, Ashwagandha and Diclofenac on leukogram of rats on 21st day of the experimental period

Parameters / Groups	T.L.C ($\times10^3$/µl)	Neutrophil ($\times10^3$/µl)	Lymphocyte ($\times10^3$/µl)	Monocyte ($\times10^3$/µl)	Eosinophil ($\times10^3$/µl)
Control	16.30±1.70^c	7.56±0.78^b	6.80±1.54^c	1.50±0.23^a	0.43±0.08^a
Ash	17.40±0.71^c	8.39±0.65^{ab}	7.19±0.96^c	1.32±0.16^a	0.50±0.08^a
Dic	17.30±1.89^c	8.19±1.72^{ab}	6.93±0.84^c	1.57±0.31^a	0.60±0.15^a
Arth	30.70±1.64^a	12.00±0.87^a	15.80±1.28^a	2.08±0.23^a	0.82±0.16^a
Arth+Ash	23.43±1.58^b	9.53±0.85^{ab}	11.45±1.38^b	1.64±0.21^a	0.81±0.22^a
Arth+Dic	20.60±1.31^{bc}	9.80±1.56^{ab}	8.57±1.25^{bc}	1.62±0.32^a	0.61±0.15^a

Values are presented as means ± SE; a-c unlike superscripts in the column itself are regarded as significant when ($P\leq0.05$)

Table (4): *Impact of Arthritis, Ashwagandha and Diclofenac on some serum biochemical parameters of rats on 21st day of the experimental period.*

Parameters / Groups	ALT (U/L)	TP (g/dl)	Albumin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	24.86±0.24^{d}	6.23±0.02^{a}	4.23±0.01^{a}	15.91±0.13^{d}	0.40±0.02^{c}
Ash	24.32±0.09^{d}	6.27±0.0^{a}	4.25±0.01^{a}	15.73±0.01^{d}	0.37±0.01^{c}
Dic	29.05±0.55^{c}	6.07±0.01^{b}	4.00±0.02^{b}	20.09±0.23^{c}	0.51±0.02^{b}
Arth	44.29±0.79^{a}	5.07±0.02^{d}	2.95±0.04^{d}	30.09±0.17^{a}	1.14±0.03^{a}
Arth+Ash	32.68±0.40^{b}	5.65±0.04^{c}	3.68±0.03^{c}	21.73±0.16^{b}	0.48±0.01^{b}
Arth+Dic	30.48±0.43^{c}	5.67±0.02^{c}	3.71±0.05^{c}	19.97±0.14^{c}	0.53±0.02^{b}

Values are presented as means ± SE; a-d unlike superscripts in the column itself are regarded as significant when (*P≤0.05)*

Table (5): *Impact of Arthritis, Ashwagandha and Diclofenac on total oxidant and total antioxidant capacities in hepatic tissue of rats on 21st day of the experimental period.*

Parameter / Group	TAC (mmol/l)	TOC (mmol/l)
Control	2.60±0.03^{b}	0.35±0.003^{d}
Ash	2.84±0.02^{a}	0.36±0.006^{d}
Dic.	2.24±0.02^{c}	0.38±0.004^{d}
Arth	0.96±0.01^{f}	0.87±0.020^{a}
Arth+Ash	1.53±0.04^{e}	0.51±0.007^{b}
Arth+Dic	1.82±0.01^{d}	0.46±0.006^{c}

Values are presented as means ± SE; a-f unlike superscripts in the column itself are regarded as significant when (*P≤0.05*)

Table (6): *Impact of Arthritis, Ashwagandha and Diclofenac on some serum inflammatory markers of rats on 21st day of the experimental period..*

Parameter / Group	IL-6 pg/ml	IL-10 pg/ml	CRP mg/dl	COX-2 ng/ml
Control	6.32±0.06^{c}	68.09±0.89^{a}	6.57±0.02^{d}	4.57±0.06^{d}
Ash	7.20±0.96^{c}	68.31±0.63^{a}	6.34±0.04^{d}	4.50±0.02^{d}
Dic.	6.13±0.03^{c}	68.22±0.51^{a}	6.27±0.05^{d}	4.48±0.04^{d}
Arth	15.19±0.19^{a}	27.07±0.99^{d}	36.94±0.84^{a}	17.84±0.33^{a}
Arth+Ash	9.82±0.19^{b}	49.72±0.76^{c}	13.16±0.52^{b}	7.84±0.19^{b}
Arth+Dic	8.61±0.07^{b}	56.21±0.94^{b}	9.93±0.24^{c}	6.74±0.11^{c}

Values are presented as means ± SE; a-d unlike superscripts in the column itself are regarded as significant when (*P≤0.05)*

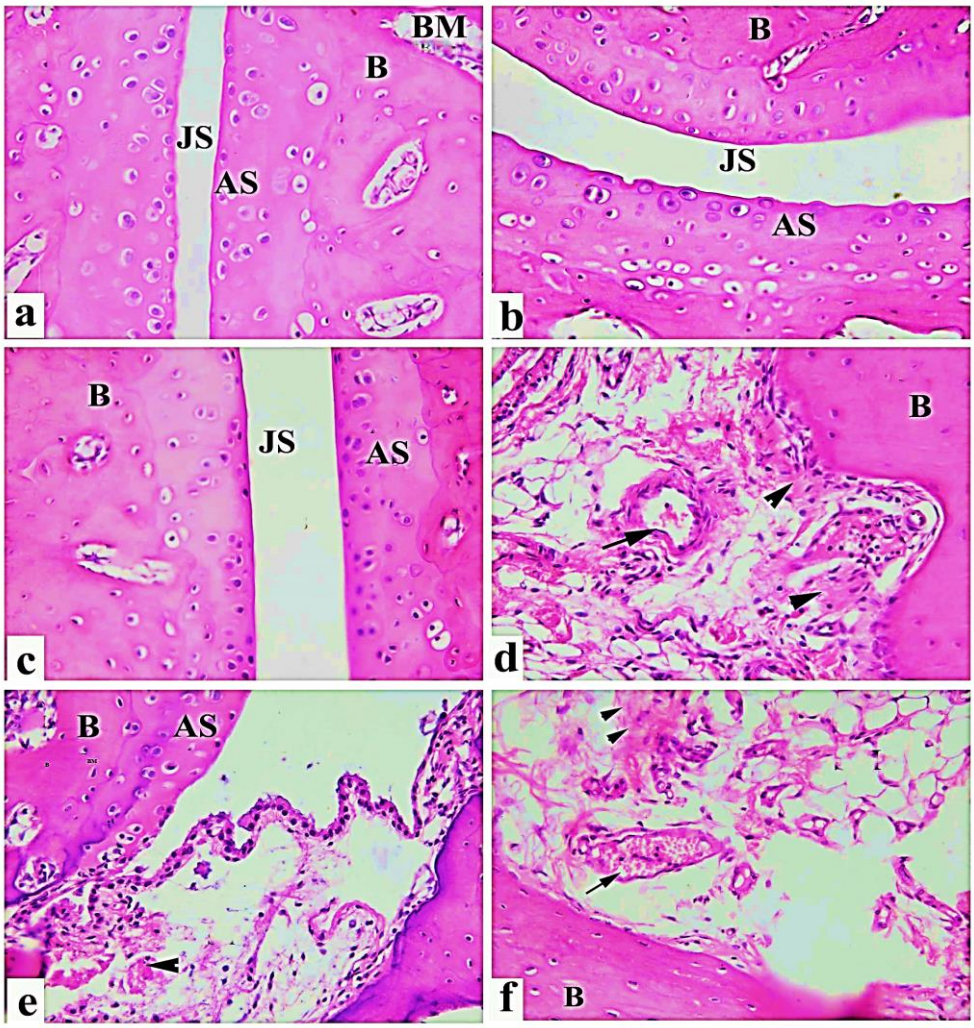

Fig. (3): Hind paw histopathological picture on 21st day of experimental period. Control rats (a), Ashwaghanda (b), diclofenac sodium (c), showing normal articular cartilage arrangement with smooth articular surface (AS), normal synovium (SM) and normal joint space (JS). (d): hind paw of arthritis group showing severe hyperplasia of synovial membrane (SM) with moderate leukocytic infiltration, edema along with deposition of collagen. (e): paw of arthritis+ashwagandha treated group, rats showing mild hyperplasia of synovial membrane with mild leukocytic infiltration, edema along with mild deposition of collagen. (f): paw of arthritis+diclofenac treated rats showing mild congestion (arrow), hyperplasia of synovial membrane with mild leukocytic infiltration, edema along with deposition of collagen (arrowhead). H&E. X 400.

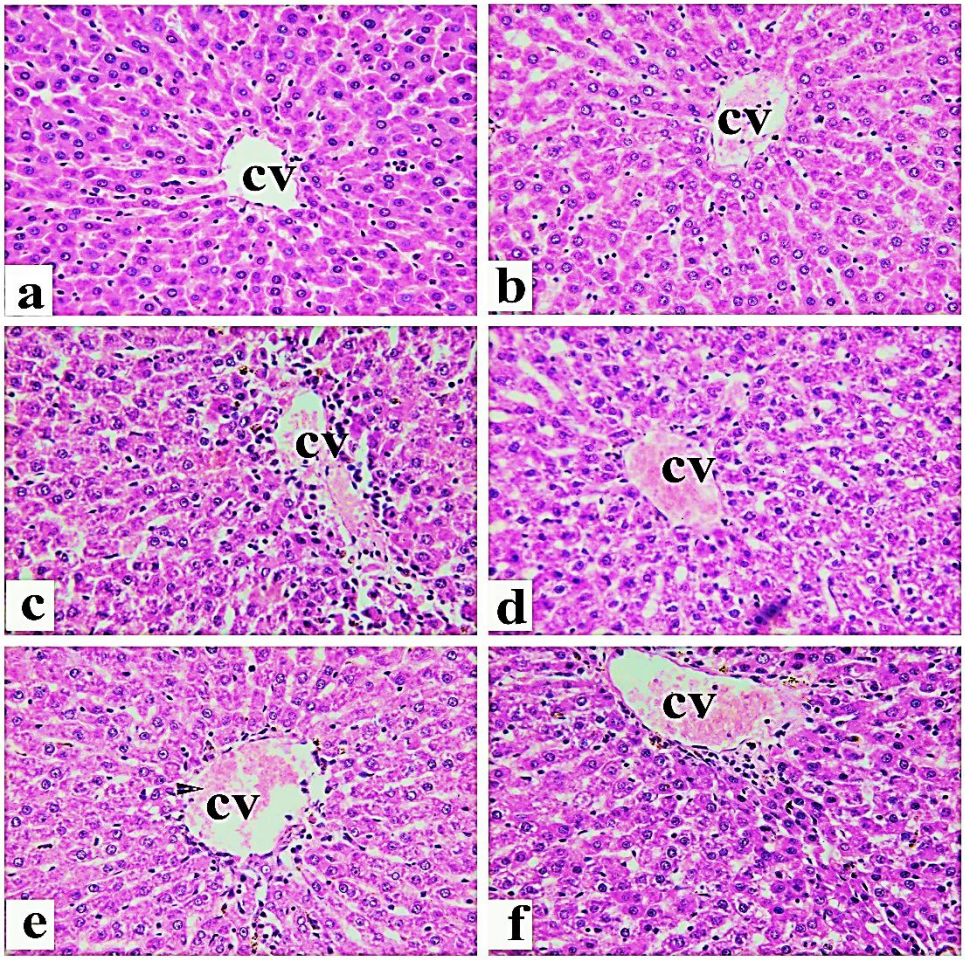

Fig. (4): Liver histopathological picture of normal control group (a) and ashwagandha group (b) exhibiting normal central vein (cv) and hepatic cells. (c): diclofenac treated group showing dilatation of central vein along with activation of Von Kupffer cells. (d) arthritis group rats revealing mild congestion of blood vessels. (e): arthritis+ashwaghanda group rats showing mild congestion of central veins. (f): arthritis+diclofenac treated group rats demonstrating mild congestion of blood vessels with activation of Von Kupffer cells. H&E. X 400.

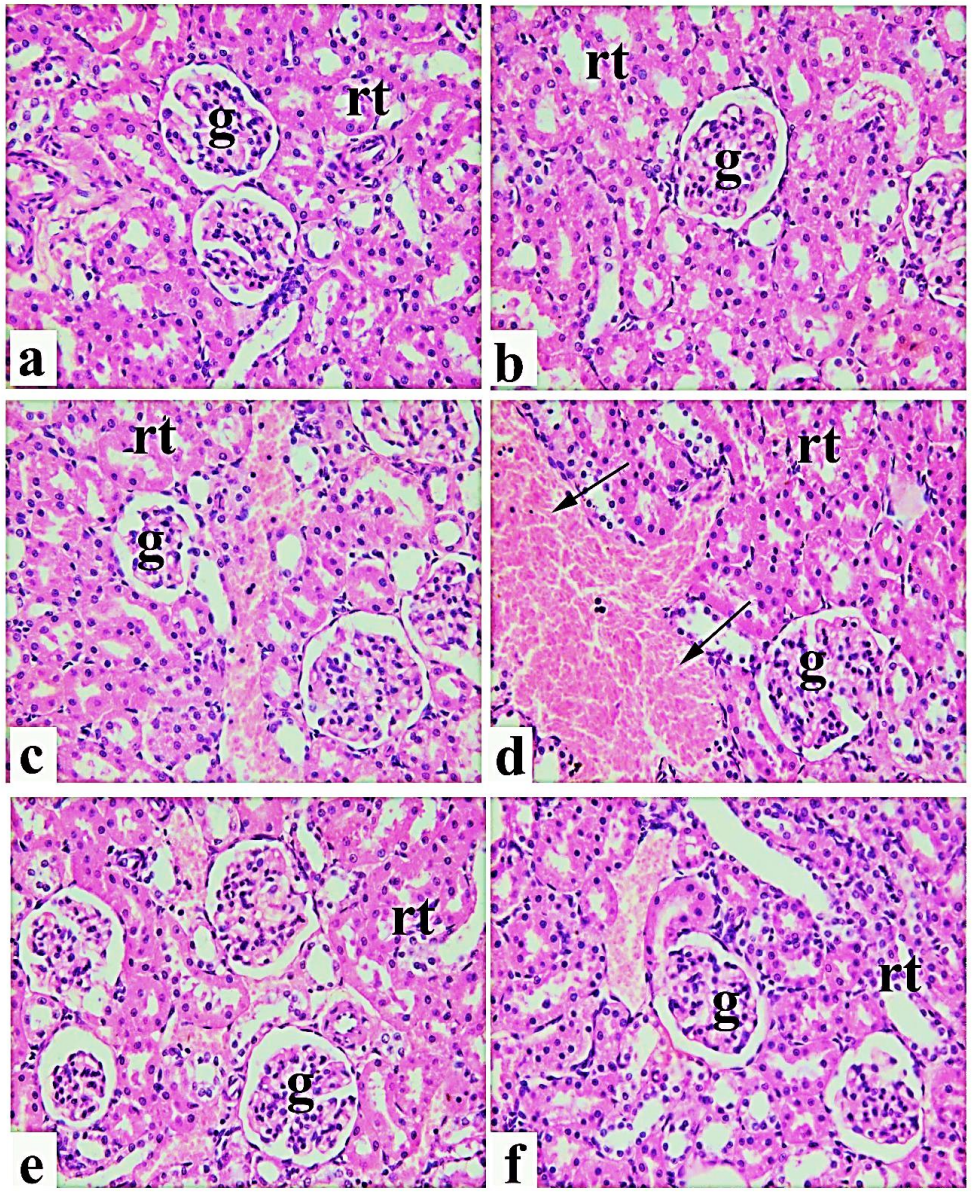

Fig. (5): Kidney histopathological picture of normal control group (a) and ashwagandha group (b) revealing normal glomeruli (g) and renal tubules (rt). (c): diclofenac treated group & (d) arthritis group rats showing mild to moderate congestion of blood vessels. (e): arthritis+ ashwaghanda group rats showing normal histological picture of both glomeruli and renal tubules. (f): arthritis+diclofenac treated group rats showing mild congestion of blood vessels. H&E. X 400.

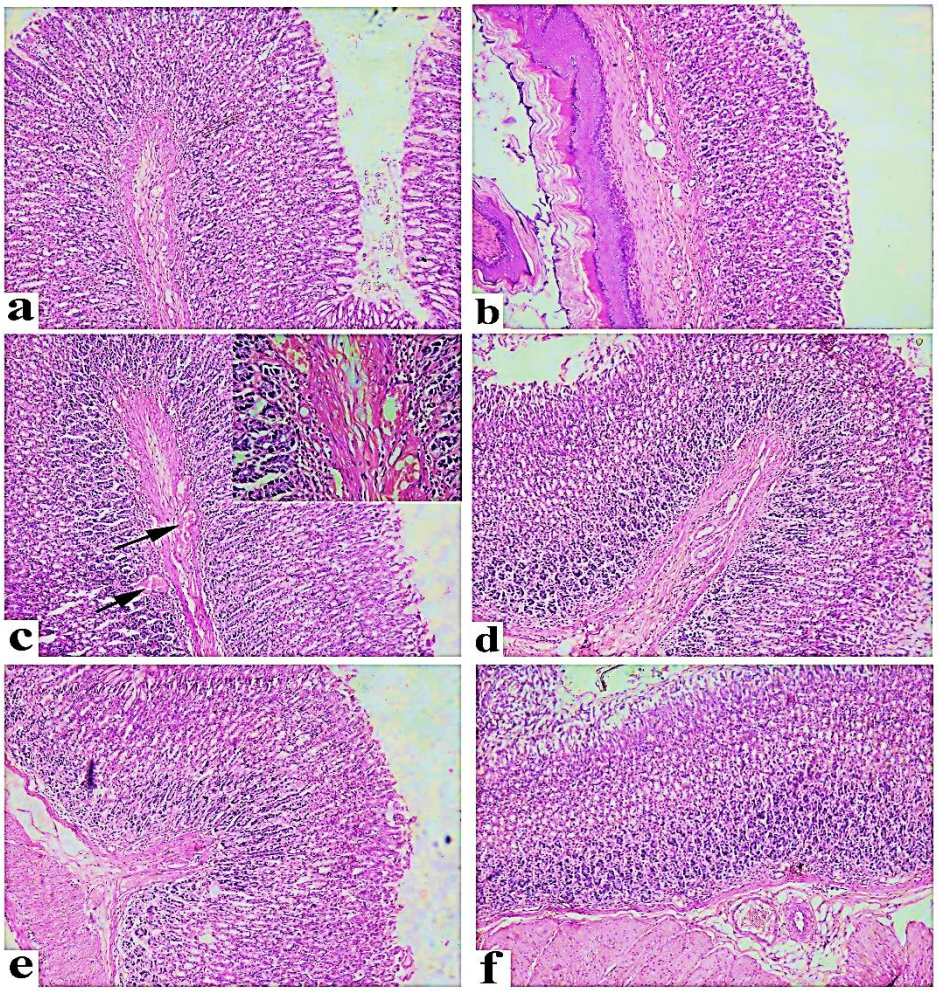

Fig. (6): Stomach histopathological picture of (a) normal control group and (b) ashwaghanda group showing normal mucosa and submucosa. (c): stomach of diclofenac treated group rats showing moderate congestion of submucosal blood vessels. (d) stomach of arthritis showing normal mucosa and submucosa. (e): stomach of arthritis+ ashwaghanda group rats showing normal histological picture of stomach with intact gastric epithelium. (f): stomach of arthritis+diclofenac treated group rats showing moderate congestion of submucosal blood vessels along with mild desquamation of gastric epithelium. H&E. X 100.

Discussion

Arthritis is a wide spread, inflammatory, immune related disorder ***(Ahsan et al., 2021)***. In developed nations, rheumatic arthritis is thought to be one of the primary causes of severe health problems ***(Mody, 2009)***. Being a

systemic illness, arthritis can influence most of the body organs, not only the joints ***(Manan et al., 2022)***. The main treatment course of arthritis is administration of synthetic NSAIDs. But, due to prolonged and continous usage of them, serious adverse reactions may develop including renal, hepatic and gastric illness ***(Bensman, 2019; Sriuttha et al., 2018; Tsujimoto et al., 2018)***. Contrary to synthetic medications, it is commonly assumed that herbal therapies have no side effects and well tolerated by patients.

The current inquiry was managed to compare the anti-inflammatory consequences belonging to ashwagandha and diclofenac sodium in rats subjected to fomalin provoked arthritis, since ashwagandha possesses an anti-inflammatory effect.

Clinical investigation of rats injected with 0.1 ml formalin 2.5% in our study, declared redness and swelling associated with abnormal movement due to pain. A resembling record was stated by ***Ahsan et al. (2021)***. Also, ***Choudhary et al. (2015)*** said that swelling, pain and bone and cartilage degeneration are the main signs of rheumatic arthritis (RA). These signs were relieved upon the treatment with Ash and Dic. Our results came in harmony with ***Gupta and Singh (2014); Maleškić Kapo et al. (2023)***.

Current results pronounced significant ($P \leq 0.05$) expansion in paw thickness of Arth group in contrast to C group. Such finding was consonant to ***(Arzi et al., 2015; Panda et al., 2021)***. Where, tumor necrosis factor alpha (TNF-α) production rises, which provokes IL-6 and IL-1β to be expressed more. These two factors generate the deteriorating enzymes that cause osteoclast differentiation, which promotes arthritic disintegration progress and increases the vascular permeability in the edematous area ***(Cheng et al., 2015)***. On the other hand, Arth+Ash and Arth+Dic groups exhibited significant decline in paw thickness counter to Arth group. This came in line with ***Gupta and Singh (2014); Khaled et al. (2022)***. This consequence might be coming from the antioxidant attributes of Ashwagandha and its bioactive ingredients. Similarly, ***Swathi et al. (2021)*** reported significant decrease in paw diameter in Wistar rats treated with diclofenac sodium. As, diclofenac can hinder COX activity, specifically COX-2 ***(Kam and See, 2000)***, which is well known to contribute in the production of prostaglandins (PGs) during inflammatory conditions ***(Yang, 2009)***.

Our hematological outcomes demonstrated normocytic normochromic anemia in Dic group contrasted to the control one. A former research recorded a significant decrease in RBC count in Wistar rats given diclofenac relative to the control ***(Alabi and Akomolafe, 2020)***. Too, ***Esteves et al. (2021)*** recorded normocytic

normochromic anemia, leukocytosis and reticulocytosis in a woman taking diclofenac with no considerable change in serum ferritin, iron, vitamin B12 and folic acid concentrations, where, NSAID-related acquired hemolytic anemia was hypothesized. Furthermore, one of the most deleterious impacts of diclofenac is the gastrointestinal seizures which may result in bleeding or iron malabsorbtion. As, NSAIDs have the potential to harm large and small intestines, they can result in enteropathy (an inflammation combined with blood and protein loss from the intestine), as well as small intestine ulcerations and incisions ***(Kołodziejska and Kołodziejczyk, 2018)***. Our histopathological assessment of stomach in Dic treated rats revealed congestion of submucosal blood vessels along with mild desquamation of gastric epithelium. More and above, remarkable instances of exposure to diclofenac or its metabolites have been shown to result in a condition known as diclofenac-induced immune hemolytic anemia because of the development of drug-independent IgG autoantibodies and antibodies which interacted with the medication and its metabolic products ***(Meyer et al., 2003)***.

In addition, Arth group disclosed normocytic normochromic anemia in contrast to control group. Rheumatic arthritis (RA) is marked by elevated IL-6 blood levels, which induces the liver to produce hepcidin ***(Dayer and Choy, 2010)***, that blocks macrophages' ability to release iron, which results in iron hiding, thus iron becomes unavailable for erythropoiesis producing anemia of inflammation ***(Ganz and Nemeth, 2009)*** or anemia of chronic diseases (secondary anemia). Moreover, the bone marrow erythroblasts in those suffering from rheumatoid arthritis are less responsive to interleukin-3, that has hemopoietic growth-promoting action ***(Jaworski et al., 2008)***.

On the contrary, Arth+Ash rats exhibited considerable improvement in Hb, PCV and RBCs in contrast to Arth rats. Equivalently, ***Ziauddin et al. (1996)*** stated significant elevation in Hb content and RBCs in myelosuppressed mice recieving ashwagandha. ***Priyanka et al. (2020)*** found that Hb content, lymphocyte%, RBCs, T.L.C, diminished glutathione (GSH), and superoxide dismutase (SOD) activities all showed significant rises over the course of the 21 days in horses receiving different doses of Ash and subjected to stress, thus expressing the hematopoietic and antioxidant impacts of Ash in animals. Therefore, Ash could express hematological influence via scavenging free radicals, so it can prevent alterations to the erythrocyte membrane, early cell death, and shortened life of erythrocyte ***(Gómez Afonso et al., 2023)***. More and above, ***Davis and Kuttan (2000)*** declared that *Withania somnifera* root extract administration in Babl/c

mice significantly increased bone marrow cells and T.L.C on 10th day, suggesting that the extract may stimulate the hemopoietic system.

Both Dic group and Arth group revealed considerable ($P \leq 0.05$) serum hypoproteinemia and hypoalbuminemia with remarkable ($P \leq 0.05$) increment in ALT activity, urea and creatinine serum levels in contrast to the control one. Same results of hepatic injury were obtained by ***Alabi and Akomolafe (2020); (Alabi et al., 2017); Comar et al. (2013)***. Increased blood concentrations of urea and creatinine indicated a possible damage in the glomerular filtration rate barrier, which could impact renal function. Serum ALT activity that was elevated suggested hepatotoxicity, which resulted in considerable damage to the liver cell membrane and the release of these enzymes into the bloodstream. ***Harirforoosh and Jamali (2005)*** noticed that rats treated with Dic had inhibited renal prostaglandin, which may have hindered renal physiology by lowering glomerular filtration rate and changing the equilibrium of electrolytes.

Furthermore, Dic can result in mitochondrial damage via interfering with immune-mediated defenses, reducing both enzymatic and nonenzymatic antioxidants in hepatic and renal tissues, and producing reactive oxygen metabolites ***(Galati et al., 2002; Gil et al., 1995)***. In addition, 4′, 5-hydroxydiclofenac, a diclofenac metabolite, can induce necrosis and neutrophil infiltration in hepatocytes ***(Alabi et al., 2017)***.

On the other side, giving Ash to rats that had been given formalin to induce arthritis revealed improvement in the detected serum biochemical markers. ***Priyanka et al. (2020)*** believed that the dropped values of AST, ALT, and creatinine indicate that ashwagandha root extract was safe and palatable for the animals and that the anti-inflammatory properties of ashwagandha were illustrated by the decreased level of IL6. ***Harikrishnan et al. (2008)*** stated decreased hepatic marker enzymes activities (ALT, AST and ALP) and blood urea and ammonia levels in rats with hyperammonemia and treated with Ash root powder, and attributed this effect to the phytochemical constituents of Ash and its antioxidant ability.

Our study explained a correlation between oxidative stress and inflammatory reactions brought on by formalin, evidenced by significantly increased TOC and decreased TAC in Arth rats correlated to the control rats. ***Berthou et al. (2015)*** claimed that oxidative stress had a part in the progress of rheumatoid arthritis. On the contrary, Arth+Ash group declared a substantial rise in hepatic TAC with a considerable drop in hepatic TOC versus Arth group. In accord with ***Pingali et al. (2013)*** who noted an improvement in TAC with *W. sominefra* administration in

healthy human. The phytochemical structure of ashwagandha is abundant, where it includes witanolides, flavonoids, alkaloids, saponins, chlorogenic acid, sterols, coumarins, glycosides, resins, carbohydrates and fatty acids ***(Mikulska et al., 2023)***. Antioxidant capability of Ash can be accounted for its ingredients such as withaferin A and its alkaloids that have been assumped as potent antioxidants through motivation of nuclear factor erythroid 2 (Nrf2) expression ***(Dutta et al., 2019; Palliyaguru et al., 2016)***.

The chemopreventive action of ashwagandha is probably aided by its anti-oxidant and anti-inflammatory features, which may account for part of its efficacy in treating a range of rheumatologic conditions ***(Prakash et al., 2002)***. Where, ***Khan et al. (2019)*** said that reactive oxygen species (ROS) and metaloproteinase-8 (MMP-8) levels were brought down to normal in arthritic rats upon oral application of 300 mg/kg of *W. somnifera* aqueous root extract, which also prevented NF-κB activity and promoted IL10 secretion. It has been demonstrated that inflammatory and autoimmune disorders are impacted by the potent anti-inflammatory cytokine, IL10, which exhibits a vast range of anti-inflammatory action ***(Khan et al., 2015a)***, it has the capability to suppress the synthesis of several proinflammatory cytokines ***(Clair, 1999)***.

From the acute phase proteins, C-reactive protein (CRP), which can be deposited during inflammation ***(Du Clos and Mold, 2004)***. ***Pepys and Baltz (1983)*** declared that plasma CRP levels mainly refer to the extent of tissue damage or inflammatory illness. CRP gene is primarily induced in hepatocytes by the action of elevated inflammatory cytokines levels, particularly IL6 ***(Boras et al., 2014)***. IL6 can promote $CD4^+$ T helper cells, which is the first step in the integration of acquired and innate immunity, and decrease the synthesis of transferrin, albumin, and fibronectin ***(Tanaka and Kishimoto, 2014)***.

The liberty of free radicals as byproducts of cell metabolism, has the potential to trigger interleukins and TNF-α output from T-cell, and subsequently affects the generation of immune cells' adhesive molecules that can lead to inflammation and tissue damage ***(Jameson, 2005)***. As, within inflammed joints, TNF-α and IL6 induce bone disintegration, joint destruction, and cell apoptosis ***(Wang and He, 2020)***. In the edematous area, IL6 stimulates the progress of bone erosion and vasodilation by producing the degrading enzymes necessary for osteoclast differentiation ***(Cheng et al., 2015)***.

Upon exposure of the cell to proinflammatory substances, prostanoids are expressed by the aid of COX, where PGs are released spontaneously in significant amounts by COX-2, which is

released by chondrocytes and synovial tissue in response to endotoxins and cytokines stimulation ***(Yu and Kim, 2013)***. NSAIDS inhibit prostaglandins synthesis via prohibition of arachidonic acid and cyclooxygenase (COX) enzyme union ***(Mehta et al., 2019)***. In response to TNF-α and IL1β, COX-2 is upregulated and evoked in inflammatory cells ***(Ueno et al., 2005)***. COX-1 suppression done by NSAIDS is associated with gastrointestinal consequences ***(Biava et al., 2011)***.

It was validated that administration of Ash, significantly decreased arthritic index, pro-inflammatory cytokine IL-6 and CRP levels in arthritic rats ***(Khan et al., 2015b)***. Another study documented a diminishing effect of ashwagandha root acqueous solution on the generation of proinflammatory cytokines and an augmenting effect towards the anti-inflammatory cytokines' generation in HaCaT human keratinocyte cell line ***(Sikandan et al., 2018)***. ***Kalpana et al. (2023)*** speculated that because of the anti-inflammatory phytochemicals present in ashwagandha that specifically block endotoxin-induced MYD88 signaling, ashwagandha provokes the MPL-like balanced TLR4 activation. Indeed, ashwagandha involves a steroidal lactone known as withaferin A, which restricts NF-κB, the primary mediator of MYD88 signaling, from being activated in order to re-establish immune stability and overcome inflammatory disorder ***(Logie and Vanden Berghe, 2020)***.

Conclusion

To sum up, *Withania sominefra* (Ashwagandha) extract has no considerable difference noticed in paw thickness, hematological, biochemical and inflammatory tested parameters correlated to the control group. Further, giving *W. sominefra* orally to arthritic rats remarkably lessened paw thickness and alleviated disordered alterations in all determined variables in a way better than diclofenac. Accordingly, *W. sominefra* has anti-arthritic and anti-inflammatory activities that promote it to be used for rheumatoid arthritis treatment with no deleterious side impacts.

Conflict of interest

As stated by the authors, the research was performed with no financial or commercial partnerships that might be interpreted as having a conflict of interest.

References

Ahsan, H., Haider, I., Mushtaq, M.N., Qaisar, M.N., Naqvi, F., and Asif, A. (2021). Pharmacological support to anti-arthritic prospective of physostigmine: a new approach. Inflammopharmacology *29*, 1119-1129.

Akpolat, M., Kanter, M., and Uzal, M.C. (2009). Protective effects of curcumin against gamma

radiation-induced ileal mucosal damage. Archives of toxicology *83*, 609-617.

Alabi, Q.K., and Akomolafe, R.O. (2020). Kolaviron Diminishes Diclofenac-Induced Liver and Kidney Toxicity in Wistar Rats Via Suppressing Inflammatory Events, Upregulating Antioxidant Defenses, and Improving Hematological Indices. Dose-response : a publication of International Hormesis Society *18*, 1559325819899256.

Alabi, Q.K., Akomolafe, R.O., Olukiran, O.S., Adeyemi, W.J., Nafiu, A.O., Adefisayo, M.A., Omole, J.G., Kajewole, D.I., and Odujoko, O.O. (2017). The Garcinia kola biflavonoid kolaviron attenuates experimental hepatotoxicity induced by diclofenac. Pathophysiology : the official journal of the International Society for Pathophysiology *24*, 281-290.

Arzi, A., Olapour, S., Yaghooti, H., and Sistani Karampour, N. (2015). Effect of royal jelly on formalin induced-inflammation in rat hind paw. Jundishapur journal of natural pharmaceutical products *10*, e22466.

Bensman, A. (2019). Non-steroidal Anti-inflammatory Drugs (NSAIDs) Systemic Use: The Risk of Renal Failure. Frontiers in pediatrics *7*, 517.

Berthou, F., Ceppo, F., Dumas, K., Massa, F., Vergoni, B., Alemany, S., Cormont, M., and Tanti, J.F. (2015). The Tpl2 Kinase Regulates the COX-2/Prostaglandin E2 Axis in Adipocytes in Inflammatory Conditions. Molecular endocrinology (Baltimore, Md) *29*, 1025-1036.

Biava, M., C Porretta, G., Poce, G., Battilocchio, C., Botta, M., Manetti, F., Rovini, M., Cappelli, A., Sautebin, L., and Rossi, A.J.C.m.c. (2011). Enlarging the NSAIDs family: ether, ester and acid derivatives of the 1, 5-diarylpyrrole scaffold as novel anti-inflammatory and analgesic agents. *18*, 1540-1554.

Boras, E., Slevin, M., Alexander, M.Y., Aljohi, A., Gilmore, W., Ashworth, J., Krupinski, J., Potempa, L.A., Al Abdulkareem, I., Elobeid, A., *et al.* (2014). Monomeric C-reactive protein and Notch-3 co-operatively increase angiogenesis through PI3K signalling pathway. Cytokine *69*, 165-179.

Cheng, X.L., Liu, X.G., Wang, Q., Zhou, L., Qi, L.W., Li, P., and Liu, E.H. (2015). Anti-inflammatory and anti-arthritic effects of Guge Fengtong Formula: in vitro and in vivo studies. Chinese journal of natural medicines *13*, 842-853.

Chinnasamy, V., Subramaniyan, V., Chandiran, S., Kayarohanam, S., Kanniyan, D.C., Velaga, V., and Muhammad, S. (2019). Antiarthritic Activity of Achyranthes Aspera on Formaldehyde - Induced Arthritis in Rats. Open access Macedonian journal of medical sciences *7*, 2709-2714.

Choudhary, M., Kumar, V., Malhotra, H., and Singh, S. (2015). Medicinal plants with potential anti-arthritic activity. Journal of intercultural ethnopharmacology *4*, 147-179.

Clair, E.W.S. (1999). Interleukin 10 treatment for rheumatoid arthritis. Annals of the Rheumatic Diseases *58*, I99.

Comar, J.F., Babeto de Sá-Nakanishi, A., de Oliveira, A.L., Marques Nogueira Wendt, M., Bersani Amado, C.A., Ishii Iwamoto, E.L., Peralta, R.M., and Bracht, A. (2013). Oxidative state of the liver of rats with adjuvant-induced arthritis. Free Radical Biology and Medicine *58*, 144-153.

Davis, L., and Kuttan, G. (2000). Immunomodulatory activity of Withania somnifera. Journal of Ethnopharmacology *71*, 193-200.

Dayer, J.M., and Choy, E. (2010). Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor. Rheumatology (Oxford, England) *49*, 15-24.

Du Clos, T.W., and Mold, C. (2004). C-reactive protein: an activator of innate immunity and a modulator of adaptive immunity. Immunologic research *30*, 261-277.

Dutta, R., Khalil, R., Green, R., Mohapatra, S.S., and Mohapatra, S. (2019). Withania Somnifera (Ashwagandha) and Withaferin A: Potential in Integrative Oncology. International journal of molecular sciences *20*.

Esteves, A., Teixeira da Silva, F., Carvalho, J., Carvoeiro, A., and Felgueiras, P. (2021). Diclofenac-Induced Immune Hemolytic Anemia: A Case Report and Review of Literature. Cureus *13*, e12903.

Feldman, B.V., Zinkl, J.G., Jain, N.C., and Schalm, O.W. (2000). Schalm's Veterinary Hematology 5th Ed. Lippincott Williams & Wilkins.

Galati, G., Tafazoli, S., Sabzevari, O., Chan, T.S., and O'Brien, P.J. (2002). Idiosyncratic NSAID drug induced oxidative stress. Chemico-biological interactions *142*, 25-41.

Ganz, T., and Nemeth, E. (2009). Iron sequestration and anemia of inflammation. Seminars in hematology *46*, 387-393.

Gil, M.L., Ramirez, M.C., Terencio, M.C., and Castell, J.V. (1995). Immunochemical detection of protein adducts in cultured human hepatocytes exposed to diclofenac. Biochimica et biophysica acta *1272*, 140-146.

Gómez Afonso, A., Fernandez-Lazaro, D., Adams, D.P., Monserdà-Vilaró, A., and Fernandez-Lazaro, C.I. (2023). Effects of Withania somnifera (Ashwagandha) on Hematological and Biochemical Markers, Hormonal Behavior, and Oxidant Response in Healthy Adults: A Systematic Review. Current nutrition reports *12*, 465-477.

Gupta, A., and Singh, S. (2014). Evaluation of anti-inflammatory effect of Withania somnifera root on collagen-induced arthritis in rats. Pharmaceutical Biology *52*, 308-320.

Harikrishnan, B., Subramanian, P., and Subash, S. (2008). Effect of Withania Somnifera Root Powder on the Levels of Circulatory Lipid Peroxidation and Liver Marker Enzymes in Chronic Hyperammonemia. E-Journal of Chemistry *5*, 589394.

Harirforoosh, S., and Jamali, F. (2005). Effect of nonsteroidal anti-inflammatory drugs with varying extent of COX-2-COX-1 selectivity on urinary sodium and potassium excretion in the rat. Canadian journal of physiology and pharmacology *83*, 85-90.

Hitchon, A., and El-Gabalawy, S. (2004). Oxidation in rheumatoid arthritis. Arthritis research & therapy *6*, 265.

Imamura, H., Hata, J., Iida, A., Manabe, N., and Haruma, K. (2013). Evaluating the effects of diclofenac sodium and etodolac on renal hemodynamics with contrast-enhanced ultrasonography: a pilot study. European journal of clinical pharmacology *69*, 161-165.

Jameson, J.L.J.H.s.p.o.i.m. (2005). Disorders of the thyroid gland. 2104-2113.

Jaworski, J., Maslinski, W., Pazdur, J., Sliwinska-Stanczyk, P., Kaminska-Tchorzewska, E., Jung, L., and Lacki, J.K. (2008). Decreased expression of integrins by hematopoietic cells in patients with rheumatoid arthritis and anemia: relationship with bone marrow cytokine levels. Journal of investigational allergology & clinical immunology *18*, 17-21.

Kalpana, K., Yap, S., Tsuji, M., and Kawamura, A. (2023). Molecular Mechanism behind the Safe Immunostimulatory Effect of Withania somnifera. Biomolecules *13*.

Kam, P.C.A., and See, A.U.-L. (2000). Cyclo-oxygenase isoenzymes: physiological and pharmacological role. *55*, 442-449.

Khaled, H., Hanna, J., Shoukry, N., Darwish, A., and Fares, N. (2022). Therapeutic Potential of Withania somnifera Extract on Experimental Model of Arthritis in Rats: Histological Study. Frontiers in Scientific Research and Technology *4*, 82-90.

Khaled, K.A., Sarhan, H.A., Ibrahim, M.A., Ali, A.H., and Naguib, Y.W. (2010). Prednisolone-loaded PLGA microspheres. in vitro characterization and in vivo application in adjuvant-induced arthritis in mice. AAPS PharmSciTech *11*, 859-869.

Khan, J., Ramadan, K., Korczeniewska, O., Anwer, M.M., Benoliel, R., and Eliav, E. (2015a). Interleukin-10 levels in rat models of nerve damage and neuropathic pain. Neuroscience Letters *592*, 99-106.

Khan, M.A., Ahmed, R.S., Chandra, N., Arora, V.K., and Ali, A. (2019). In vivo, Extract from Withania somnifera Root Ameliorates Arthritis via Regulation of Key Immune Mediators of Inflammation in Experimental Model of Arthritis. Anti-

inflammatory & anti-allergy agents in medicinal chemistry *18*, 55-70.

Khan, M.A., Subramaneyaan, M., Arora, V.K., Banerjee, B.D., and Ahmed, R.S. (2015b). Effect of Withania somnifera (Ashwagandha) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats. Journal of complementary & integrative medicine *12*, 117-125.

Kołodziejska, J., and Kołodziejczyk, M. (2018). Diclofenac in the treatment of pain in patients with rheumatic diseases. Reumatologia *56*, 174-183.

Levesque, R. (2005). SPSS programming and data management: a guide for SPSS and SAS users (Spss).

Logie, E., and Vanden Berghe, W. (2020). Tackling Chronic Inflammation with Withanolide Phytochemicals-A Withaferin a Perspective. Antioxidants (Basel, Switzerland) *9*.

Maleškić Kapo, S., Rakanović-Todić, M., Burnazović-Ristić, L., Kusturica, J., Kulo Ćesić, A., Ademović, E., Loga-Zec, S., Sarač-Hadžihalilović, A., and Aganović-Mušinović, I. (2023). Analgesic and anti-inflammatory effects of diclofenac and ketoprofen patches in two different rat models of acute inflammation. Journal of King Saud University - Science *35*, 102394.

Manan, M., Saleem, U., Ahmad, B., Aslam, N., Anwar, A., and Zafar, A. (2022). Anti-arthritic and toxicological evaluation of ethanolic extract of Alternanthera bettzickiana in rats. Frontiers in pharmacology *13*, 1002037.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature *454*, 428-435.

Mehta, S.S., Ayers, E.R., and Reid, M.C.J.E.T.f.P.i.t.O.P. (2019). Effective Approaches for Pain Relief in Older Adults. 1-11.

Meyer, O., Hoffmann, T., Aslan, T., Ahrens, N., Kiesewetter, H., and Salama, A. (2003). Diclofenac-induced antibodies against RBCs and platelets: two case reports and a concise review. Transfusion *43*, 345-349.

Mikulska, P., Malinowska, M., Ignacyk, M., Szustowski, P., Nowak, J., Pesta, K., Szeląg, M., Szklanny, D., Judasz, E., Kaczmarek, G., *et al.* (2023). Ashwagandha (Withania somnifera)-Current Research on the Health-Promoting Activities: A Narrative Review. Pharmaceutics *15*.

Mody, G.M. (2009). Reflections on rheumatoid arthritis in selected sub-Sahara African countries. East African medical journal *86*, 201-203.

Palliyaguru, D.L., Chartoumpekis, D.V., Wakabayashi, N., Skoko, J.J., Yagishita, Y., Singh, S.V., and Kensler, T.W. (2016). Withaferin A induces Nrf2-dependent protection against liver injury: Role of Keap1-independent mechanisms. Free Radic Biol Med *101*, 116-128.

Panda, S.P., Panigrahy, U.P., Mallick, S.P., Prasanth, D., and Raghavendra, M.J.F.J.o.P.S. (2021). Screening assessment of trimethoxy flavonoid and - (-)-epigallocatechin-3-gallate against formalin-induced arthritis in Swiss albino rats and binding properties on NF-κB-MMP9 proteins. *7*.

Parihar, S. (2022). A literature review on pharmacological activities of Withania somnifera. *02*, 147-154.

Paul, S., Chakraborty, S., Anand, U., Dey, S., Nandy, S., Ghorai, M., Saha, S.C., Patil, M.T., Kandimalla, R., Proćków, J., *et al.* (2021). Withania somnifera (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie *143*, 112175.

Pepys, M.B., and Baltz, M.L. (1983). Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Advances in immunology *34*, 141-212.

Pingali, U., Pilli, R., and Fatima, N.J.C.T.i.N.R. (2013). Effect of Withania somnifera extract on mental stress induced changes in hemodynamic properties and arterial wave reflections in healthy subjects. *11*, 151.

Prakash, J., Gupta, S.K., and Dinda, A.K. (2002). Withania somnifera Root Extract Prevents DMBA-Induced Squamous Cell Carcinoma of Skin in Swiss Albino Mice. Nutrition and Cancer *42*, 91-97.

Priyanka, G., Anil Kumar, B., Lakshman, M., Manvitha, V., and Kala Kumar, B. (2020). Adaptogenic and Immunomodulatory Activity of Ashwagandha Root Extract: An Experimental Study in an Equine Model. Frontiers in veterinary science *7*, 541112.

Sikandan, A., Shinomiya, T., and Nagahara, Y. (2018). Ashwagandha root extract exerts anti-inflammatory effects in HaCaT cells by inhibiting the MAPK/NF-κB pathways and by regulating cytokines. International journal of molecular medicine *42*, 425-434.

Sriuttha, P., Sirichanchuen, B., and Permsuwan, U. (2018). Hepatotoxicity of Nonsteroidal Anti-Inflammatory Drugs: A Systematic Review of Randomized Controlled Trials. International journal of hepatology *2018*, 5253623.

Svensson, M.N.D., Zoccheddu, M., Yang, S., Nygaard, G., Secchi, C., Doody, K.M., Slowikowski, K., Mizoguchi, F., Humby, F., Hands, R., *et al.* (2020). Synoviocyte-targeted therapy synergizes with TNF inhibition in arthritis reversal. Science advances *6*, eaba4353.

Swathi, K.P., Jayaram, S., Sugumar, D., and Rymbai, E. (2021). Evaluation of anti-inflammatory and anti-arthritic property of ethanolic extract of

Clitoria ternatea. Chinese Herbal Medicines *13*, 243-249.

Tanaka, T., and Kishimoto, T. (2014). The biology and medical implications of interleukin-6. Cancer immunology research *2*, 288-294.

Todd, P.A., and Sorkin, E.M. (1988). Diclofenac sodium. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs *35*, 244-285.

Tsujimoto, S., Mokuda, S., Matoba, K., Yamada, A., Jouyama, K., Murata, Y., Ozaki, Y., Ito, T., Nomura, S., and Okuda, Y. (2018). The prevalence of endoscopic gastric mucosal damage in patients with rheumatoid arthritis. PloS one *13*, e0200023.

Ueno, N., Takegoshi, Y., Kamei, D., Kudo, I., Murakami, M.J.B., and communications, b.r. (2005). Coupling between cyclooxygenases and terminal prostanoid synthases. *338*, 70-76.

Wang, K., Zhang, D., Liu, Y., Wang, X., Zhao, J., Sun, T., Jin, T., Li, B., and Pathak, J.L. (2018). Traditional Chinese medicine formula Bi-Qi capsule alleviates rheumatoid arthritis-induced inflammation, synovial hyperplasia, and cartilage destruction in rats. Arthritis research & therapy *20*, 43.

Wang, T., and He, C. (2020). TNF-α and IL-6: The Link between Immune and Bone System. Current drug targets *21*, 213-227.

Yang, H.W. (2009). [COX-2 regulation of prostaglandins in synaptic signaling]. Sheng li ke xue jin zhan [Progress in physiology] *40*, 317-320.

Yasmeen, T., Qureshi, G.S., and Perveen, S. (2007). Adverse effects of diclofenac sodium on renal parenchyma of adult albino rats. JPMA The Journal of the Pakistan Medical Association *57*, 349-351.

Yu, S.-M., and Kim, S.-J. (2013). Production of reactive oxygen species by withaferin A causes loss of type collagen expression and COX-2 expression through the PI3K/Akt, p38, and JNK pathways in rabbit articular chondrocytes. Experimental Cell Research *319*, 2822-2834.

Ziauddin, M., Phansalkar, N., Patki, P., Diwanay, S., and Patwardhan, B. (1996). Studies on the immunomodulatory effects of Ashwagandha. J Ethnopharmacol *50*, 69-76.

دراسات باثولوجية إكلينيكية مقارنة حول التأثير المضاد للالتهاب للأشواغاندا وديكلوفيناك في الفئران

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الملخص العربي

التهاب المفاصل هو مرض التهابي مؤلم، يتميز بتدمير المفاصل والعجز. يتم استخدام عقاقير مضادات الالتهاب غير الستيروتيدية بشكل كبير كمسكنات للألم، إلا أن استخدامها لفترات طويلة يمكن أن ينتج عنه آثار جانبية ضارة لأعضاء الجسم المختلفة. لقد جذبت الأشواغاندا (*Withania somnifera*) نظر الباحثين بحكم استخداماتها الطبية المتعددة. بحثت الدراسة الحالية التأثير المضاد للالتهابات للأشواغاندا مقابل ديكلوفيناك الصوديوم من خلال تقييم سمك المخلب وفحص الدم والمؤشرات الكيميائية الحيوية والالتهابية والتغير النسيجي المرضي في الفئران. تم توزيع الفئران إلى 6 مجموعات والاحتفاظ بها لمدة 21 يوما: المجموعة الضابطة؛ تلقت محلول الملح عن طريق الفم، مجموعة الأشواغاندا (Ash)؛ تلقت عن طريق الفم الأشواغاندا (200 مجم/كجم من وزن الجسم)، مجموعة ديكلوفيناك (Dic) ؛ تناولت عن طريق الفم ديكلوفيناك الصوديوم (2 ملجم/كجم من وزن الجسم)، مجموعة التهاب المفاصل (Arth) ؛ تعرضت الفئران للحقن التحت أخمصي من 0.1 ملي من الفورمالين (2.5 ٪) في المخلب الأيمن الخلفي في اليوم الأول وتلقت محلول الملح عن طريق الفم، مجموعة (Arth+Ash)؛ تم إحداث التهاب المفاصل كما هو موضح في مجموعة (Arth) ثم عولجت بالأشواغاندا (بنفس جرعة مجموعة (Ash)عن طريق الفم)، مجموعة (Arth+Dic)؛ تم إحداث التهاب المفاصل كما هو موضح في مجموعة (Arth) ثم عولج بديكلوفيناك (بنفس جرعة مجموعة (Dic)عن طريق الفم). زاد سمك المخلب بشكل ملحوظ في الفئران (Arth) مقارنة بالفئران الضابطة في أوقات مختلفة على مدار التجربة، إلا أنه قد تم تسجيل انخفاض كبير في مجموعات (Arth + Ash) و (Arth+Dic). عند الانتهاء من البحث (21 يوما)، تم تجميع عينات الدم والأنسجة. أظهرت نتائج الدم عن فقر الدم في الفئران المصابة بالالتهاب، وتم تخفيف هذا التأثير عند إعطاء الأشواغاندا. بينما، كان لدى ديكلوفيناك تأثيرات سلبية في الدم. وأظهرت مستويات ALT واليوريا والكرياتينين و IL-6 و CRP و COX2 و TOC الكبدي زيادة كبيرة في مصل الدم إلى جانب انخفاض كبير في مستوى IL-10 في مصل الدم وأيضا TAC الكبدي في مجموعة (Arth). وفي الوقت نفسه، أظهرت مجموعات (Arth+Ash) و(Arth+Dic) تحسنا معنويا في هذه المؤشرات مقارنة بمجموعة (Arth). بالإضافة إلى ذلك، أوضح تقييمنا النسيجي المرضي الآثار السلبية لديكلوفيناك على أنسجة جسم الفئران المتنوعة وأن الأشواغاندا أظهرت تأثيرا آمنا. وأخيرا، إن تناول مستخلص *W. sominefra* عن طريق الفم للفئران المصابة بالتهاب المفاصل الناجم عن الفورمالين، قلل بشكل ملحوظ من سمك المخلب وخفف من التغيرات غير المرغوبة في جميع المؤشرات المقاسة بطريقة أفضل من ديكلوفيناك. وبالتالي، يمكن استخدام *W. sominefra* لعلاج التهاب المفاصل الروماتويدي دون التسبب في أي ضرر لأنه يمتلك تأثيرات مضادة للالتهابات.

الكلمات الدالة: أشواجندا، التهاب المفاصل، ديكلوفيناك، خلايا الدم الحمراء، المؤكسد، الالتهاب، الفئران.