Hematological Studies on the Effect of Some Agents Against Arthritis Induced in Rats

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Abstract;
This study was designed to investigate the effect of formaldehyde-induced arthritis in rat model and evaluate the potency of flaxseed oil (FO) and graviola leaf extract (GrLE) as anti-arthritic agents. For the study, we used sixty male albino rats which were split up randomly into six equal groups as following; Group I: Normal control rats received 0.5 ml/kg b.wt of 9% saline, orally for 2 weeks, Group II: Injected with FO at a dose of 3.0 ml/kg b.wt by i.p route for 2 weeks, Group III: Received GrLE at an oral dose of 100 mg/kg b.wt for 2 weeks, Group IV: Arthritic control group which induced to arthritis by sub planter injection of 0.1 ml of 2% formaldehyde solution v/v through the left hind paw on day 1 and day 3 of the experiment, Group V: Arthritic rats treated with FO, Group VI: Arthritic rats treated with GrLE. The results showed that arthritic untreated rats had signs of macrocytic hypochromic anemia. Also, they showed a significant leukocytosis, neutrophilia, lymphocytosis, monocytosis and eosinophilia. Moreover, they showed a significant thrombocytosis. However, arthritic rats treated with FO and GrLE showed improvement in the hematological parameters when compared to the arthritic untreated group. This suggested that FO and GrLE have anti-arthritic effect against formaldehyde-induced arthritis, likely due to their anti-inflammatory and antioxidant properties.

Keywords:
Formaldehyde, hematology, flaxseed oil, graviola, rats.

Introduction

Arthritis is a widespread, immunological disease which is affected by genetic and environmental factors ***(Ahsan et al., 2021)***. Presence of arthritis seems to increase the impact of oxidative stress due to free radical releasing ***(Sun et al., 2017)***. It also, stimulates secretion of many pro-inflammatory mediators like TNF-α, IL-1β, and IL-6 which play a

major role in the pathogenesis and the clinical signs of this disorder ***(Qasim et al., 2021)***.

Flaxseeds are rich in lignans ***(Hendawi et al., 2016*)**, which are known to be natural antioxidants ***(Naqshbandi et al., 2013)***. Flaxseed oil was found to have anti-inflammatory and anti-arthritic effects as it contains high variety proportions of unsaturated fatty acids including oleic acid, linoleic acid and linolenic acid ***(Kaithwas and Majumdar, 2012).***

Annona muricata, which also known as graviola, soursop or guanabana ***(Coria-Téllez et al., 2016)***, has anti-nociceptive, anti-inflammatory and anti-arthritic effect through inhibition of TNF-α and IL-1β in affected tissue. Also, *A. muricata* was proved to contain enzymatic and non-enzymatic antioxidants that attenuate releasing of ROS such as nitric oxide, decrease lipid peroxidation and act as buffer against oxidative stress ***(Moghadamtousi et al., 2015)***.

This study aimed to investigate the impact of formaldehyde-induced arthritis in rat model and evaluate the potency of flaxseed oil (FO) and graviola leaf extract (GrLE) as anti-arthritic agents.

Material and methods

Lab animals

Sixty normal male Wistar albino rats, with average weight (90-120) gm, were procured from the laboratory animal house; Faculty of Veterinary Medicine, Suez Canal University. The animals were acclimatized for one week at the laboratory animal house before the start of the experiment. They were kept at a consistent temperature range of 23±2 C^o and on 12-hour light-dark cycle. They were given a decent meal and unlimited access of water while been housing under normal healthy conditions.

Drugs and Chemicals

Flaxseed oil was purchased from Zongle Therapeutics, USA, while, graviola leaves were obtained from NOW FOODS, USA. Formaldehyde was supplied from El-Nasr Pharmaceutical Chemicals Co., Egypt.

Experimental design and grouping of rats

At the start of the study, the animals were weighed and then separated randomly into 6 equal groups; NC group kept as control and received 0.5 ml/kg b.wt of 9% saline, orally, for 2 weeks, FO group was injected with 3.0 ml/kg b.wt of FO by i.p route for 2 weeks, while, GrLE group received 100 mg/kg b.wt of GrLE, orally for 2 weeks. ArC group was given 0.1 ml of 2% formaldehyde solution (v/v) injected in sup planter region of left hind paw on day 1 and day 3 of the experiment to induce arthritis. ArC+FO group induced to arthritis and treated with 3.0 ml/kg b.wt of FO (i.p) for 2 weeks. ArC+GrLE group induced to arthritis and rats treated with 100 mg/kg b.wt of GrLE, orally, for 2 weeks.

Sampling

Rats were sacrificed twice; after one week then after two weeks of the experiment, respectively. Five rats/group were sacrificed each time. Under the effect of tetrahydrofuran inhalation anesthesia, blood samples were drawn from the retro-orbital venous plexus of rats for hematological estimation.

Hematological parameters estimation

The estimated hematological parameters were RBCs count, Hb conc., HCT, erythrocytic indices, total and differential leukocytic count, as well as, platelets.

Statistical Analysis

Data were collected and analyzed using SPSS, 22 and Duncan's Multiple Range test for analysis of variance (ANOVA), and the results were marked as significant at a $(P \leq 0.05)$ probability level. Effect of treatments on hematological parameters was assessed by the analysis of variance. Values are presented as means and standard errors.

Results

Data stated in **Table (1)** showed that after one and two weeks of the experiment, there were non-significant differences in Hb concentration, RBCs count or HCT % in FO and GrLE groups in comparison to normal. In ArC group, Hb concentration, RBCs count and HCT % showed a significant reduction when compared with normal. Compared to ArC group, the values were significantly increased in Ar+FO and Ar+GrLE groups.

As illustrated in **Table (1)**, after one week of the experimental period, there were non-significant differences in MCV, MCH and MCHC in all treated groups when compared with normal group.

Two weeks after the experimental period, there were non-significant differences in MCV, MCH and MCHC between FO, GrLE and normal groups. ArC group had a significant elevation in MCV and MCH values, while, MCHC value was significantly reduced when compared to normal group. Ar+FO and Ar+GrLE groups showed a significant decrease in MCV and MCH values and a significant increase in MCHC when compared to ArC group.

After one and two weeks of the experimental period, there were non-significant differences in RDW in all treated groups when compared with normal group.

As presented in **Table (2)**, after one and two weeks of the experimental period, results showed non-significant changes in WBCs count, neutrophils, lymphocytes, monocytes and eosinophils of FO and GrLE groups when compared to normal group. They were significantly elevated in ArC group when compared to normal group. Both Ar+FO and Ar+GrLE groups had a significant improvement in their values as compared to ArC

group.

Data shown in **Table (2)** indicated that, after one and two weeks of the experimental period, platelets count showed non-significant change in FO and GrLE groups when compared to normal group. There was a significant thrombocytosis in ArC group when compared to normal group. There was a significant decline in PLT count of Ar+FO and Ar+GrLE groups when compared to ArC group.

Table (1): *Effect of formaldehyde, flaxseed oil (FO) and graviola leaf extract (GrLE) on erythrogram of rats after one and two weeks of the experimental pperiod.*

Groups / Parameters	NC	FO	GrLE	ArC	Ar+FO	Ar+GrLE
After one week						
Hb (g/dl)	15.13±0.19^{a}	14.83±0.20^{a}	14.74±0.14^{a}	10.00±0.09^{c}	13.61±0.15^{b}	13.44±0.15^{b}
RBCs (x10^{6}/µl)	7.22±0.17^{a}	7.14±0.07^{a}	7.15±0.12^{a}	4.56±0.14^{c}	6.03±0.07^{b}	5.95±0.15^{b}
HCT (%)	46.06±0.56^{a}	45.50±0.61^{a}	46.02±0.42^{a}	30.00±0.26^{c}	40.08±0.44^{b}	39.45±0.46^{b}
MCV (fl)	63.80±0.79^{a}	63.73±1.45^{a}	64.36±1.44^{a}	65.79±1.30^{a}	66.47±1.66^{a}	66.30±1.54^{a}
MCH (pg)	20.96±0.25^{a}	20.77±0.48^{a}	20.62±0.47^{a}	21.93±0.75^{a}	22.57±0.26^{a}	22.59±0.88^{a}
MCHC (%)	32.85±0.01^{a}	32.59±0.01^{a}	32.03±0.01^{a}	33.33±0.01^{a}	33.96±0.61^{a}	34.07±0.68^{a}
RDW (%)	15.09±0.30^{a}	14.80±0.12^{a}	15.63±0.38^{a}	15.70±0.26^{a}	15.07±0.15^{a}	15.50±0.15^{a}
After two weeks						
Hb (g/dl)	15.07±0.19^{a}	14.73±0.35^{a}	14.41±0.22^{ab}	9.47±0.18^{d}	13.89±0.04^{bc}	13.45±0.27^{c}
RBCs (x10^{6}/µl)	7.01±0.19^{a}	7.23±0.13^{a}	6.94±0.11^{ab}	3.65±0.12^{d}	6.56±0.13^{bc}	6.20±0.12^{c}
HCT (%)	46.20±0.56^{a}	45.20±1.06^{a}	44.22±0.65^{ab}	32.40±0.53^{d}	42.61±0.33^{bc}	40.40±1.06^{c}
MCV (fl)	65.91±1.28^{b}	62.52±2.40^{b}	63.72±0.97^{b}	88.77±1.71^{a}	64.95±1.12^{b}	65.16±1.63^{b}
MCH (pg)	21.50±0.42^{b}	20.37±0.79^{b}	20.76±0.32^{b}	25.95±0.54^{a}	21.17±0.36^{b}	21.69±0.35^{b}
MCHC (%)	32.62±0.01^{a}	32.59±0.02^{a}	32.59±0.01^{a}	29.23±0.02^{b}	32.60±0.00^{a}	33.29±0.29^{a}
RDW (%)	15.20±0.00^{a}	14.80±0.32^{a}	14.63±0.29^{a}	15.27±0.02^{a}	15.40±0.03^{a}	15.07±0.29^{a}

Mean ± SE is used to express values.

In every raw, means with different superscripts are considered significant at (P≤0.05).

Table (2): *Effect of formaldehyde, flaxseed oil (FO) and graviola leaf extract (GrLE) on leukogram and platelets of rats after one and two weeks of the experimental period.*

Groups / Parameters	NC	FO	GrLE	ArC	Ar+FO	Ar+GrLE
After one week						
WBCs ($x10^3/\mu l$)	7.93±0.11^c	7.77±0.27^c	7.74±0.15^c	17.36±0.83^a	13.58±0.44^b	13.33±0.52^b
Neutrophils ($x10^3/\mu l$)	1.38±0.09^c	1.55±0.13^c	1.76±0.17^c	4.22±0.24^a	3.20±0.21^b	3.21±0.27^b
Lymphocytes ($x10^3/\mu l$)	6.21±0.10^c	5.93±0.24^c	5.58±0.14^c	11.92±0.51^a	9.52±0.24^b	9.43±0.39^b
Monocytes ($x10^3/\mu l$)	0.26±0.05^c	0.21±0.03^c	0.32±0.02^c	1.05±0.15^a	0.72±0.03^b	0.56±0.07^b
Eosinophils ($x10^3/\mu l$)	0.08±0.00^c	0.08±0.00^c	0.08±0.00^c	0.17±0.01^a	0.14±0.00^b	0.13±0.00^b
PLT ($x10^3/\mu l$)	591.67±5.55^c	592.00±4.04^c	598.33±6.12^c	831.00±5.86^a	721.33±3.48^b	727.33±6.74^b
After two weeks						
WBCs ($x10^3/\mu l$)	8.05±0.37^c	7.60±0.45^c	7.31±0.22^c	21.20±0.83^a	11.98±0.41^b	12.10±0.49^b
Neutrophils ($x10^3/\mu l$)	2.34±0.36^c	1.98±0.40^c	1.76±0.08^c	10.46±0.64^a	4.77±0.16^b	4.62±0.19^b
Lymphocytes ($x10^3/\mu l$)	5.40±0.16^c	5.32±0.16^c	5.23±0.34^c	9.65±0.61^a	6.60±0.40^b	6.89±0.38^b
Monocytes ($x10^3/\mu l$)	0.23±0.04^c	0.22±0.03^c	0.24±0.05^c	0.88±0.14^a	0.50±0.05^b	0.49±0.02^b
Eosinophils ($x10^3/\mu l$)	0.08±0.00^c	0.08±0.00^c	0.08±0.00^c	0.21±0.01^a	0.11±0.00^b	0.10±0.00^b
PLT ($x10^3/\mu l$)	605.00±5.86^c	606.00±2.31^c	603.67±4.70^c	989.67±6.64^a	633.00±4.36^b	635.33±6.96^b

Mean ± SE is used to express values.
In every raw, means with different superscripts are considered significant at (P≤0.05).

Discussion

Arthritis is a systemic disease that affects not only joints but, its effect could expand to any other organs in the body ***(Manan et al., 2022)***. Formaldehyde-induced arthritis is the most commonly used model for experimental arthritis ***(Kumar et al., 2008)*** due to its resemblance to human arthritis ***(Uttra, 2017)***. Therefore, this study aimed to study the impact of formaldehyde-induced arthritis in rat model and evaluate the potency of FO and GrLE as anti-arthritic agents.

The present results revealed that, after one and two weeks of the experiment, formaldehyde-induced arthritic rats showed a significant alteration in different hematological parameters than normal control. After one week of the experiment, Hb conc., RBCs count and HCT % were significantly reduced. While, MCV, MCH and MCHC showed non-significant change in all treated groups when compared to normal that resulted in normocytic normochromic anemia. After two weeks, both MCV and MCH values were significantly elevated, and MCHC % was significantly

decreased when compared to normal control and that resulted in macrocytic hypochromic anemia. The results agreed with ***Farrukh et al., (2022)*** and ***Manan et al., (2022).***

Formalin has oxidative effect on RBCs that causes acute intravascular hemolysis, as formaldehyde can be noxious to RBCs and causes hemolysis. Also, there were reports of a secondary hemolysis in hemodialysis patients when formalin is used for disinfection of equipment ***(Yazdi et al., 2012)***. Many studies showed that the mechanism of action of formaldehyde, as a potent reducing agent, entails converting of NAD into NADH. The resulting modification of the redox reactions of the erythrocyte causes suppression of glycolysis and thus, sharp decline in ATP stored in the cells ***(Feldman et al., 2000; Latimer et al., 2003)***.

The decreased Hb and RBCs concentrations in arthritic rats caused anemia that may increase as a result of dysfunction of erythrocyte and decreased production of erythropoietin. Also, IL-1β gene -which proved to be elevated in case of arthritis- has a role in sustaining the regular blood physiology ***(Uttra and Hasan, 2017)***. Also, reactive oxygen species (ROS) can cause direct oxidative impairment to bone marrow and enhance hemoglobin glycation which causes erythrocyte fragility ***(Niforou et al., 2014)***.

Since anemia of chronic diseases is immune driven, cytokines and the reticulo-endothelial system cells contribute to its pathogenesis through inducing changes in iron homoeostasis, proliferation of erythroid progenitor cells, decreased erythropoietin production and short life span of red blood cells ***(Weiss and Goodnough, 2005)***. Another explanation by ***Safer et al., (2005)*** indicated that these reduction in of RBCs, Hb and HCT values may be due to that formaldehyde-induced arthritis generates secretion of toxins in the body of diseased animals that results in defect in thyroid gland' performance and it is well known that thyroid hormones has an indirect impact on metabolic processes of the body and bone marrow ability to produce erythrocytes.

WBCs count and DLC including neutrophils, lymphocytes, monocytes and eosinophils were significantly elevated in formaldehyde-induced arthritic rats than normal control. These results were in accordance with ***Farrukh et al., (2022)***. This increase could be attributed to the internal inflammatory processes that result in releasing of certain pro-inflammatory cytokines which enhance WBCs proliferation ***(Hassan and Jassim, 2010)*** or immune system stimulation which increase production of pro-inflammatory cytokines ***(Farrukh et al., 2022)***.

Our results also, indicated a significant thrombocytosis in untreated arthritic rats than normal control. These results were in agreement with ***Manan et al., (2022)***. This increase could be due to release of IL-1 and TNF-α in arthritic state that cause rise in platelet count ***(Maria et al., 1983)***. Moreover, the major impact of cytokines particularly IL-6, which increase maturation of generated PLT cells and thus, increase PLT production **(Pamuk et al., 2008)**.

Administration of FO to normal rats had non-significant effect on the hematological parameters when compared to normal control rats during the experimental period. These result were similar to a previous study by ***Hendawi et al., (2016)***. After one and two weeks of the experiment, formaldehyde-induced arthritic rats treated with FO seemed to significantly ameliorate the altered hematological parameters when compared with untreated arthritic rats. These results were supported by previous studies of ***El-shater et al., (2022).*** This improvement was in response to flaxseed oil that has antioxidant activities and abilities to scavenge the free radical and protect the erythrocyte membrane from the generated MDA. Also, the decreased WBCs count and DLC may be due to presence of antioxidant components like β-carotene and tocopherols which have protective effect ***(Hendawi et al., 2016)***. The decrease in platelets could be due to the mild antiplatelet effect of FO with collagen-induced platelet aggregation ***(El-shater et al., 2022)***.

Administration of GrLE to normal rats had non-significant effect on the hematological parameters when compared with normal control group during the experimental period. Our findings were similar to ***Usunobun and Okolie, (2016)***. After one and two weeks of the experiment, formaldehyde-induced arthritic rats treated with GrLE seemed to significantly ameliorate the altered hematological parameters when compared with untreated arthritic rats. These findings coincided with ***Shukry et al., (2020)*** and ***Olude et al., (2023)***. ***Ejere et al., (2013)*** attributed this improvement to the ability of graviola to restore body fluids and stimulate erythropoietin production. Also, ethanol extract of *A. muricata* could scavenge free radicals and superoxide, as well as, preserving the function of several antioxidant enzymes, including catalase ***(Olude et al., 2023)***. ***Tsai et al., (2017)*** attributed the protective capacity *A. muricata* to the phenolic chemicals present its leaves. These compounds may be responsible for the anti-hemolytic activity they exhibited since they not only stabilize free radicals but also make erythrocytes more resistant to oxidative stress.

Conclusion

We recommended the usage of FO & GrLE as anti-arthritic agents that

can aid in relieving the hematological perturbations of arthritis with no adverse side effects.

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دراسات دموية على تأثير بعض المواد المستخدمة ضد التهاب المفاصل في الجرذان

الملخص العربي

صممت الدراسة الحالية لدراسة تأثير التهاب المفاصل الناجم عن الفورمالدهيد في الجرذان وتقييم مدى فعالية زيت بذور الكتان ومستخلص أوراق القشطة الشائكة كعوامل مضادة لالتهاب المفاصل. تم استخدام ستين جرذ في الدراسة وتقسيمهم على ٦ مجموعات. أظهرت النتائج أن الجرذان التي تم حقنها بالفورمالدهيد أصيبت بفقر الدم الناقص الصباغ كبير الكريات وكثرة الصفيحات. أيضا، أظهروا زيادة كبيرة في كرات الدم البيضاء وعدد الكريات البيضاء التفاضلية بما في ذلك العدلات والخلايا الليمفاوية والوحيدات والحمضيات.

ومع ذلك، أظهرت الفئران المعالجة بزيت بذر الكتان ومستخلص أوراق القشطة الشائكة تحسنًا في هذه القياسات مقارنة بالمجموعة غير المعالجة.