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THE ROLE OF PROPHETIC MEDICINE USING *LAWSONIA INERMIS* ON INFLAMMATORY PAIN IN EXPERIMENTAL ANIMALS MEDIATED THROUGH INCREASE IN CORTISOL LEVEL

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ABSTRACT

Background: The crude ethanolic extract of *Lawsania Inermis* produced significant and dose-dependent anti-inflammatory, analgesic and antipyretic effects in rats, while the butanol and chloroform fractions of the extract showed significantly more anti-inflammatory, analgesic and antipyretic and effects than the crude and aqueous extracts. This suggests that constituents of the extracts differ from one to the other in their pharmacological properties. Furthermore, the exact mechanism of action as anti-inflammatory, antipyretic or analgesic activity has not been investigated. **Methods:** The leaves of *Lawsonia Inermis* (500g) were subjected to successive extraction with deferent solvents in the following order: light petroleum ether, chloroform, methanol and distilled water. Three models were used to test anti-inflammatory activity Carrageenan-induced paw edema in normal and adrenalectomized rats and six-day air pouch in rats representing the acute state and the adjuvant induced arthritis representing the chronic one. Cortisol level was measured in rats challenged for paw edema test. Two models were used to investigate the analgesic activity of different extracts: The acetic acid-induced writhing model and Randell-Selitto model. **Results:** The four extract of *Lawsonia Inermis* decreased paw edema in normal and adrenalectomized rats. In addition decrease in the volume of exudates and leukocytic number induced by carrageenan in air pouches. Furthermore, tested extracts showed decrease in the hind paw diameters induced by Complete Freund's adjuvant injection. The four extracts decreased the number of writhing movement in mice and pain hypersensitivity to mechanical stimuli in rats. All the four extract increased the cortisol level which is a novel effect.

KEYWORDS

Lawsonia Inermis, Anti-inflammatory activity, Analgesic activity and Cortisol.

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INTRODUCTION

Inflammation involves a series of vascular events that serve as a defense mechanism including clotting mechanism activation, increased blood flow, increased capillary permeability and enhanced influx of phagocytic cells (Elgert, 2009)[1].

During inflammation, several cell types such as mast cells, platelets and leukocytes are responsible for the release of inflammatory mediators which play an important role in the development of inflammation (Theoharides *et al*, 2012)[2]. Inflammatory pain is characterized by an increased sensitivity to stimuli, which does not cause pain under normal conditions (Woolf and Costigan, 1999)[3].

Inflammatory response induces the synthesis of different mediators that finely control the inflammatory process. Arachidonic acid releases from cell membrane phospholipids by the action of phospholipase A_2.Free arachidonic acid may be metabolized by 5-lipoxygenase to form leukotrienes or by one of the isoforms of cyclooxygenase (COX) to form prostaglandins (PG_S) (Wang *et al*, 2021)[4].

Cytokines are major determinants of cellular infiltrate, and the systemic response to inflammation (Wallace, 2007[5], Resch, 2005)[6]. Several cytokines play key roles in mediating acute inflammatory reactions, namely interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) (Hubbard and Giardina, 2000[7], Ren and Torres, 2009[8]).

Activated phagocytes release highly reactive species such as superoxide anion ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) and have also been involved in the pathogenesis of many chronic inflammatory disorders like rheumatoid arthritis (Sharma *et al,* 2007[9], Weinberg *et al*, 2007[10]).

Cortisol is the most potent glucocorticoid produced by the human adrenal cortex in response to inflammation. Cortisol is synthesized from cholesterol through a series of enzymatically mediated steps. In plasma, the major portion of cortisol is bound with affinity to corticosteroid-binding globulin (CBG), transcortin, with most of the remainder loosely bound to albumin. Cortisol act through specific intracellular receptors and has effects in numerous physiologic systems, including immune function, glucose metabolism, vascular tone and bone metabolism (Teblick *et al*, 2019[11]).

Lawsonia inermis, Linn (Family: Lythraceae) which is commonly known as henna. The pharmacological studies showed that *Lawsonia inermis* showed antibacterial, antifungal, antiparasitic, antioxidant, hepatoprotective, antipyretic, wound and burn healing, immunomodulatory, antiurolithiatic, antidiabetic, hypolipidemic, antiulcer, antidiarrhoeal, diuretic, anticancer and many other pharmacological (Mikhaeil *et al*, 2004)[12].

The Prophet may God's prayers and peace be upon him, used henna in the field of medicine. He would not get a sore or a thorn without putting henna on it. Ibn Majah narrated in his Sunan that when the Prophet, peace and blessings be upon him, would wrap his head in henna and say (it is beneficial, God willing, from headaches) SunanIbn Majah (2/1158) and Imam Ahmad included it in his (Musnad) (6/462).

MATERIAL AND METHODS

Animals

The animals used in this study were male albino mice weighing 20-25g and male Sprague-Dawley rats weighing 150-200g each. The animals were purchased from the animal house, (National Research Center (NCR), Cairo, Egypt). They were kept at the animal facility of the NRC at an ambient temperature of 25°C with alternating 12h light and dark cycles and a constant humidity 1 week before experimentation. Animals were allowed free access to food (standard pellet diet) and water *ad libitum*. All animal procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Drugs and chemicals

Plant material

The air-dried leaves of *Lawsonia inermis* were purchased from the local herbal stores in Cairo. They were identified and authenticated by to Prof. Dr. Nagwa Ammar, Department of Pharmacognosy, National Research Center, Giza, Egypt.

Acetic acid, Chloroform, Gum acacia, Methanol, and Petroleum ether were purchased from El-Nasr Pharmaceutical Company, Egypt. Carrageenan was

purchased from Sigma-Aldrich, Italy. Complete Freund's adjuvant was purchased from Difco Laboratories (Detroit, MI, USA). Diclofenac potassium was purchased from Novartis Pharma., Egypt. Cortisol level was determined with a commercially available ELISA kit (MonobindInc, USA).

Selection of doses was based on preliminary screening. The dose of diclofenac potassium was 5mg/kg of diclofenac (El-Ghazaly, 1996)[13]. The dose of the test extracts were also chosen after carrying out preliminary tests on carrageenan-induced edema and writhing test using 500 and 750mg/kg test extracts. The dose of 750mg/kg was selected.

Experimental Design

Preparation of *Lawsonia inermis* extracts

The air dried powdered leaves of *Lawsonia inermis* (500g) were subjected to successive extraction in a continuous extraction apparatus (Soxhlet) with solvents of increasing polarity in the following order: light petroleum ether, chloroform, methanol and distilled water. The solvent of each extract was removed by distillation under reduced pressure at a low temperature not exceeding 40ºC using a vacuum rotatory evaporator. Successive extracts were physically examined and tested for their phytochemical constituents. Lawsone content in the four extracts was estimated using a colorimetric method.

Six groups each of 6 rats (150-200g) were used and treated as follow:

Group 1

Vehicle (25% gum acacia solution) treated group and served as control group and injected carrageenan.

Group 2

Treated with Diclofenac Potassium (5mg/kg) orally.

Group 3

Treated with *Lawsonia inermis* Methanolic extract (750mg/kg) orally.

Group 4

Treated with Chloroformic extract (750mg/kg) orally.

Group 5

Treated with Petroleium ether extract (750mg/kg) orally.

Group 6

Treated with Water extract (750mg/kg) orally.

All test substances were freshly prepared. The drugs dissolved or suspended in water in the form of emulsion using 25% gum acacia as a solvent.

Screening of anti-inflammatory activity

Carrageenan-induced rat paw-edema. One hour following the previously mentioned treatments, paw-edema was measured according to Winter *et al*, (1962)[14]. Paw swelling was induced by a subcutaneous (S.C.) injection of 0.1ml of 1% carrageenan suspension in saline into the right hind paw. Edema was quantified by measuring the hind paw edema volume immediately before carrageenan injection and at selected time intervals thereafter (1, 2, 3 and 4 h post carrageenan administration) plethysmographically using Plethysmometer (UGO Basile, Italy) in normal and adrenalectomized rats.

Cortisol level was measured in rats challenged for paw edema test and bilateral adrenalectomy was carried out in anaesthetized rats using dorsal approach to study the effect of the adrenal gland on the therapeutic action of the test extracts and to determine the anti-inflammatory effect of the test drugs in the absence of the adrenal gland.

Blood sample was taken early in the morning at baseline before carrageenan injection and 2h and 4h after for assay of cortisol in normal rats (Catelan *et al*, 2006[15], Li *et al*, 2007[16]). Serum cortisol level was determined with a commercially available ELISA kit (MonobindInc, USA) using the procedure recommended by the manufacturer.

Bilateral adrenalectomy was carried out in rats (ADX) using dorsal approachunder anesthesia with sodium pentobarbital (50mg/kg,i.p) (Li *et al*, 2007)[16]. The procedure was performed via a median incision of the muscular wall, allowing visualization of the glands, which were then stripped of the surrounding adipose tissue and resected (Catelan *et al*, 2006)[15]. The rats were given 0.9% Na Cl solution instead of drinking water to compensate for sodium loss and (ADX) animals

were used to study the effect of the adrenal gland on the therapeutic action of the tested drugs. Animals were allowed to recover for 5 days before the experiment (Li *et al*, 2007)[16].

On day 6 paw edema test was performed to determine the anti-inflammatory effect of the tested drugs in the absence of the adrenal gland. The distribution of the different groups was the same as that described above under control rats. Paw volume (water displacement) was determined plethysmographically using Plethysmometer (UGO Basile, Italy) and the results of the treated rats were compared with the control animals (Li *et al*, 2007)[16].

The increase in volume will be taken as the volume (ml^3) of edema and will be determined for each rat. The percentage of edema inhibition in treated animals versus control was calculated as follows:

% Inhibition $= (V_t - V_0)_{control} - (V_t - V_0)_{treated} / (V_t - V_0)_{control} \times 100$

Six-Day Air-Pouch Test

The method used was carried out according to the method described by Sedgwick *et al*. (1986b)[17]. On the first day of the experiment, 20ml of sterile air were injected subcutaneously in the back of each rat. Two days later, another 10ml of air were injected at the same site. On the fifth day after the first injection, a further 10ml of air was injected into the pouch. Twenty four hr later, carrageenan (2ml of 1% saline solution) was injected into the air pouch.

The tested drug was given orally on the fifth and the sixth days 1 hr before carrageenan injection. Six hr after carrageenan injection, the animals were lightly anaesthetized with ether, 2ml of heparinized saline were injected into the pouch, the pouches were lightly massaged for 3 s before aspirating the pouch contents using a pasture pipette and transferring the fluid into graduated plastic tubes kept in ice. The volume of exudates was measured and leukocytic content was determined using the hematocytometer (Cheesbrough, 2006)[18].

White blood cells were calculated as follow:

White blood cells (per liter) = cells counted x 20 x 10^6 / 4 x 0.1

Where 20= 1 in 20 dilution of blood

4= mm^2 area counted

0.1= mm depth of chamber

Adjuvant induced arthritis

The method used was carried out according to the method described by Cicala *et al*, (2000)[19]. Arthritis was induced by injecting 100ul of complete Freund's adjuvant (CFA) into the left hind paw. Hind paw volume was measured by a hydroplethysmometer immediately before arthritis induction (Basal value) and at 3, 7, 11, 14, 17 and 21 days thereafter. Hind paw swelling was expressed as an increase in hind paw volume in ml calculated by subtracting the Basal value from the hind paw measured at all times considered. Drugs were given daily by oral gavages to rats following therapeutic regimen of dosing, from day 13 to 21. Control group received the same volume of water. The animals were sacrificed on day 21.

Screening of analgesic activity

Writhing test

Writhing test was carried out according to the method of Zeashan *et al*, (2009)[20]. Male mice weighing between 20 and 25g were used. Acetic acid in a concentration of 0.6% solution was injected intraperitoneally. The animals were chosen as follows, on the first day the animals were injected acetic acid intraperitoneally then those who writhe were taken and on the second day (Zeashan *et al*, 2009)[20.]

Test animals were given the drug 1 h before acetic acid injection. Five min were allowed to elapse then the mice were then observed for a period of 30 min and the number of writhing movement (contraction of the abdominal muscles, twisting of the trunk, accompanied by stretching of the hind limbs) were recorded for each animal (Zeashan *et al*, 2009)[20].

% Inhibition =100-(mean writhes of test/mean writhes of control ×100)

Effective analgesic compounds are those, which inhibit writhing more than 70%. Compounds with less than 70% inhibition are considered to have minimal activity.

Randall-Selitto test (Pain in inflamed tissue)

Pain hypersensitivity to mechanical stimuli was assessed using an analgesy-meter. Male rats were wrapped loosely in a towel with the hind paw placed between the analgesy-meter cone shaped pusher and the plinth. A linearly increasing force at a loading rate 30gm/sec was then applied. The endpoint reached upon hind paw withdrawal and the corresponding force was recorded as the withdrawal threshold (Randall and Selitto, 1957)[21]. The site at which the force was applied was marked with a pin before the test started to insure that all measurements were taken from the same site. Three readings for each paw were taken at 5 min intervals and averaged (Anseloni *et al*, 2003)[22].

The animals were chosen as follows, each animal was tested for its control pain threshold. Any animal with a control pain threshold greater than 80 g was eliminated and replaced (Randall and Selitto, 1957)[21]. The animals were trained for 14 days before the test to stabilize the base line and increase the test sensitivity (Anseloni *et al*, 2003)[22]. Acute inflammation was induced by intraplantar injection of 0.1ml of carrageenan solution (0.5% in distilled water) into the left hind paw.

Both test extracts and standard drug were given orally 30 min before carrageenan injection. The mechanical threshold was measured for left and right paws before and after four hours of carrageenan injection, at 15, 30, 60, 90 and 120 min. Nociception is defined as the weight at which the rat withdraws its paw. The cut off value was 0.5kg maximum.

% Analgesia = (100 B/A) - 100

Where A-pressure (in g) at the time (0), B-pressure (in g) 15, 30, 60, 90 and 120 min after treatment (Bujalska, 2003[23], Kayser, 2007[24]).

Statistical analysis

In the present study, all results were expressed as mean± standard error of the mean. Data of 6-day air pouch model and writhing test were analyzed using One-way ANOVA followed by LSD multiple range test. Data of carrageenan-induced rat paw edema, adjuvant-induced arthritis, Randall-Selitto test and cortisol level were analyzed using Two-way ANOVA to test for interaction between time and grouping.

RESULTS AND DISCUSSION

Estimation of the active constituents in *Lawsonia Inermis* extracts

Lawsone content in the four extracts were estimated using colorimetric method, based on the maximum absorptivity at different wave length against authentic substance. Final results obtained from calibration curve and Final results were expressed as milligrams of lawsone equivalents per gram of dried weight of each extract as seen in Table No.1.

Anti-inflammatory activity

Effect of *Lawsonia inermis* extracts on carrageenan-induced rat paw edema

Results are shown in Table No.2. Subplanter injections of carrageenan suspension showed marked, time-related and progressive increase in the hind paw volume of the control untreated rats when compared with that of zero time paw diameter. Maximal swelling occurred approximately 3 hr following carrageenan administration.

Pretreatment with *Lawsonia inermis* extracts significantly inhibited the carrageenan-induced inflammatory edema by 73.95%, 68.98%, 58.31% and 55.58% for methanol, chloroform, petroleum ether and water extract respectively at the 4thhr when compared with the control non-treated group.

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using Two-way ANOVA.

*Statistically significant from the control values at ($P<0.05$).

@Statistically significant from diclofenac treated group at the corresponding time at ($P<0.05$).

In ADX rats pretreatment with *Lawsonia inermis* extracts significantly inhibited the carrageenan-induced inflammatory edema by 51.85%, 44.44%, 44.44% and 22.22% for methanol, chloroform, petroleum ether and water extract respectively at the 4thhr when compared with the control non-treated group. Results are shown in Table No.3.

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using Two-way ANOVA.

*Statistically significant from the control values at (P<0.05).

@Statistically significant from diclofenac treated group at the corresponding time at (P<0.05).

All extracts of *Lawsonia inermis* caused a significant increase in serum cortisol level when compared with control untreated rats (P<0.05) which is a novel effect. The methanolic extract was the most powerful in increasing the serum level of cortisol reaching about 38 fold that of the control value (P<0.05). The effects were also significant (P<0.05) when compared with that of diclofenac as shown in Table No.4.

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using Two-way ANOVA.

*Statistically significant from the control values at (P<0.05).

@Statistically significant from diclofenac treated group at the corresponding time at (P<0.05).

Petroleum ether extract, chloroformic extract, methanolic extract caused significant decrease in the volume of exudates induced by carrageenan in air pouches (P<0.05). The effect of the water extract however was not significant when compared with that of control (P<0.05). Diclofenac under the same condition caused a significant decrease in the volume of exudates compared with the control untreated rats (P<0.05) as shown in Table No.5.

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using One-way ANOVA.

*Statistically significant from the control values at (P<0.05).

@Statistically significant from diclofenac treated group at the corresponding time at (P<0.05).

Petroleum ether extract, chloroformic extract, methanolic extract caused significant decrease in the leukocytic number induced by carrageenan in air pouches (P<0.05). The effect of the water extract however was not significant when compared with that of control (P<0.05). Diclofenac under the same condition caused a significant decrease in the volume of exudates compared with the control untreated rats (P<0.05) as shown in Table No.6.

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using One-way ANOVA.

*Statistically significant from the control values at (P<0.05).

@Statistically significant from diclofenac treated group at the corresponding time at (P<0.05).

Results are shown in Table No.7. Subplanter injections of Complete Freund's adjuvant suspension showed marked, time-related and progressive increase in the hind paw diameters of the control untreated rats when compared with that of zero time paw diameter. Pretreatment with *Lawsonia inermis* extracts significantly inhibited the paw edema by 81.21%, 76.97%, 73.94%, and 54.55% for methanol, chloroform, petroleum ether and water extracts respectively at day 21 when compared with the control non-treated group (P<0.05). The standard diclofenac showed significant inhibition by 68.96% at da

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using Two-way ANOVA.

*Statistically significant from the control values at (P<0.05).

@Statistically significant from diclofenac treated group at the corresponding time at (P<0.05).

The effect of *Lawsonia inermis* extracts on the writhing response of mice was shown in Table No.8. The writhing response induced by acetic acid significantly inhibited by *Lawsonia inermis* extracts by 77%, 75%, 74% and 68% for methanol, chloroform, petroleum ether and water extracts respectively. Diclofenac significantly inhibited the writhing response by 84%.

All *Lawsonia inermis* extracts showed significant effect when compared to control untreated group and standard drug diclofenac at ($p < 0.05$).

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using One-way ANOVA.

*Statistically significant from the control values at ($P<0.05$).

@Statistically significant from diclofenac treated group at the corresponding time at ($P<0.05$).

Antinociceptive effect of *Lawsonia Inermis* extracts using Randall-Selittomodel (pain in inflamed tissue) was measured using withdrawal threshold which is defined as the weight at which the rat withdraws its paw. The cut off value was 0.5kg maximum. The effect of *Lawsonia inermis* extracts on the rat withdrawal threshold was shown in Table No.9, Table No.10.

The two extract methanol and chloroform showed significant effect marked than that of diclofenac ($p < 0.05$) in all time intervals while water extract showed less effect in all time intervals. The petroleum ether extract showed marked significant effect at 30 minutes and less effect at all the other time intervals.

All *Lawsonia inermis* extracts showed significant effect when compared to control untreated group and standard drug diclofenac at ($p < 0.05$).

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using Two-way ANOVA.

*Statistically significant from the control values at ($P<0.05$).

@Statistically significant from diclofenac treated group at the corresponding time at ($P<0.05$).

Results are expressed as mean± SEM (n=6). The statistical comparison of difference between the control and the treated groups was carried out using Two-way ANOVA.

*Statistically significant from the control values at ($P<0.05$).

@Statistically significant from diclofenac treated group at the corresponding time at ($P<0.05$).

Discussion

Lawsonia inermis leaves extract has been examined to clarify its possible action as an anti-inflammatory agent by three models of inflammation: Paw edema and six-day air pouch in rats representing the acute state and the adjuvant induced arthritis representing the chronic one.

Carrageenan, a sulfated mucopolysacharide derived from Irish Sea moss is the agent of choice for testing the anti-inflammatory drugs as it is not known to be antigenic (Di Rosa, 1972)[25]. The subcutaneous injection of carrageenan into the rat hind paw produces a three distinct phases; namely an initial phase, mediated by release of histamine and serotonin (from 0 to 1h); a second phase, mediated by kinins (from 1.5 to 2.5h) and finally a third phase dependent on PGs and leukotrienes release (from 2.5 to 6 h) (Tratsk *et al*, 1997)[26]. The peak of inflammatory response with carrageenan was obtained at 3h (Arya and Kumar, 2004) and the plateau reached from 3 to 4h. Carrageenan injection induces expression of cyclooxygenase enzymes (2 to 3 h), which is associated, with an elevation of PGE_2 (Vinegar *et al*, 1969[27], Sametz *et al*, 1985[28]). The anti-inflammatory effect of the oral administration of the four *Lawsonia inermis* extracts was evident in the first hour of carrageenan-injection and lasted till the fourth hour, suggesting interference with the actions of histamine, serotonin, kinins and PGs biosynthesis. These effects are in agreement with other plant extracts showing anti-inflammatory activity and contain some of *Lawsonia inermis* constituents.

A number of flavonoids contained in *Lawsonia inermis* are reported to possess anti-inflammatory activity. Isoplumbagin and lawsaritol, isolated from the stem bark and roots of this medicinally-used species, showed anti-inflammatory activity against carrageenan-induced paw oedema in rats (Gupta *et al*, 1993b)[29]. *Lawsonia inermis* are reported to exhibite free radical scavenging activity (Hsouna *et al*, 2011)[30].

Apigenin and luteolin have been reported to exhibit anti-inflammatory activity (Ismaili *et al*, 2004)[31]. They also showed analgesic and anti-inflammatory

activities in rats with hot plate test, paw pressure and carrageenan induced paw edema (Silva *et al*, 2005[32], Nsonde-Ntandou *et al*, 2010[33]).

Viji and Helen, (2008)[34] reported that luteolin significantly inhibited 5-LOX and COX-2 activities in rat monocytes *in vivo*. Luteolin and luteolin-7-glucoside, exerted inhibitory effects on TNF-α, IL-6, IFN-γ and Il-1production (Ha *et al*, 2006)[35]. Apigenin contained in *Lawsonia inermis* enhanced the inhibition of inducible COX and iNOS synthase (Liang *et al*, 2001)[36]. In addition, scopoletin inhibited eicosanoid-release from macrophages (Silvan *et al*, 1996)[37].

Lupeol which is one of *Lawsonia inermis* constituents showed anti-inflammatory behavior similar to indomethacin (Theophile *et al*, 2006)[38].

Since the methanolic and chloroformic extracts gave more pronounced effect than diclofenac and the four extracts of *Lawsonia inermis* showed central analgesic effect in increasing the withdrawal threshold in non-inflammed paw in randall-selitto model, a hypothesis was made that the oral administration of *Lawsonia inermis* extracts may stimulate corticosteroid release from the adrenal glands or increase the corticotropine releasing factor which stimulate opioid release (Rittner *et al*, 2005)[39]. This hypothesis was tested using ADX rats.

The oral administration of the four *Lawsonia inermis* extracts caused a reduction in the intensity of the inflammatory response which was less in ADX rats than normal rats challenged for paw edema. This suggests that increased corticosteroid release may play a role in the anti-inflammatory activity of *Lawsonia inermis*. This suggestion was supported by the increase in cortisol level measured at 2 and 4 hr after carrageenan injection in paw.

Glucocorticoids are capable of exerting a shift of membrane lipid metabolism. Post-transcriptional inhibition of COX_2 synthesis by glucocorticoids assists this mechanism by suppressing the synthesis of pro-inflammatory PGs (Malcher-Lopes *et al*, 2008)[40].

Fecho *et al*, (2007)[41] reported that there is a positive relationship between hypothalamic–pituitary–adrenal (HPA) axis activity and acute inflammation and inflammatory pain. This was evident by the role of the HPA axis in the immune processes and pain behavior associated with the carrageenan model of acute hind paw inflammation.

Six-day air pouch model in rats is another way to test the effect of anti-inflammatory drugs. The injection of air subcutaneously into the dorsal surface of a rat induced the formation of a connective tissue cavity lined with cells which both structurally and functionally resembled synovial lining cells six day after air injection. The histopathological features of such change closely resemble changes occurring in rheumatoid synovitis, it can be induced with carrageenan when this irritant is injected into the 6-day air pouch. This is manifested both by the volume of exudates and its cellular content in the six-day old air pouch (Khayyal *et al*, 2005)[42].

The exudates contained inflammatory cells, mainly polymorphs and mononuclear cells as well as PGE_2, Leukotriene LTB_4 and cytokines (IL-1, IL-6, TNF-α) (Khayyal *et al*, 2005)[42].

The present study documented that treatment of the animals with *Lawsonia inermis* extracts inhibited carrageenan induced acute inflammation. This was manifested by decrease in fluid exudation and the numbers of exudates leucocytes cells compared to standard drug, diclofenac potassium.

The effect of *Lawsonia inermis* extracts are in agreement with other investigators who suggested that apigenin content of propolis extract responsible for part of the anti-inflammatory activity of the extract (El-Ghazaly, 1996)[13].

Experimental models of adjuvant-arthritis in rats share many histological and immunological features of human rheumatoid arthritis. CFA induced arthritis manifested by an acute phase that was followed by a second chronic one, showing a prominent edematous response, an effect that was observed up to day 21 (Schopf *et al*, 2006)[43]. The acute phase of edema is a non-specific inflammatory response identified at the third day while the chronic one is a specific immunological

type starting at day 10 post injection (Schopf *et al*, 2006)[43].

The clinical phase of adjuvant arthritis (10-21 day) characterized by edematous changes of the hind paws. This stage of arthritis typically includes chronic activation of HPA axis and pro-inflammatory cytokines produced from activated monocytes and macrophages (IL6 at day14 showed elevated level and TNF-α from day 10 onwards) (Stofkova *et al*, 2006)[43]. Measurement of paw edema volume was shown to be a relevant index for disease progression in the adjuvant arthritis model as well as for the efficacy of its treatment (Schopf *et al*, 2006)[43].

The present study revealed that *Lawsonia inermis* extracts administered curatively were capable of abolishing the progressive increase of the paw dimensions, observed in control group during the chronic phase (10-21 days) after adjuvant administration. These results are in agreement with similar results obtained from hydroalcoholic extract of *Lawsonia inermis* suppressed the swelling of the paw edema and decreased the paw volume in both acute and chronic phase of CFA arthritis (Kore *et al*, 2011)[44]. Luteolin, a flavonoid, contained in *Lawsonia inermis* inhibited the proliferation of synovial fibroblasts in collagen-induced arthritic rats. Treatment with luteolin also decreased the secretion of MMP-1 and -3 and the expression of IL-6. Luteolin inhibited the proliferation and partially blocked the pathogenic function of synovial fibroblasts in RA (Hou *et al*, 2009)[45]. Luteolin also inhibited nuclear factor-κB activity in rat fibroblasts (Kim *et al*, 2003)[46].

Luteolin and apigenin reduced the level pro-inflammatory cytokines (TNF-α and IL1β) and lysosomal enzymes in arthritic rats (Prakash-Babu *et al*, 2011)[47]. Apigenin attenuate the osteoclast function through inhibition of TNF-α and IL-6 (Bandyopadhyay *et al*, 2006)[48].

Lupeol decreased the activity of lysosomal enzymes, glycoproteins and collagen in adjuvant induced arthritis (Latha *et al*, 2001)[49]. Lupeol reduced MPO activity which is an indicator of leukocytes migration to inflamed areas and showed similar activity to those of indomethacin (Fernandez *et al*, 2001)[50]. Lupeol showed antiarthritic activity (Agarwal and Rangari, 2003)[51]. Arumugam *et al*, (2008)[52] reported that Luteolin and apigenin were found to inhibit acute and chronic inflammation induced in *Wistar albino* rats.

Lupeol is a triterpene found in *Lawsonia inermis* extract and its ester is lupeollinoleate. The effects of lupeol and lupeollinoleate on the development of complement in adjuvant arthritis in rats were compared with those of indomethacin. The effect of lupeollinoleate in reducing the foot-pad thickness and complement activity in arthritic rats was even greater than that of unesterifiedlupeol and indomethacin.

In the present study, writhing test was selected to investigate the peripheral anti-nociceptive activity. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. It is known as a visceral pain model of nociception (Vogel, 2008b)[53]. Several mediators such as kinins, acetylcholine, substance-P, Calcitonin-gene-related peptide (CGRP) and PGs are involved in visceral pain model of nociception and transmission of nociception from the viscera (Seidel *et al*, 2007)[54]. Moreover, the nociceptive mechanism of abdominal writhing induced by acetic acid, involves the process or release of arachidonic acid metabolites via COX and PGs biosynthesis (Reinold *et al*, 2005)[55].

The oral administration of the four *Lawsonia inermis* extracts showed significant analgesic effect. The antinociceptive activity may be due to the presence of flavonoid luteolin and apigenin as reported in the antinociceptive action of *Buddleja globosa* methanol extracts which contained luteolin-7-*O*-glucoside and exhibited analgesic activity in writhing test (Backhouse *et al*, 2008)[56]. Zeashan *et al*, (2009)[20] showed that luteolin possess significant and dose dependant central and peripheral analgesic activity. Luteolin possessed antinociceptive activity when examined using writhing test (Block *et al*, 1998)[57]. Scopoletin

exhibited analgesic activity (Park *et al*, 2004)[58]. The antinociceptive activity results of *Lawsonia inermis* extracts agree with the results obtained by Randall-Selitto assay that quantifies the withdrawal response elicited by application of a linearly increasing mechanical force to the dorszum of the rat's hind paw. This assay evaluates the ability of analgesic agent to affect response thresholds to mechanical pressure stimulation of inflamed tissues (Anseloni *et al*, 2003)[22]. The assay based on the principle that inflammation increases the sensitivity to pain and that this sensitivityzc is susceptible to modification by analgesics. Inflammation decreases the pain reaction threshold and the threshold is readily elevated by analgesics. Peripherally acting analgesics such as the non-steroidal anti-inflammatory drugs increase only the threshold of the inflamed paw, whereas centrally acting analgesic increase also the threshold of the intact paw (Vogel, 2008c)[59].

The test involves a short period of stimulation on somatic rather than visceral sites of stimulation where a pressure of measurable increasing force is applied gradually to the hind paw. The pressure measured in term of force which indicate the threshold of pain (weight in grams), when the pressure increases, the animal withdraw the paw, attempt to bite, struggle and squeal which indicate spinal reflex and supraspinal reflex (Cicala *et al*, 2000)[19].

The observed anti-nociceptive activity may be due to the extracts content as proved by Muthuraman *et al*, (2008)[60] who found that luteolin reduced the withdrawal threshold in Randall-Selitto model. Moreover luteolin exhibited better anti-nociceptive activity than celecoxib in writhing and carrageenan-induced paw pressure (Kang *et al*, 2010)[61].

Table No.1: Estimation of active constituent in *Lawsonia Inermis* extracts

S.No	Extract type	Lawsone content (mg%) at λ 453nm	Luteolin content (mg%) at λ 351nm	Apigenin content (mg%) at λ 338nm
1	Methanolic extract	1.73	1.48	1.18
2	Chloroformic extract	1.63	1.26	0.93
3	Petroleum ether extract	0.81	0.62	0.53
4	Water extract	0.47	0.3	0.27

Table No.2: Effect of *Lawsonia inermis* extracts on carrageenan-induced rat paw edema percentage inhibition

S.No	Parameters	% Inhibition (Mean±SEM)			
	Treatments	1hr	2hr	3hr	4hr
1	Diclofenac (5mg/kg)	14.00±0.09	55.14±0.08*	58.57±0.07*	68.24±0.07*
2	Petroleum ether extract (750mg/kg)	35.66±0.05	53.64±0.04*	55.57±0.05*	58.31±0.04*
3	Chloroform extract (750mg/kg)	61.00±0.03*@	62.00±0.03*	67.10±0.03*	68.98±0.02*
4	Methanol extract (750mg/kg)	62.13±0.01*@	69.16±0.03*	74.29±0.03*	73. 95±0.03*
5	Water extract (750mg/kg)	8.80±0.04	42.43±0.06*	53.14±0.10*	55.58±0.13*

Table No.3: Effect of *Lawsonia inermis* extracts on carrageenan-induced rat paw edema percentage inhibition in adrenalectomized rats

S.No	Parameters	%Inhibition (Mean± SEM)			
	Treatments	1hr	2hr	3hr	4hr
1	Diclofenac (5mg/kg)	14.28±0.02	26.32±0.03	30.00±0.04	40.00±0.07
2	Petroleum ether extract (750mg/kg)	28.57 ±0.05*@	31.58±0.07*	36.67±0.06*	44.44±0.04*
3	Chloroform extract (750mg/kg)	42.86±0.02	42.11±0.03	43.33±0.05	44.44±0.07*
4	Methanol extract (750mg/kg)	42.86±0.04	47.37±0.06	50.00±0.05*	51.85±0.09*
5	Water extract (750mg/kg)	7.14±0.03	18.42±0.04	23.33±0.06	22.22±0.05

Table No.4: Effect of *Lawsonia inermis* extract on serum cortisol level in carrageenan induced rat paw edema model

S.No	Parameters	Change in Cortisol level (ug/dl) (Mean± SEM)	
	Treatments	2hr	4hr
1	Control (25% gum acacia)	0.18±0.005	0.26±0.006
2	Diclofenac (5mg/kg)	0.1±0.051	0.19±0.016
3	Methanol extract (750mg/kg)	8.88±0.356*@	9.95±0.372*@
4	Chloroform extract (750mg/kg)	5.66±0.181*@	8.45±0.303*@
5	Petroleum ether extract (750mg/kg)	4.66±0.225*@	5.29±0.342*@
6	Water extract (750mg/kg)	2.78±0.178*@	3.63±0.236*@
7	Authentic lawsone (0.013mg/kg)	3.33±0.235*@	3.66±0.191*@

Table No.5: Effect of *Lawsonia inermis* extracts on the volume of the inflammatory exudates induced by Six day air pouch model

S.No	Parameters	Volume of exudates (Mean±SEM)	% Inhibition
	Treatments		
1	Control(25% gum acacia)	5.08±0.15	-
2	Diclofenac (5mg/kg)	2.76±0.21*	45.9*
3	Pet. Ether extract (750mg/kg)	2.93±0.28*	42.5*
4	Chloroform extract (750mg/kg)	2.63±0.62*	48.4*
5	Methanol extract (750mg/kg)	2.53±0.13*	50.4*
6	Water extract (750mg/kg)	3.41±0.48*	32.9*

Table No.6: Effect of *Lawsonia inermis* extracts on leukocytic number of inflammatory exudates induced by Six day air pouch model

S.No	Parameters / Treatments	Leukocytes number/ml (Mean± SEM)	% Inhibition
1	Control (25% gum acacia)	292 x 10^6 ± 27.3	-
2	Diclofenac (5mg/kg)	91.8 x 10^6 ± 6.7*	68.6*
3	Pet. Ether extract (750mg/kg)	129.3 x 10^6 ± 2.8*	55.7*
4	Chloroform extract (750mg/kg)	50.6 x 10^6 ± 4.3*	82.7*
5	Methanol extract (750mg/kg)	20 x 10^6 ± 4.6*	93.2*
6	Water extract (750mg/kg)	187.8 x 10^6 ± 10.1*	35.7*

Table No.7: Effect of *Lawsonia inermis* extract on percentage inhibition of rat paw edema using adjuvant-induced arthritis

S.No	Parameters	% Inhibition of paw edema (Mean±SEM)		
	Treatments	Day 14	Day 17	Day 21
1	Diclofenac (5mg/kg)	22.24%*	50.22%*	68.96%*
2	Petroleum ether extract (750mg/kg)	48.59%*@	63.50%*	73.94%*
3	Chloroform extract (750mg/kg)	49.10%*@	65.69%*	76.97%*
4	Methanol extract (750mg/kg)	60.74%*@	72.99%*@	81.21%*
5	Water extract (750mg/kg)	17.75%	42.34%*	54.55%*

Table No.8: Effect of *Lawsonia inermis* extracts on acetic acid-induced abdominal contraction of mice (writhing)

S.No	Parameters / Treatments	Acetic acid-induced abdominal contraction (n/30 min)	%Inhibition
1	Control (25% gum acacia)	135.5±0.9	-
2	Diclofenac (5mg/kg)	21.6±0.6*	84%*
3	Pet. ether extract (750mg/kg)	33.5±0.5*@	75%*@
4	Chloroform extract (750mg/kg)	34.3±0.5*@	74%*@
5	Methanol extract (750mg/kg)	30.8±0.6*@	77%*@
6	Water extract (750mg/kg)	43.3±0.5*@	68%*@

Table No.9: Antinociceptive effect of *Lawsonia Inermis* extracts using Randall-Selitto model (pain in inflamed tissue)

S.No	Mean force (g)												
	Parameters	Zero time		15 min		30 min		60 min		90 min		120 min	
	Treatments	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	Control (25% gum acacia)	21± 0.96	22±0	20± 1.06	22±0	18± 0.93	20±0	16± 0.63	19± 0.1	15± 0.63	17±0	13± 0.77	17±0
2	Diclofenac (5mg/gk)	24± 0.48	23± 0.12	81± 0.96*	61± 0.26*	102± 2.26*	84± 0.37*	129± 1.52*	112± 0.34*	142± 1.56*	132± 0.56*	170± 5.77*	180± 0.74*
3	Pet. Ether extract (750mg/kg)	24± 0.47	20± 0.05	77± 1.41*@	50± 0.51*@	106± 1.93*@	62± 0.56*@	124± 4.73*	70± 0.64*@	140± 1.77*	75± 0.59*@	166± 4.86*	84± 0.79*@
4	Chloroform extract (750mg/kg)	20± 1.06	24± 0.22	82± 2.26*@	65± 0.86*@	116± 2.01*@	75± 1.08*@	136± 3.93*@	82± 1.53*@	150± 3.01*@	90± 1.81*@	187± 1.96*@	108± 2.2*@
5	Methanol extract (750mg/kg)	22± 0.57	28± 0.25	90± 1.26*@	142± 1.79*@	162± 1.26*@	196± 3.06*@	192± 0.52*@	216± 2.85*@	214± 2.08*@	242± 3.27*@	238± 1.63*@	292± 2.31*@
6	Water extract (750mg/kg)	21± 0.44	20±0	44± 1.29*@	40± 0.18*@	62± 1.15*@	47± 0.11*@	76± 0.93*@	48± 0.08*@	88± 0.57*@	63± 0.11*@	106± 0.58*@	77± 0.17*@

Table No.10: Effect of *Lawsonia Inermis* extracts on percentage inhibition of nociception using Randall-Selitto model (pain in inflamed tissue)

S.No	Parameters	Percentage inhibition of nociception				
	Treatments	15 min	30 min	60 min	90 min	120 min
1	Diclofenac (5mg/kg)	25.3%*	34.5%*	46%*	52.2%*	64.6%*
2	Pet. Ether extract (750mg/kg)	23%*@	36.3%*@	44%*	51%*	63%*
3	Chloroform extract (750mg/kg)	26.1%*@	41.7%@	50.4%*@	56.5%*@	72.6%*@
4	Methanol extract (750mg/kg)	29.8%*@	61.4%*@	74.6%*@	84.2%*@	94.7%*@
5	Water extract (750mg/kg)	10%*@	18%*@	24%*@	29.3%*@	37%*@

CONCLUSION

In conclusion, Lawsonia inermis extracts significantly reduced the carrageenan-induced paw edema, inhibited the formation of inflammatory exudates in six-day air pouch model, inhibited the formation of inflammatory exudates in six-day air pouch model, decreased the infiltration of leukocytes in the carrageenan-induced air pouch, and possessed antiarthritic activity in adjuvant-induced arthritis model. Furthermore, Lawsonia inermis extracts induced cortisol secretion. In addition, Lawsonia inermis extracts possess peripheral analgesic activity which was evident in the inhibition of writhing movement and exhibited central analgesic activity which was proved by increase in paw withdrawal threshold.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

BIBLIOGRAPHY

1. Klaus D. Elgert. Immunology: Understanding the immune system, *Wiley-Blackwell,* 2nd Edition, 2009, 1-25.
2. Theoharides T C, Alysandratos K D, Angelidou A, Delivanis D A, Sismanopoulos N, Zhang B and Kalogeromitros D. Mast cells and inflammation, *Biochimicaet Biophysica Acta (BBA)-Molecular Basis of Disease,* 1822(1), 2012, 21-33.
3. Woolf C J, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain, *Proc Natl Acad Sci USA,* 96(14), 1999, 7723–7730.
4. Wang B, Wu L, Chen J, Dong L, Chen C, Wen Z and Wang D W. Metabolism pathways of arachidonic acids: Mechanisms and potential therapeutic targets, *Signal Transduction and Targeted Therapy,* 6(1), 2021, 1-30.
5. Wallace V. Cytokines, Regulation in inflammation. In: Schmidt R.F. and Willis W.D. Encyclopedia of pain, *Berlin; Springer*, *New York,* 2007, 523-526.
6. Resch K. Cytokines In: Nijkamp F P, Parnham M J. (Eds). Principle of Immunopharmacology, *Birkhauser Verlag, Basel; Boston,* 2nd Edition, 2005, 45-61.
7. Hubbard A K, Giradina C. Regulation of ICAM-1 expression in mouse macrophages, *Inflammation,* 24(2), 2000, 115-125.
8. Ren K, Torres R. Role of interleukin-1β during pain and inflammation, *Brain Research Reviews,* 60(1), 2009, 57-64.
9. Sharma J N, Al-Omran A, Parvathy S S. Role of nitric oxide in inflammatory diseases, *Inflammopharmacology,* 15(6), 2007, 252-259.

10. Randall E Harris, Weinberg B, Fermor B, Guilak F. Nitric oxide synthase and cyclooxygenase interactions in cartilage and meniscus, Inflammation in the pathogenesis of chronic diseases: The COX-2 controversy, *Springer, New York,* 1st Edition, 42, 2007, 323.
11. Teblick, A, Langouche L and Van den Berghe G. Anterior pituitary function in critical illness, *Endocrine Connections,* 8(8), 2019, R131-R143.
12. Mikhaeil B R, Badria F A, Maatooq G T, Amer M M A. Antioxidant and immunomodulatory constituents of henna leaves, *Zeitschrift Fur Naturforschung C, Journal of Biosciences,* 59(7-8), 2004, 468-476.
13. El-Ghazaly M A. The effect of aqueous propolis extract on cellular migration, prostaglandins and histamine release in irradiated rats, *J. Egypt. Soc. Toxi,* 16, 1996, 69-73.
14. Winter C A, Risley E A, Nuss G W. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs Proc, *Soc. Exp. Biol. Med,* 111(3), 1962, 544-547.
15. Catelan S C, Belentani R M, Marques L C, Silva E R, Silva M A, Caparroz-Assef S M, Cuman R K N, Bersani-Amado C A. The role of adrenal corticosteroids in the anti-inflammatory effects of the whole extract of Harpagophytum procumbens in rats, *Phytomedicine,* 13(6), 2006, 446-451.
16. Li A, Zhang R X, Wang Y, Zhang H, Ren K, Berman B M, Tan M, Lao L. Corticosterone mediates electroacupuncture-produced anti-edema in a rat model of inflammation, *BMC Complementary and Alternative Medicine,* 7, 2007, 27-33.
17. Sedgwick A D, Lees P. A comparison of air pouch, sponge and pleurisy models of acute carrageenan inflammation in the rat, *Agents and Actions,* 18(3-4), 1986b, 439-446.
18. Cheesbrough M. District Laboratory Practice in Tropical Countries, *Cambridge University Press, Cambridge, New York,* 2nd Edition, 2006, 313-319.
19. Cicala C, Ianaro A, Fiorucci S, Calignano A, Bucci M, Gerli R, Santucci L, Wallace J L, Cirino G. NO-naproxen modulates inflammation, nociception and down regulates T cell response in rat Freund's adjuvant arthritis, *British journal of Pharmacology,* 130(6), 2000, 1399-1405.
20. Hussain Zeashan, G Amresh, Chandana Venkateswara Rao, Satyawan Singh. Antinociceptive activity of Amaranthus spinosus in experimental animals, *J Ethnopharmacol,* 122(3), 2009, 492-496.
21. Randall L O, Selitto J J. A method for measurement of analgesic activity on inflamed tissue, *Arch. Int. Pharma-codyn,* 111(4), 1957, 409-419.
22. Anseloni V C Z, Ennis M, Lidow M S. Optimization of the mechanical nociceptive threshold testing with the Randall-Selitto assay, *Journal of Neuroscience Methods,* 131(1-2), 2003, 93-97.
23. Bujalska M. Effect of cyclooxygenase and NO synthase inhibitors administered centrally on antinociceptive action of acetaminophen (Part II), *Polish Journal of Pharmacology,* 55(6), 2003, 1001-1011.
24. Kayser V. Randell-Selitto paw pressure test. In: Schmidt R.F. and Willis W D. Encyclopedia of pain, *Springer-Verlage Berlin Heide,* 1st Edition, 2007, 2091-2093.
25. Di Rosa M. Biological properties of carrageenan, *J Pharm Pharmacol,* 24(2), 1972, 89-102.
26. Tratsk K S, Campos M M, Vaz Z R. Filho V C, Schlemper V, Yunes R A. Calixto J B. Anti-allergic effects and oedema inhibition caused by the extract of Drymiswinteri, *Inflamm. Res,* 46(12), 1997, 509-514.
27. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats, *J Pharmacol Exp Ther,* 166(1), 1969, 96-103.

28. Sametz W, Juan H. Influence of a diet rich in eicosapentaenoic acid on the development of rat paw oedema and on the formation of prostaglandins I_2 and E_2, *Agents and Actions,* 17(2), 1985, 214-219.
29. Gupta S, Ali M, Pillai K.K, Alam M S. Evaluation of anti-inflammatory activity of some constituents of *Lawsonia Inermis*, *Fitoterapia,* 64(4), 1993b, 365-366.
30. Hsouna A B, Trigui M, Culioli G, Blache Y, Jaoua S. Antioxidant constituents from *Lawsonia inermis* leaves: Isolation, structure elucidation and antioxidative capacity, *Food Chemistry,* 125(1), 2011, 193-200.
31. Ismaili H, Milella L, Fkih-Tetouani S, Ilidrissi A, Camporese A, Sosa S, Altinier G, Loggia R D, Aquino R. *In vivo* topical anti-inflammatory and *in vitro* antioxidant activities of two extracts of Thymus satureioides leaves, *Journal of Ethnopharmacology,* 91(1), 2004, 31-36.
32. Silva H G. De O, Pires A J V, Silva F F da, Veloso C M, Carvalho G G P de, Cezario A S, Santos C C. Effects of feeding cocoa meal (Theobroma cacao L.) and palm kernel cake (Elaeis guineensis, Jacq) on milk intake and yield for lactating goats, *Rev. Bras. Zootec,* 34(5), 2005, 1786-1794.
33. Nsonde Ntandou G F, Banzouzi J T, Mbatchi B, Elion-Itou R D G, Etou-Ossibi A W, Ramos S, Benoit-Vical F, Abena A A, Ouamba J M. Analgesic and anti-inflammatory effects of Cassia siamea Lam, Stem bark extracts, *Journal of Ethnopharmacology,* 127(1), 2010, 108-111.
34. Viji V., Helen A. Inhibition of lipoxygenases and cyclooxygenase-2 enzymes by extracts isolated from Bacopamonniera (L.) wettst, *Journal of Ethnoph,* 118(2), 2008, 305-311.
35. Ha C L, Weng C Y, Wang L, Lian T W, Wu M J. Immunomodulatory effect of Glossogynetenuifolia in murine peritoneal macrophages and splenocytes, *Journal of Ethnopharmacology,* 107(1), 2006, 116-125.
36. Liang Y C, Tsai S H, Tsai D-C, Lin-Shiau S Y, Lin J K. Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activated receptor-γ by flavonoids in mouse macrophages, *FEBS Let,* 496(1), 2001, 12-18.
37. Silvan A M, Abad M J, Bermejo P, Sollhuber M, Villar A. Anti-inflammatory activity of coumarins from Santolinaoblongifolia, *J. Nat Prod,* 59(12), 1996, 1183-1185.
38. Theophile D, Laure N E, Benoît N T, Anatole A G B, Emmanuel A A, Paul T V, Pierre K. Antinociceptive and anti-inflammatory effects of the ethyl acetate stem bark extract of Brideliascleroneura (Euphorbiaceae), *Inflammopharmacology,* 14(1-2), 2006, 42-47.
39. Rittner H L, Machelska H, Stein C. Leukocytes in the regulation of pain and analgesia, *Journal of Leukocyte Biology,* 78(6), 2005, 1215-1222.
40. Malcher-Lopes R, Franco A, Tasker J G. Glucocorticoids shift arachidonic acid metabolism toward endocannabinoid synthesis: A non- genomic anti-inflammatory switch, *Eu. J. Phar,* 583(2-3), 2008, 322-339.
41. Fecho K, Manning E L, Maixner W, Schmitt C P. Effects of carrageenan and morphine on acute inflammation and pain in Lewis and Fischer rats, Brain, *Behavior and Immunity,* 21(1), 2007, 68-78.
42. Khayyal M T, El-Ghazaly M A, Abdallah D M, Okpanyi S N, Kelber O, Weiser D. Mechanisms involved in the anti-inflammatory effect of a standardized Willow bark extract, Arzneim, Forsch, *Drug res,* 55(11), 2005, 677-687.
43. Schopf L R, Anderson K, Jaffee B D. Rat models of arthritis: Similarities, differences, advantages and disadvantages in identification of novel therapeutics, In: Stevenson, C S, Marshall L A, Morgan D W. (Eds.), *In vivo* models of inflammation, *Birkhauser, Basel; Boston,* 2nd Edition, 1, 2006, 1-34.

44. Kore K J, Shete R V, Desai N V. Anti-arthritic activity of hydroalcoholic extract of *Lawsonia Inermis*, *International Journal of Drug Development and Research,* 3(4), 2011, 217-224.
45. Hou H Y, Heffer A, Anderson W R, Liu J, Bowler T, Pick L. Stripy Ftz target genes are coordinately regulated by Ftz-F1, *Dev. Biol,* 335(2), 2009, 442--453.
46. Kim S H, Shin K J, Kim D, Kim Y H, Han M S, Lee T G, Kim E, Ryu S H, Suh P G. Luteolin inhibits the nuclear factor-kB transcriptional activity in Rat-1 fibroblasts, *Biochemical Pharmacology,* 66(6), 2003, 955-963.
47. Prakash Babu N, Pandikumar P, Ignacimuthu S. Lysosomal membrane stabilization and ant-inflammatory activity of *Clerodendrum phlomidis* L. F, A traditional medicinal plant, *Journal of Ethnopharmacology,* 135(3), 2011, 779-785.
48. Bandyopadhyay S, Lion J M, Mentaverri R, Ricupero D A, Kamel S, Romero J R, Chattopadhyay N. Attenuation of osteoclastogenesis and osteoclast function by apigenin, *Biochemical Pharmacology,* 72(2), 2006, 184-197.
49. Latha R M, Lenin M, Rasool M, Varalakshmi P. A novel derivative pentacyclictriterpene and ω3 fatty acid (lupeol-EPA) in relation to lysosomal enzymes glycoproteins and collagen in adjuvant induced arthritis in rats, Prostaglandins, *Leukotrienes and Essential Fatty Acids,* 64(2), 2001, 81-85.
50. Fernandez A, Alvarez A, Garcia M D, Saenz M T. Anti-inflammatory effect of Pimentaracemosa var, ozua and isolation of the triterpenelupeol, *It Farmaco,* 56(4), 2001, 335-338.
51. Agarwal R B, Rangari V D. Anti-inflammatory and antiarthritic activities of lupeol and 19α-H lupeol isolated from strobilanthus callosus strobilanthus ixiocephala roots, *Indian Journal of Pharmacology,* 35(6), 2003, 384-387.
52. Arumugam P, Priya N G, Subathra M, Ramesh A. Anti-inflammatory activity of four solvent fractions of ethanol extract of Menthaspicata L. investigated on acute and chronic inflammation induced rats, *Environmental Toxicology and Pharmacology,* 26(1), 2008, 92-95.
53. Vogel H G. Adjuvant arthritis in rats, in: Vogel H G. Drug discovery and evaluation, *Springer Berlin Heidelberg,* 2008, 1162-1165.
54. Seidel M F, Tsalik J, Vetter H, Muller W. Substance P in rheumatic diseases, *Current Rheumatology Reviews,* 3(1), 2007, 17-30.
55. Reinold H, Ahmadi S, Depner U B, Layh B, Heindl C, Hamza M, Pahi A, Brune K, Narumiya S, Muller U, Zeilhofer H U. Spinal inflammatory hyperalgesia is mediated by prostaglandin E receptors of the EP2 subtype, *Journal of Clinical Investigation,* 115(3), 2005, 673-679.
56. Backhouse N, Rosales L, Apablaza C, Goïty L, Erazo S, Negrete R, Theodoluz C, Rodriguez J, Delporte C. Analgesic, anti-inflammatory and antioxidant properties of Buddlejaglobosa, Buddlejaceae, *Journal of Ethnopharmacology,* 116(2), 2008, 263-269.
57. Block L C, Santos A R S, De Souza M M, Scheidt C Yunes R A, Santos M A, Monache F D, Filho V C. Chemical and pharmacological examination of antinociceptive constituents of Wedeliapaludosa, *Journal of Ethnopharmacology,* 61(1), 1998, 85-89.
58. Park J M, Brady H, Ruocco M G, Sun H, Williams D, Lee S J, Kato T, Richards N, Chan K, Mercurio F, Karin M, Wasserman S A. Targeting of TAK1 by the NF-kappa B protein Relish regulates the JNK-mediated immune response in Drosophila, *Genes Dev,* 18(5), 2004, 584-594.
59. Vogel H G. Pain in inflamed tissue (Randell-Selitto-Test), In: Vogel, H.G. Drug discovery and evaluation, *Springer Berlin Heidelberg,* 2008, 1032-1035.

60. Muthuraman A, Diwan V, Jaggi A S, Singh N, Singh D. Ameliorative effects of Ocimum sanctum in sciatic nerve transection-induced neuropathy in rats, *Journal of Ethnopharmacology,* 120(1), 2008, 56-62.
61. Kang M, Jung I, Hur J, Kim S H, Lee J H, Kang J Y, Jung K C, Kim K S, Yoo M C, Park D S, Lee J D, Cho Y B. The analgesic and anti-inflammatory effect of WIN-34B, a new herbal formula for osteoarthritis composed of Lonicera japonica Thunb and Anemarrhena asphodeloides BUNGE *in vivo*, *Journal of Ethnopharmacology,* 131(2), 2010, 485-496.
62. Elgert K D. Immunology: Understanding the immune system, *Wiley-Liss, Network,* 1996, 6-7.
63. Park J H, Son K H, Kim S W, Chang H W, Bae K, Kang S S, Kim H P. Anti-inflammatory activity of Synurusdeltoides, *Phytother Res,* 18(11), 2014, 930-933.
64. Stofkova A, Skurlova M, Tybitanclova K, Veselsky L, Zelezna B, Jurcovicova J. Relationship among nitric oxide, leptin, ACTH, corticosterone and IL-1β, in the early and late phases of adjuvant arthritis in male long Evans rats, *Life Sci,* 79(26), 2006, 2486-2491.
65. Zeashan H, Amresh G, Rao C V, Singh S. Anti-nociceptive activity of Amaranthusspinosus in experimental animals, *Jour of Ethnophar,* 122(3), 2003, 155-158.

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