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# *IN-VITRO* SELECTIVE INHIBITION OF COX-2 ACTIVITY BY (3β)-LUP-20(29)-EN-3-OL A PENTACYCLIC TRITERPENE ISOLATED FROM *C. ADANSONII* STEM BARK EXTRACT

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**Keywords:**

(3β)-lup-20(29)-en-3-ol, Cyclooxygenase-1 and -2, *Crateva adansonii*, Anti-inflammatory activity

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**ABSTRACT:** This study is aimed at investigating/elucidating the molecular structure and the biochemical mechanism of anti-inflammatory action of an isolated but yet unknown bioactive compound CA1 from *Crateva adansonii* stem bark extract. Qualitative phytochemical tests on the isolated bioactive anti-inflammatory compound gave positive reaction to terpenoid only, while the structural elucidation indicates that CA1 is (3β)-lup-20(29)-en-3-ol, a pentacyclic triterpene with molecular weight of 426.70 g, and molecular formular of $C_{30}H_{50}O$. The molecular structure of this pentacyclic triterpene was established using nuclear magnetic resonance (NMR), ultra-violet/visible spectrophotometer, infra-red, gas and mass spectrophotometers. The (3β)-lup-20(29)-en-3-ol was subjected to *in-vivo* anti-inflammatory activity study using egg-albumin induced rat hind paw edema method. However, (3β)-lup-20(29)-en-3-olwas investigated for its selective COX-1 and COX-2 inhibitory activities. An *in-vitro* model of inflammation using a colourimetric COX (ovine) inhibitor screening assay method was used as a model to investigate the effect of CA1 on $PGE_2$ production. (3β)-lup-20(29)-en-3-ol at the tested concentrations significantly ($P < 0.05$) and selectively inhibited the inducible form of cyclooxygenase, cyclooxygenase-2 enzyme, but exhibited no inhibitory activity against COX-1.

**INTRODUCTION:** Inflammation, a complex physiological/biochemical process, is the body response to cellular and tissue release of pro-inflammatory mediators caused by infectious agents or physical and chemical stimuli. It is characterized by vasodilation (increase in blood flow due to reduction in intracellular calcium concentration, inhibition of myosin light chain kinase *via* NO-dependent process, or dephospho-rylation of myosin by phosphatase), increased vascular permeability and cellular recruitment to the inflammatory site [11].

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These biochemical and cellular alterations are mediated and regulated by inflammatory mediators such as prostanoids (metabolic products of cyclooxygenase enzymes) [2]. Both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are therapeutic agents used to checkmate the biosynthesis of these prostanoids, while nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs in the management of pain, fever, redness and edema the characteristic features of an inflammatory disorder.

Researches have shown that both therapeutic and adverse effects of NSAIDs are directly linked to cyclooxygenase (COX) inhibition. The three major isoforms of cyclooxygenase are classified into constitutive COX-1, inducible COX-2 and COX-3 [5-37]. COX-1 catalyzes formation of cytoprotective

prostaglandins in the, kidney, lung, stomach, ileum, colon, cecum [17], thrombocytes, vascular endothelium, pancrease, langerhans islets, seminal vesicles and brain [6]. Cyclooxygenase-2 plays pivotal role in the progression of tissue inflammation through the synthesis of series-2 prostanoids from arachidonic acid catabolism. The metabolic intermediate Prostaglandin $H_2$ ($PGH_2$) is eventually converted into prostaglandin $E_2$ ($PGE_2$) by catalytic action of $PGE_2$-synthase enzyme [35]. Induction of COX-2 by various growth factors, pro-inflammatory agents, endotoxins, mitogens, and tumor agents indicates that this COX-isoform has a critical role in initiation of pathological processes such as inflammation. It is a well-established fact that COX inhibitors are associated with a number of side effects including gastrointestinal erosions, renal and hepatic insufficiencies. These critical adverse reactions are mostly dependent on COX-1 inhibition [15].

Induction of COX-2 is associated with hyperplastic response, increased myeloperoxidase activity and leukocyte infiltration into the skin [41]. However, this induction of pro-inflammatory enzyme is an adaptive tool in maintaining homeostasis. Both normal physiological roles and adverse effects of pro-inflammatory prostanoids are directly proportional to their concentrations at site of action, which in turn is directly dependent on the rate of biosynthesis and elimination. However, to maintain physiological homeostasis and for proper functioning of the cells/tissues of the body, the levels of pro-inflammatory prostanoids (thromboxane, prostacyclin, prostaglandin $E_2$) must be maintained at their normal physiological concentrations.

In order to achieve this and avoid over production of these pro-inflammatory mediators, COX-2 evolutionarily became an inducible enzyme. To further potentiate the reduction in the toxicological significance of bioavailability of COX-2 metabolic activities, expression of COX-2 is directly dependent on and proportional to the stimulatory strength of the stress stimuli/tissue assaults. However, the consequences of high/over production of pro-inflammatory prostanoids during tissue exposure to the inflammatory stimuli/assault/infection is an increase in the reactive metabolite production. Moreover, inducible cyclooxygenase-2, a major source of pro-inflammatory prostanoids ($PGE_2$ and $PGI_2$) and pain hypersensitivity is significantly increased in inflamed tissues [31]. The activation of $EP_2$ and IP receptors by these prostanoids markedly enhanced edema formation, increase vascular permeability, leukocyte infiltration by promoting blood flow and pain through indirect nociceptor excitation via transient receptor potential ankyrin-1 (TRPA1) activation [22, 26].

CA1 is a pentacyclic triterpene ((3β)-lup-20(29)-en-3-ol) isolated from the dichloromethane: methanol stem bark extract of *C. adansonii* [7]. Rathinavel, *et al*., 2021 [30] reported the effect of lupeol fraction from *Crateva adansonii* on TNF-α, an important inflammatory cytokine marker that activates the expression of other cytokines, chemokines, adhesion molecules, and neutrophil causes significant reduction of TNF-α, IL-1, and IL-6 cytokine levels which could inhibit the infiltration and activation of macrophage at inflammatory sites. It has been reported that (3β)-lup-20(29)-en-3-ol at its effective therapeutic doses exhibited no toxicity to normal cells and tissues (Saleem, 2009) [33], but displayed low cytotoxicity on healthy cells and acted synergistically when used in combined therapies [39].

Oral administration of (3β)-lup-20(29)-en-3-ol for consecutive 18 days did not produce any mortality or systemic toxicity in rats [12]. Preetha *et al*., 2006, [29] showed that oral administration of (3β)-lup-20(29)-en-3-olat a higher dose for 7 days did not cause mortality or any systemic toxicity in mice. Various pharmacological activities exhibited by (3β)-lup-20(29)-en-3-olunder *in-vitro* and *in-vivo* conditions include its beneficial activities against inflammation, cancer, arthritis, diabetes, heart diseases, renal and hepatic toxicities. Some available experimental evidence showed that extracts of *C. adansonii* leaves caused moderate anti-trypanosomal activity (Igoli *et al*., 2012) [16] and marked inhibition of xanthine oxidase activity [1]. A comprehensive study conducted by Fernández *et al.,* [8] showed that topical application of (3β)-lup-20(29)-en-3-ol in the ear alleviated 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in an ear mouse model [8]. Their study further indicated that topical application of (3β)-lup-20(29)-en-3-ol decreased myeloperoxidase

levels [neutrophil specific marker] thus causing reduction in cell infiltration into inflamed tissues in mice [8]. The anti-inflammatory potential of (3β)-lup-20(29)-en-3-ol could be assessed from the observation that its' pretreatment significantly reduced prostaglandin $E_2$ ($PGE_2$) production in A23187-stimulated macrophages [8]. Another study by Fernández *et al.,* [8] has shown that (3β)-lup-20(29)-en-3-ol-rich extract of *Pimenta racemosa* plant (which is widely used by country doctors in Caribbean region to treat inflammatory ailments) exhibits significantly high anti-inflammatory activity in animal models [8].

Their finding indicates that the anti-inflammatory behavior of the (3β)-lup-20(29)-en-3-ol-rich extract was similar to that exhibited by the selective cyclooxygenase inhibitor (Indomethacin) [8, 25] isolated and characterized lupeol (a pentacyclic triterpenoid) and stigmasterol from ethyl acetate fraction of methanol root extract of *Combretum hypopilinum*, a plant known for its wide medicinal applications in traditional setting for pain, infertility and tuberculosis treatment. Lupeol-based cream enhances foot ulcers healing process in complicated diabetes via anti-inflammatory effect of NF-*κ*B signaling pathways, as suggested by a reduction of inflammation-associated mediators, such as IL-6 (proinflammatory cytokine), elevated IL-10 levels (anti-inflammatory cytokine), faster neovasculogenesis and proliferation of fibroblasts [3].

Beserra *et al.,* 2019 [3] further reported that lupeol minimized the oxidative stress and improved the antioxidant status through increased mRNA expression of heme oxygenase-1and superoxide dismutase. The increased induction of COX-2 in inflamed cells and tissues provided the rationale for development of selective COX-2 inhibitors with the realization that prolong use of currently available synthetic non-steroidal anti-inflammatory drugs are fraught with several drawbacks or limitations linked to increased incidence of cancer, gastric disorder, and renal related diseases [13, 23]. Although specific inhibitors of cyclooxygenase-2 are associated with cardiovascular toxicity, synthetic pharmaceutical agents continue to dominate the market with COX-2 inhibitors due to remarkable reduction of adverse gastrointestinal and renal effects associated with conventional NSAIDs as well as to their potential therapeutic benefits in several diseases, including certain types of cancer [31]. Moreover, increasing attention has been directed to natural products with the hope of discovering anti-inflammatory compounds devoid of these undesirable side effects. Within Enugu metropolis (Eastern Nigeria), the stem bark extract of *C. adansonii* is used in the treatment of arthritis, a disorder of chronic inflammation as well as soft tissue swellings and pains. *C. adansonii*, a well-known Nigerian medicinal plant, has been used by traditional medicine practitioners in the management of chronic diseases such as rheumatism, arthritis, and gout [22]

## MATERIALS AND METHODS:

**Plant Material:** The isolated anti-inflammatory compound CA1, a pentacyclic triterpene (3β)-lup-20(29)-en-3-ol) from our previous research work was used for this analysis.

**Physical Properties of CA1:** (3β)-lup-20(29)-en-3-ol). A known quantity (5 mg) of CA1, a white crystalline solid was weighed out in three batches of triplicates each. Batch A, B and C were dissolved in 1ml of water, acetone and chloroform at room temperature 25 ºC respectively.

**Studies on Acute Inflammation:**

**Systemic Acute Edema of the Rat Paw:** The effect of (3β)-lup-20(29)-en-3-olon systemic acute edema was studied using the rat paw edema method of Winter *et al*. (1962) [40]. Acute inflammation was measured in terms of change in volume of the rat hind paw induced by subplantar injection of fresh egg albumin. The animals (n = 4) received 100 mg/kg of (3β)-lup-20(29)-en-3-ol (Lupeol), while the control groups received either equivalent volume of the vehicle (3% Tween-80) or Ibuprofen (100 mg/kg). Treatments were administered intraperitoneal. Edema formation was assessed in terms of the difference in the zero time paw volume of the injected paw and its volume at the different times after egg-albumin injection, using mercury displacement method. Percentage inhibition of edema was calculated using the relation (Perez, 1996):

$$\text{Inhibition of Edema (\%)} = 100[1- (a-x) / (b-y)]$$

Where, a = mean paw volume of treated rats after egg albumin injection, x = mean paw volume of treated rats before egg albumin injection, b = mean

paw volume of control rats after egg albumin injection, y = mean paw volume of control rats before egg albumin injection,

Inflammation = Average paw volume at time t - Average paw volume at zero time

% Inflammation = (Average inflammation of treated group at time T) / (Average inflammation of control at same time) × 100

**Cyclooxygenase (COX) Inhibitor Assay:** The inhibitory activities of isolated compound (3β)-lup-20(29)-en-3-ol(Lupeol) against COX-1 and COX-2 were determined using a colourimetric COX (ovine) Inhibitor Screening Assay (Cayman Chemical Company) according to the manufacturer's protocol [18].

The inhibitory activities of the compounds were measured by monitoring the production of oxidized N, N, N', N'- tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. Three background wells were prepared by adding 160 µl of assay buffer and 10 µl of heme to each well, while 2 sets of three 100% initial activity and 15 inhibitor wells were prepared by adding 150 µl of assay buffer, 10 µl of heme and 10 µl of enzyme (COX-1 and COX-2) to each well. Ten microliter of different concentrations of (3β)-lup-20(29)-en-3-ol (1, 2, 4, 10 and 14 mg/ml) were added to three inhibitor wells each. Ten microliter of solvent (the same solvent used for dissolving (3β)-lup-20(29)-en-3-ol) was added to all the wells. They were properly mixed and incubated for 5 min at 25°C. Then 20 µl of colorimetric substrate (TMPD) and arachidonic acid solutions were added to all the wells. The wells were properly mixed and incubated for 2 min at 25°C. Exactly 2 min after the incubation the absorbance were read at 590 nm.

The COX-inhibiting activity was calculated according to the equation.

$$\text{COX inhibiting activity (\%)} = [(A_2 - A_0) - (A_1 - A_0) / (A_2 - A_0)] \times 100$$

Where, $A_0$ is the absorbance of the background wells, $A_1$ is the absorbance in the presence of the test compound, $A_2$ is the absorbance of 100% initial activity wells.

**Membrane Stabilization Activity:** The membrane stabilization activity of the fraction and (3β)-lup-20(29)-en-3-ol (Lupeol) was studied *in-vitro* using hypotonicity-induced heamolysis of human red blood cells.

**Preparation of Erythrocyte Suspension:** Fresh whole ox-blood (10 ml) was collected, transferred to heparinized centrifuge tubes, centrifuged at 3000 rpm for 5 min, and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). The composition of the buffer solution (g/L) was $NaH_2PO_4$ (0.2), $Na_2HPO_4$ (1.15) and NaCl (9.0) [36].

**Hypotonicity-Induced Haemolysis:** The hypotonic solutions (distilled water) (5 ml) containing 250, 500 and 1000 µg/ml concentrations of the isolated compound (3β)-lup-20(29)-en-3-olwere put in 2 pairs (per concentration) of centrifuge tubes. Control tubes contained 5 ml of the vehicle (distilled water) or Diclofenac (250, 500 and 1000 µg/ml). While the isotonic buffer solutions (5 ml) containing 250, 500 and 1000 µg/ml concentration of isolated compound(3β)-lup-20(29)-en-3-olwere put in 2 pairs (per concentration) of centrifuge tubes and isotonic control tubes contained 5 ml of buffer solution or Diclofenac (250, 500 and 1000 µg/ml). Erythrocyte suspension (0.05 ml) was added to each tube and after gentle mixing, the mixtures were incubated for 1 h at room temperature (31°C). After incubation, the reaction mixture was centrifuged for 3 min at 1300 rpm and the absorbance (OD) of the supernatant measured at 540 nm using Spectrophotometer. The inhibition (%) of haemolysis was calculated using the relation [36].

$$\text{Inhibition of haemolysis (\%)} = 100\,[1-\{(OD_2 - OD_1) / (OD_3 - OD_1)\}]$$

Where, $OD_1$ = absorbance of test sample in isotonic solution, Where $OD_2$ = absorbance of test sample in hypotonic solution, Where $OD_3$ = absorbance of control sample in hypotonic solution.

**Characterization of Pentacyclic Triterpene (Elucidation of Structure of CA1):** The UV spectra were obtained with a shimadzu 3101 PC instrument and IR spectra determined with a jasco FT-IR 410 apparatus. $^1H$ (400.6MHz) and $^{13}C$ (100.13 MHz), NMR spectra were recorded in $CDC_{13}$ (with its signals at δ 7.25 and 77.0 ppm as

reference), TLC was carried out on silica gel 60 $GF_{254}$ pre-coated plates with detection by UV light or by spraying with 50% $H_2SO_4$ followed by heating at 100 °C. The structure of CA1 was identified by comparing the spectra obtained with those of published library of compounds.

**Statistical Analysis:** The data were recorded as means ± standard deviation and analyzed by SPSS. One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. $p < 0.05$ was regarded as significant and $p > 0.05$ was non-significant.

## RESULTS:

**Physical Properties of (3β)-lup-20(29)-en-3-ol (Pentacyclic triterpene) Lupeol:** Lupeol, a white crystalline solid, is highly insoluble in water, but soluble in acetone and chloroform.

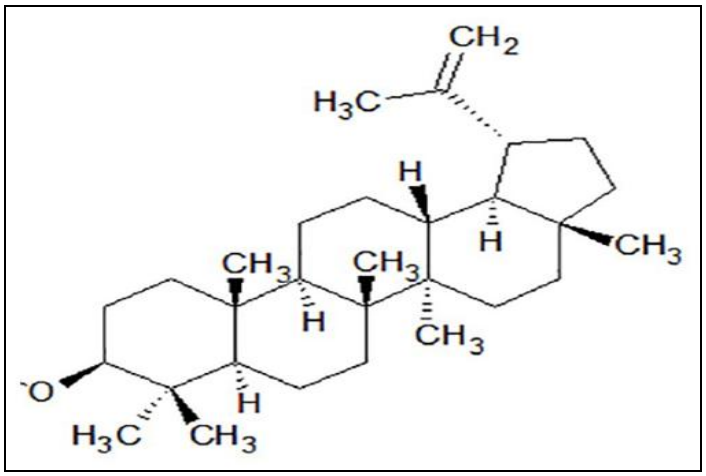


**FIG. 1: THE MOLECULAR STRUCTURE OF THE PURE ISOLATE (3B)-LUP-20(29)-EN-3-OL (LUPEOL)**

**Effect of Isolated Pentacyclic Triterpene (3β)-lup-20(29)-en-3-ol) on Systemic Acute Edema:** Intraperitoneal administration of (3β)-lup-20(29)-en-3-ol caused significant ($p<0.05$) inhibition of systemic acute edema within 0.5 – 3.5 h **Table 1.**

**TABLE 1: EFFECT OF LUPEOL ((3B)-LUP-20(29)-EN-3-OL) ON SYSTEMIC ACUTE EDEMA**

| Treatment | Dose (mg/kg) | Edema (ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 h | 1 h | 1.5 h | 2 h | 2.5 h | 3 h | 3.5 h | 4 h |
| Lupeol | 100 | 0.31±0.05$^{\beta}$ (60%) | 0.38±0.17 $^{\beta}$ (53%) | 0.30±0.19 $^{\beta}$ (61%) | 0.29±0.11$^{\beta}$ (59%) | 0.26±0.06$^{\beta}$ (62%) | 0.22±0.13$^{\beta}$ (64%) | 0.18±0.19$^{\beta}$ (66%) | 0.18±0.15 (55%) |
| Ibuprofen | 100 | 0.55±0.08 (30%) | 0.57±0.04 (29%) | 0.53±0.21 (30%) | 0.51±0.03 (28%) | 0.48±0.23 (29%) | 0.42±0.10 (31%) | 0.35±0.20 (34%) | 0.28±0.19 (30%) |
| Control | - | 0.78±0.12 | 0.80±0.12 | 0.76±0.18 | 0.71±0.23 | 0.68±0.27 | 0.61±0.15 | 0.53±0.10 | 0.40±0.02 |

Results are expressed in mean ± SD; n = 4. The mean values with beta ($^{\beta}$) as superscripts across the column compared with (3% Tween-80-I.P.) are considered significant ($P<0.05$).

**Effect of Isolated Pentacyclic Triterpene (3β)-lup-20(29)-en-3-ol) on Cyclooxygenase Activity:** As shown in **Table 2**, at concentrations of 2, 4, 10 and 14 mg/ml, (3β)-lup-20(29)-en-3-olshowed significant ($P<0.05$) selective inhibitory activity against inducible COX-2. The inhibitory effects were non-concentration-dependent, with the lower concentration (2 mg/ml) exhibiting the highest inhibition (58.85%). At concentration of 1 mg/ml, the selective inhibition was mild, although non-significant when compared with control group. (3β)-lup-20(29)-en-3-olshowed no inhibitory activity against COX-1.

**TABLE 2: INHIBITORY EFFECT OF LUPEOL ((3B)-LUP-20(29)-EN-3-OL) ON CYCLOOXYGENASE (COX-2) ACTIVITY**

| Treatment | Concentration (mg/ml) | Absorbance | Inhibition (%) |
|---|---|---|---|
| Lupeol | 1 | 0.597±0.03 | 10.29 |
| '' | 2 | 0.479±0.03 $^{\beta}$ | 58.85 |
| '' | 4 | 0.508±0.02 $^{\beta}$ | 46.91 |

| | | | |
|---|---|---|---|
| ‘’ | 10 | 0.521±0.01 $^{\beta}$ | 41.56 |
| ‘’ | 14 | 0.554±0.04 $^{\beta}$ | 27.99 |
| 100% Activity | - | 0.622±0.01 | 00.00 |
| Background | - | 0.379±0.01 | - |

Results are expressed in mean ± SD; n = 3. The mean values with beta **($^{\beta}$)** as superscripts across the column compared with control group (100% Activity) are considered significant (P<0.05).

**Effect of Isolated Pentacyclic Triterpene (3β)-lup-20(29)-en-3-ol) on Hypotonicity-Induced Haemolysis:** The isolated compound (3β)-lup-20(29)-en-3-olcaused significant ($p < 0.05$) concentration-related inhibition of hypotonicity-induced haemolysis of red blood cells.

The greatest inhibition was afforded by the highest dose 1000 µg/ml of the isolated compound, (3β)-lup-20(29)-en-3-ol. **Table 3** shows that the percentage inhibition of haemolysis by (3β)-lup-20(29)-en-3-olwere concentration dependent.

At doses of 500 and 1000 µg/ml percentage inhibition of haemolysis found to decrease in this order: (3β)-lup-20(29)-en-3-ol> Diclofenac.

**Characterization of Pentacyclic Triterpene(3β)-lup-20(29)-en-3-ol):** A comparison of the spectra obtained from the $^{1}$D NMR, $^{1}$H NMR, $^{13}$C NMR, $^{2}$D NMR involving DEPT, COSY, HMOC and HMBC of (3β)-lup-20(29)-en-3-ol with the published library of compounds established the identity of the isolate CA1 as (3β)-lup-20(29)-en-3-ol or Lupeol **Fig. 1.**

**TABLE 3: EFFECT OF LUPEOL ((3B)-LUP-20(29)-EN-3-OL) ON HYPOTONICITY-INDUCED HAEMOLYSIS**

| Treatment | Concentration (µg/ml) | Absorbance of Hypotonic Solution (nm) | Absorbance of Isotonic Solution (nm) | % Inhibition |
|---|---|---|---|---|
| Lupeol | 250 | 0.13±0.00 | 0.11±0.00 | 07.22 |
| | 500 | 0.21±0.00 | 0.12±0.00 | 33.68 |
| | 1000 | 0.32±0.00 | 0.13±0.00 | 73.87 |
| Diclofenac | 250 | 0.34±0.00 | 0.13±0.00 | 16.74 |
| | 500 | 0.35±0.00 | 0.14±0.00 | 15.52 |
| | 1000 | 0.32±0.00 | 0.17±0.00 | 30.52 |
| Hypotonic Control | -- | 0.38±0.00 | -- | -- |
| Isotonic Control | -- | -- | 0.18±0.00 | -- |

Results are expressed in mean ± SD; n = 4

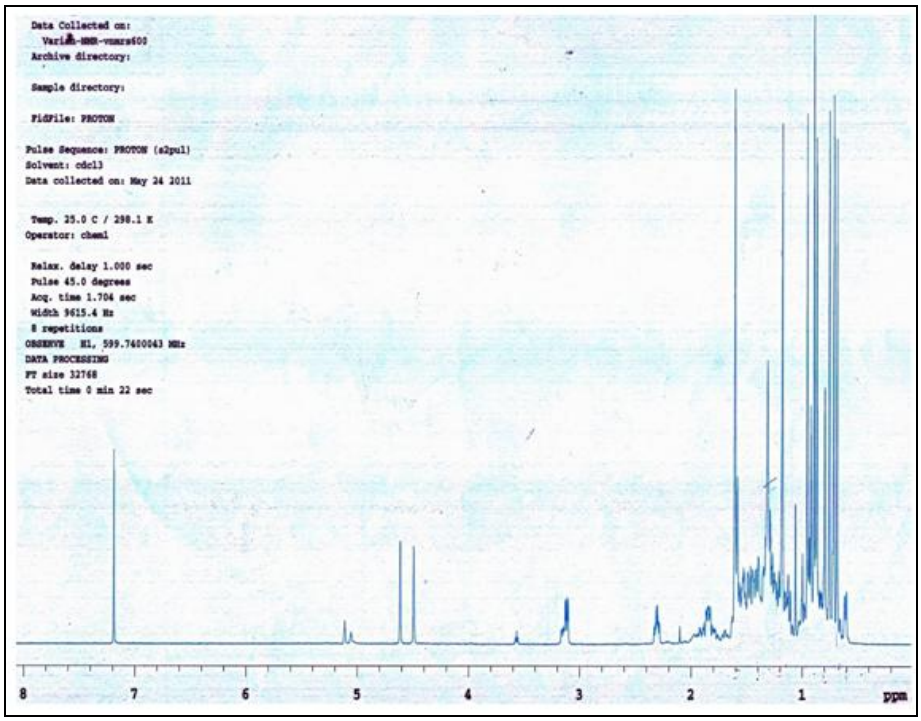


**FIG. 2: NMR DATA OF LUPEOL**

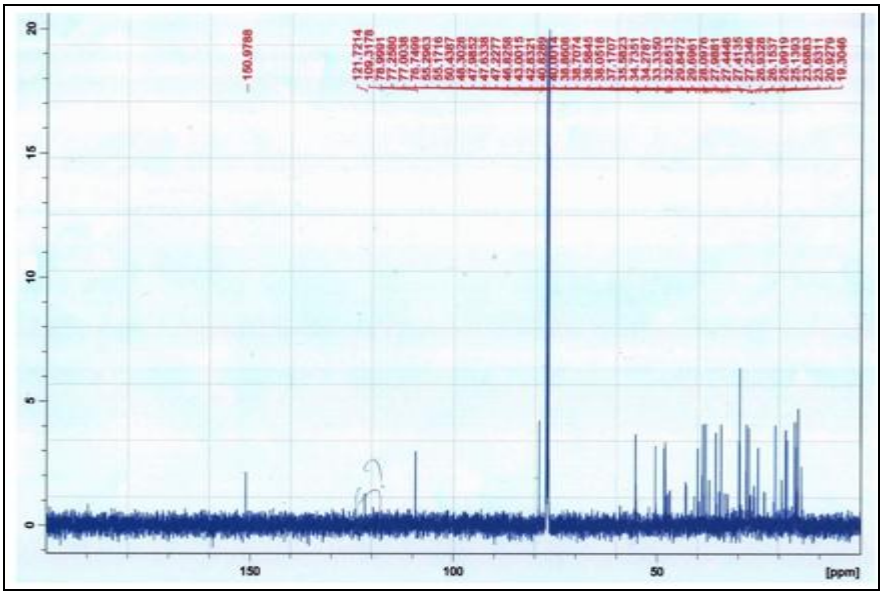


**FIG. 3: NMR DATA OF LUPEOL**

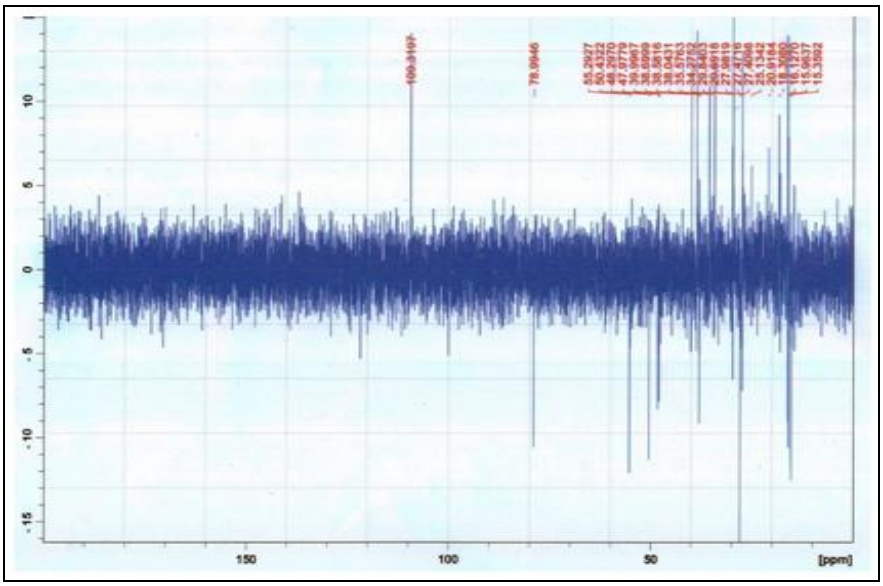


**FIG. 4: NMR DATA OF LUPEOL**

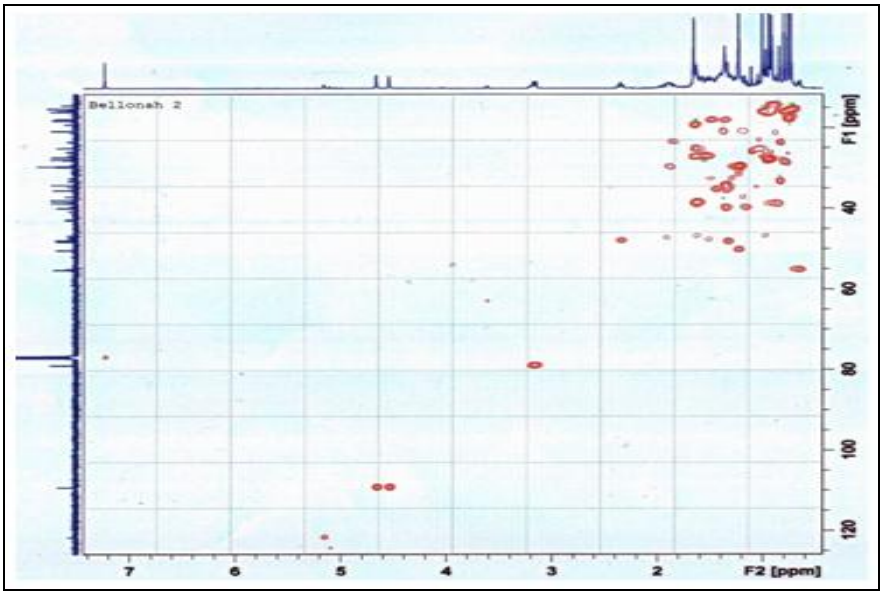


**FIG. 5: NMR DATA OF LUPEOL**

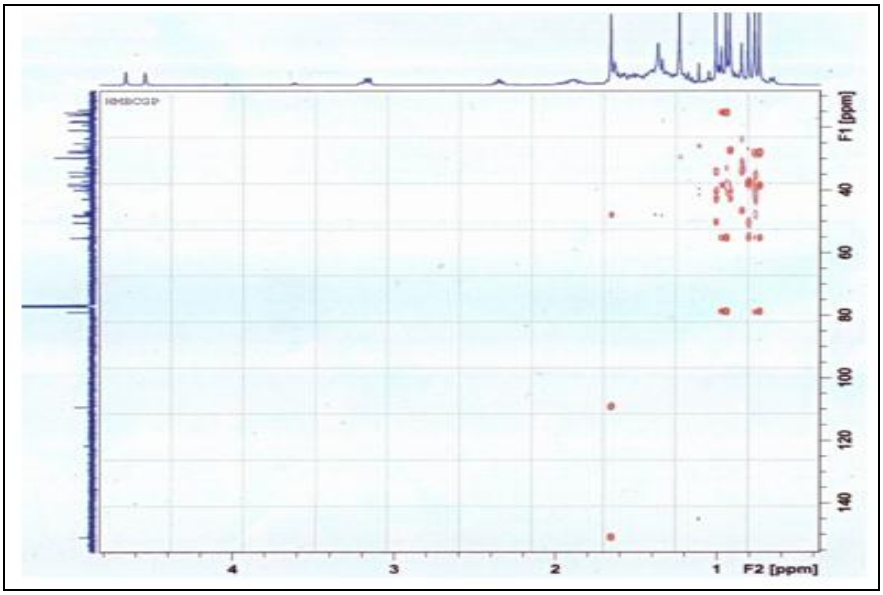


**FIG. 6: NMR DATA OF LUPEOL**

**DISCUSSION:** Anti-inflammatory activity studies technique, using egg-albumin induced rat hind paw edema method of Winter *et al*., (1962) [40], was employed in this study to investigate the effect of (3β)-lup-20(29)-en-3-ol (Lupeol) on systemic acute edema. Our observation from this present research reveals that i.p administration of the (3β)-lup-20(29)-en-3-ol (Lupeol) significantly inhibited the mean paw volume within 0.5-3.5 hours. This observed significant inhibition of increase in paw thickness and volume by (3β)-lup-20(29)-en-3-ol (Lupeol) is in agreement with previous studies which established that lupeol treatment (10-100 µM) decreased the generation of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-β (IL-1β) in lipopolysaccharide-treated macrophages (Fernandez *et al.,* 2001b) [9], which are the most potent NF-kB activators that causes rapid phosphorylation of kappa Bs at two sites within their N-terminal regulatory domain. Other metabolic effects of lupeol were observed to be associated with its potential to modulated signaling pathways such as nuclear factor kappa B (NF-kB) and the phosphatidylinositol-3- kinase [$Pl_3$k]/Akt (protein kinase B pathway), which are reported to play an important role in inflammation [34].

Stabilization of the membranes of these inflammatory cells inhibits lysis and subsequent releases of the cytoplasmic contents which in turn inhibits the tissue damage and exacerbation of the inflammatory response (Okoli *et al*., 2008). *In-vitro* assessment of the effect of (3β)-lup-20(29)-en-3-ol (Lupeol) on membrane stabilization showed that it inhibited hypotonicity- induced lysis of oxygenated red blood cells. Our results regarding the stability of cell membrane is consistent with previous studies by Geetha and Varalakshmi, 1999 [12] and Latha *et al*., 2001 [19, 20], who reported that treatment of arthritic rats with lupeol and its linoleate and eicosapentaenoate esters decreased the level of glycoproteins and lysosomal enzymes, suggesting a reduction of endocytosis by leucocytes and/or stabilization of the lysosomal membrane. Membrane glycopoteins are largely responsible for the physical properties of the cell membrane and contribute to the regulation of the volume and water content of cells by controlling ion exchange across the membrane [32].

Since, pathological conditions can alter surface volume ratio of the cell through loss of membrane surface or gain in volume [32], the physical integrity of the treated cell membranes may have been enhanced by the (3β)-lup-20(29)-en-3-ol (Lupeol) to hinder cell lysis caused by hypotonic solutions that cause the cell to swell and rupture. The stability of biological membrane is also affected by reactive oxygen species generated by activated neutrophils, monocytes and other cells during inflammatory processes. The result of the present study may suggest that one of the ways (3β)-lup-20(29)-en-3-ol (Lupeol) exhibits anti-inflammatory effect is by inhibition of neutrophil recruitment/ infiltration into the inflamed tissue.

This result agrees with of those of (Fernandez *et al.*, 2001b [9]; Gutierrez-lugo *et al*., 2004) [14] who showed that lupeol decreases myeloperoxidase levels (neutrophil specific marker) thus causing reduction in cell infiltration into inflamed tissues in mice and inhibit the activity of soybean lipoxygenase-1 (15-sLO).

The structure of CA1 was identified as (3β)-lup-20(29)-en-3-ol or Lupeol by comparing the spectra obtained with those of published library of compounds. This compound is an established constituent of *Crateva* species and exhibited selective inhibition of inducible COX-2 activity in this study. Lupeol a dietary pentacyclic triterpene found in certain fruits, vegetables and medicinal plants has potent anti-inflammatory, antioxidant, anti-carcinogenic, anti-mutagenic, and anti-malarial activities [23]. Triterpenes are important structural components of plant membrane and free triterpenes serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes [36]. This significant therapeutic effect exhibited by (3β)-lup-20(29)-en-3-ol (Lupeol) may be attributed to its high plasma concentration following intraperitoneal administration which is due to its physiochemical nature, a white solid crystal soluble in acetone and chloroform. Lipid-water partition coefficient is among the major factors that control the rate of drug absorption. The lipophilic nature of (3β)-lup-20(29)-en-3-ol (lupeol) has a major role to play in its absorption and distribution after intraperitoneal administration as it can easily traverse the membrane by dissolving in the lipid bilayers.

This present *in-vitro* COX inhibitory assay indicates that Lupeol a dietary pentacyclic triterpene at varied concentrations exhibited significant selective inhibitory activity against COX-2, but had no inhibitory effect against COX-1. Since, (3β)-lup-20(29)-en-3-ol(lupeol) exhibited no inhibitory activitiy on COX-1, it therefore implies that (3β)-lup-20(29)-en-3-ol (lupeol) favours the resident peritoneal macrophages and a wide variety of cell types which express only cyclooxygenase-1 (constitutively) that synthesizes (from either endogenous or exogenous arachidonic acid) a balance of four major prostanoids; prostacyclin $I_2$, thromboxane $A_2$, prostaglandin $D_2$ and 12-hydroxy heptadecatrienoic acid, with prostaglandin $E_2$ as a minor fifth product. The present *in-vitro* study, indicates that the anti-inflammatory properties of (3β)-lup-20(29)-en-3-ol (Lupeol) could be attributed to its significant reduction in synthesis and release of pro-inflammatory prostanoids, which is in agreement with Fernandez *et al*., 2001 [9], who reported that the anti-inflammatory potential of lupeol could be assessed from the observation, that lupeol pretreatment significantly reduced prostaglandin $E_2$ ($PGE_2$) production in $A_{231}$ 87-stimulated macrophages. However, our result is in accordance with another study by Fernandez *et al*., 2001b [9] which shows that lupeol rich extract of *Pimenta racemosa* plant (which is widely used by country doctors in Caribbean region to treat inflammatory ailments) exhibits significant anti-inflammatory activity in animal models [8]. He further reported that their study showed that the anti-inflammatory behaviour of the lupeol-rich extract was similar to that exhibited by the selective cyclooxygenase-2 inhibitor, indomethacin. This present research finding, that (3β)-lup-20(29)-en-3-ol (lupeol) apentacyclic triterpene is a selective inhibitor of an inducible COX-2 validates these reports of Fernandez *et al*. (2001a) (2001b) [9].

Induction of cyclooxygenase-2 with lipopolysaccharide, TNF-α and cytokines leads to a shift in products of arachidonic acid metabolism to preferential synthesis of two prostanoids, prostacyclin $I_2$ and prostaglandin $E_2$ [4]. In inflammation, redness and edema result from increased blood flow into the inflamed tissue through $PGE_2$-mediated augmentation of arterial dilation and increased microvascular permeability, while pain results from the action of $PGE_2$ on peripheral sensory neurons and on central sites within the spinal cord and the brain [11]. The observation from this study indicates that this preferential synthesis of $PGE_2$ and $PGI_2$the two major pro-inflammatory prostanoids was attenuated by (3β)-lup-20(29)-en-3-ol lupeol (a selective COX-2 antagonist). One major anti-inflammatory properties of (3β)-lup-20(29)-en-3-ol (lupeol) could be attributed to its selective inhibition of inducible COX-2, (Rathinavel, *et al*., 2021) [30] which results to significant reduction in synthesis and release of its two major pro-inflammatory prostanoids; prostacyclin $I_2$ and prostaglandin $E_2$.

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