Bioactive compounds in Magnifera indica demonstrates dose-dependent anti-inflammatory effects

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Magnifera indica (MI) is one of the notable medicinal plants use for the treatment of inflammatory diseases in folkloric medicine. Recent studies by present author demonstrated anti-inflammatory effects of Mangifera indica to have dose-dependent effects, pointing out its dose-dependent effects to be relevant in therapeutics investigations. However, as far as we know, Mangifera indica has one or no translational drugs and products already established in clinical trials or in pharmaceutical practice. This is rather a concern to drug discovery and optimization of research findings, particularly in case of Magnifera indica plant which has various medicinal efficacies already ascribed to its properties. It has been investigated that, Mangiferin a very potent bioactive compound found inside extracts of Magniferaindica, is a polyphenolic and a glucosylxanthone which has strong antioxidant and anti-inflammatory properties. Hence, various effects such as anti-bacterial, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, anti-microbial, gastroprotective have been ascribed to the medicinal use of this plant. However, some other potent natural phytochemicals found in Mangiferaindica which include saponin, tannins, steroids, flavoniod, alkaloids, cardiac glycosides, reducing sugar and anthraquinone, have been discovered to be useful in herbal medicine and cosmetics productions. Thus, this paper focus on the need to encourage translational studies in order to improve lead optimization as well as need to adequately promote further research that may formulate bioactive compounds in Magnifera indica for the purpose of treating inflammatory diseases and stress related disorders. Also, this paper emphasized on the necessity in conducting more clinical trials using Mangifera indica bioactive compounds for variety of conditions in human volunteers.

Keywords: Mangifera indica; Mangiferin; Anti-inflammatory

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Introduction

Man has relied over the years on the art of treating diseases with the use of natural products, especially within his immediate environment. [1] Over the years, natural products from plants have indeed offered humanity clinically useful drugs such as aspirin, reserpine, physostigmine, quinine and ginseng [2]. Magnifera indica (MI) is one of the notable medicinal plants for the treatment of inflammatory diseases in folkloric medicine. However, as far as we know, Magnifera indica has one or no translational drugs and products already established in clinical trials or in pharmaceutical settings. Thus, it is rather a concern in drug discovery and optimization, particularly for magnifera indica plant which has various medicinal efficacies been ascribed to its properties in folkloric medicine.

Inflammation

Inflammation has been described as series of well
coordinated dynamic events that mostly depend on sequential arrival of inflammatory leukocytes from the blood to the site of inflammation, where neutrophils are the first cells to migrate into tissues in response to noxious stimuli. It is assumed that neutrophil infiltration or its related event might be crucial for subsequent macrophage infiltration. [3]

The discovery of some efficacious drugs such as sodium salicylate, acetyl salicylic acid aspirin, cortisone, gold salts and phenylbutazone for the treatment of inflammatory disorders and related conditions is an important milestone in the development of clinically useful anti-inflammatory agents [4] and the newer ones like selective cyclooxygenase enzymes (COX- 2) inhibitors such as celecoxibs. As well as the currently used major groups of anti-inflammatory drugs which include non steroidal anti-inflammatory drugs (NSAIDs), the glucocorticoids and disease modifying anti-rheumatic drugs [5]. However, many of these available treatments against inflammatory conditions are associated with serious adverse effects and have also been found to have their own limitations. Before now, it has been estimated that about 34-46% of the users of NSAIDs will sustain gastrointestinal damage due to the inhibition of the protective COX enzymes in gastric mucosa[6]. The identification of the two isoforms of cyclooxygenase as led to the understanding of mucosa protection from gastric injury, however, because NSAIDs inhibit both cyclooxygenase enzymes when administered at doses effective to reduce the progress of inflammation and pain disorder, they cause ulceration and ulcers. For the recently developed selective cyclooxygenase enzymes COX-2 inhibitors which do not damage the gastrointestinal tract or neither cause any ulceration, but they have a potential adverse effect of prothrombotic tendency, and unproven hypothesis still exist in literatures which described selective COX-2 inhibitors such as celecoxibs when administered at high doses significantly inhibited gastric prostaglandin E2 (PGE2) production in the stomach. And NSAIDs have also been shown to decrease rate of blood flow in the GIT as part of their mechanisms of injury. The introduction of glucocorticoids in clinical practice is effective, mainly use to treat certain inflammatory conditions but, have also been shown to produce an array of side-effects upon chronic administration.

While acute inflammation has been described as the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes especially neutrophil from the blood into the injured tissues, thereafter, series of biochemical events propagate and initiate the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue [7]. Chronic Inflammation is described by the progressive shift in the type of mediators present at the site at which the inflammation is taking place which is characterized by simultaneous and prolong biochemical destructions [7]. In all cases, the anti-inflammatory drugs owe their end point effects with their ability to inhibit synthesis of inflammatory mediators at the inflammatory sites. These inflammatory mediators that are synthesis in the body drive the progress and severity of inflammation and established its signs and symptoms. The mediators of inflammation are bradykinin, plasmin, thrombin, histamine serotonin interferon, oxygen derived free radicals, nitric oxide, platelet activating factor, interleukins, NF kappa B, leukotrenes, TNF Alpha, interleukins, tumor necrosis factor, prosthогlandins, lipoxins, thromboxane A2, substance P, and lysosomal enzymes [8]. However, because of severe adverse effects associated with NSAIDs and glucocorticoids in the treatments of inflammatory disorders, there is rising need for the development of newer and safer anti-inflammatory drugs particularly of plants origin [9]. During the last decade, it became increasingly clear that inflammatoryreactions can be profoundly different depending on the initial stimulus evoking the response. In certain conditions, microbial infections elicit a classical or the so called type 1 inflammatory responses often characterized by neutrophil and macrophage recruitment and the release of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-a). Furthermore, during these inflammatory responses and the subsequent T-cell responses, which are dominated by T helper 1 (TH1) cells, macrophages are activated to become more efficient at killing the infectious agents. Macrophages do this together with the enhancement of other macrophage effectors’ function induced under these circumstances. The macrophage activation process is described as type 1, classical or macrophage response. In contrast, infections with large extracellular parasites, such as helminths, do elicit an innate immune reaction characterized by recruitment of eosinophils and basophils which release the cytokine interleukin-4 (IL-4), subsequently eliciting a T helper 2 (TH2) response [10].

Under these conditions, there is also enhanced accumulation of macrophages at the site of infection, which simply concentrate at high number at the infection site so as to help fight the parasite, thus, due to the different cytokines production; these macrophages exhibit a distinct phenotype which differentiates them at the site of infection [11,12]. Instead of an increased killing capacity by the innate cell, these alternatively activate type 2 reaction which actually display decreased phagocytic function and microbikilling capacity, this process express a different set of cellular markers and are associated with healing processes and protective responses to helminth infection [10,11]. Up till now, type 2 inflammation is less well characterized than the prototypic type 1 inflammatory response; it is not clear
whether the mechanistic principles known for type 1 inflammatory reaction that lead to pathogen killing can be simply applied to type 2 responses, which seems to be more connected with healing processes and tissues repair.\textsuperscript{11} However, increasing knowledge in the biochemical and molecular bases of inflammation has led to possible clearfindings into discovery of newer compounds from plant origin in which some of this plants have been successfully proven to target the progress of systemic inflammatory disorders, and with the aid of bioactive polyphenols such as flavonoids, mangiferin, leotulin, naringenin which are found in most plants have provide beneficiary anti-inflammatory effect in ethanopharmacology research.

**Mangifera Indica**

Alami et al.\textsuperscript{13} described that the decoction of dried bark of mango is used for treating diabetes. Chhabra et al.\textsuperscript{14} found that the decoction of dried stem bark of mango is used for toothache and is taken orally for treatment of malaria. Garcia et al.\textsuperscript{15} illustrated the effects of standard aqueous extract of MI used in Cuba under the brand name of Vimang®.

*Mangifera indica* is a commonly used plant in folkloric medicine. Various studies indicate mango possesses anti-diabetic, anti-oxidant, anti-inflammatory properties. Several effects like anti-bacterial, anti-pyretic, anti-diarrhoeal, anti-allergic, immunomodulation, anti microbial and gastro-protective have also been ascribed to medicinal use of *Mangifera indica*. These studies are very encouraging and indicate this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects\textsuperscript{16} Mangoes belong to genus *Mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae, varied medicinal properties are attributed to different parts of mango tree. Most parts of the tree are used medicinally and the bark also contains tannins, which are used for the purpose of dyeing \textsuperscript{16}.

**Significance of bioactive compounds in Mangifera**

It has been discovered that *mangiferin* a very potent bioactive compound found inside extracts of *Mangifera indica*, being a polyphenolic and a glucosylxanthone, possess strong antioxidant properties due to its ability to reduce lipid peroxidation in cell and enhances glutathione levels, apart from that, it has also be shown to produce immunomodulation, wound healing and antidiabetic activities for this reason, major phytochemicals constituents of *Mangifera indica* are always of an interest. Emphases have been made towards the different chemical constituents of this plant, especially the polyphenolics flavonoids, triterpenoids, isomangiferin, tannins & gallic acid derivatives. In which they have all proved to be therapeutically useful. The stem bark is reported to contain protocatechic acid, catechin, mangiferin, alanine, glycine, \(\gamma\)-aminobutyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3\(\beta\),26-diol, 3-ketodammar-24 (E)-en-20S,26-diol, C-24 epimers of cycloart-25 en 3\(\beta\),24,27-triol and cycloarten-3\(\beta\),24,27-triol. Indicoside A and B, manghopanale, friedelin, cycloarten-3\(\beta\)-30-diol and derivatives, mangsterol, manglupenone, mangocoumarin, n-tetacosane, n-heneicosane, n-triacontane and mangiferolic acid methyl ester and others isolated from stem bark of MI. Mangostin, 29-hydroxy mangiferonic acid and *mangiferin* have been isolated from the stem bark together with common flavonoids \textsuperscript{16}.

However, the bioactive compound *mangiferin* can be found in many plant species, among which the mango tree (*Mangifera indica*) is one of the primary sources. Further studies have shown that *Mangiferin* is also present in some medicinal herbs, influencing their therapeutic and preventive properties, and also in honeybush (*Cyclopia sp.*), a popular South African herbal tea. *Mangiferin* easily dissolves in water, so it can well be extracted into infusions and decoctions; this ability alone makes it suitable for its use as available medicinal plant to relief fever and aches. However, some investigations have also described *Mangiferin* to be an efficient iron chelator.

*In vitro* and *in vivo* pharmacological studies, demonstrated many other activities of *mangiferin*, which are neuroprotective, memory improving, as well as radioprotective against X-ray, gamma, and UV radiation. Similar studies done in *in vitro* and *in vivo* indicated also its ability to inhibit carcinogenesis and cancer cells growth by apoptosis induction. Manufacturing industries also used it in cosmetics, due to antioxidant and UV-protecting properties\textsuperscript{17} Further studies, unveiled other notable phytochemicals found in *Mangifera indica* which includes; saponin, tannins, steroids, flavonoid, alkaloids, cardiac glycosides, reducing sugar and anthraquinone. All these bioactive chemicals have been found to be useful in herbal medicine and cosmetics.

In Cuba, Vimang® is an aqueous extract of *Mangifera indica* L. traditionally in used for its analgesic, anti-inflammatory, antioxidant and immunomodulatory properties. Vimang® has *mangiferin* been its major component. Preclinical studies demonstrated that these products prevented tumor necrosis factor \(\alpha\) -induced IxB degradation and the binding of nuclear factor \(\kappa\)B to DNA, which induces the transcription of genes implicated in the
expression of some mediators and enzymes involved in inflammation, pain, oxidative stress and synaptic plasticity\[18\]. *Mangifera indica* aqueous extract Vimang® restored the redox balance in Type-I diabetic patients in cuba\[19\]. Further studies by Garrido Suarez et al reported cases in patients with acute herpetic neuralgia using Vimang® in an hospital in cuba, patients that received a daily dose of 1800 mg of extract (two coated Vimang® tablets 300 mg each, three times daily before meals, results from their findings suggest that vimang® supplementation might be beneficial to prevent and treat neuropathic pain. However, this product is probably known to this region alone.

**Test for Mangifera indica’s anti-inflammatory effects**

In an attempt to further validate the anti-inflammatory properties of MI, we performed experimental procedures as described; some fresh variety of mango stem bark (*Mangifera indica* Linn) was collected from a cultivated field from the Abraka community, Delta State Nigeria. It was authenticated at the Department of Pharmacology and Therapeutics, Delta State University Abraka for the purpose of this study. MI stem bark was dried in the oven (Laboratory Oven, Gallenkomp, UK) at 60°C to reduce the water content; the dried stem bark later weighed (825 g) and was blended into a fine powder. The powered MI was soaked in 1000 mL of water for 2 days and was later filtered through Watman 2.0 filter paper. The dried filtrate was placed in the Soxhlet apparatus (Laborato 4000 efficient HB, Germany). Another 500 mL of distilled water was allowed to run continuously through it in a heater for a period of 24 hours. The solution obtained was evaporated to dryness in a Rotary Evaporator (Lavorota 4000 Efficient HB, Germany) at 40°C. The yield of the extract was 10.2% with reference to the powdered material, the LD50 of mangiferin in Wistar rats was discovered at 365 mg/kg from which doses of 50, 100 and 200 mg/kg were used in the animal experiments.

Male Wistar rats weighing between (150-180g) were obtained from the animal house of the college of Health Sciences, Delta State University Abraka and were used in this study. The animals were allowed to aclimatize to their new environment for 2 weeks before the commencement of the experiments. Throughout the study period, they had free access to clean drinking water ad libitum and were fed with pelleted rat feeds. They were placed under hygienic conditions at room temperature (22°C–26°C) and 12h light and darkness exposure. Relative humidity was normal. The animals were divided into five groups with five animals to each group, they respectively received the dosage of as 50 mg/kg, 100mg/kg and 200mg/kg of the MI, 50mg/kg and 100mg/kg of acetylsalicylic acid (Sigma Aldrich, USA) to serve as positive control group and the negative control group was given 3mL of normal saline orally. After the determination of the initial right hind paw size in the rats in the various groups, the extract groups were treated with 50, 100 and 200mg/kg of the MI, respectively; the second group was treated with 50 and 100mg/kg of acetylsalicylic acid and the last group was treated with 3mL of normal saline, respectively, using an oral canula.

Half an hour later, inflammatory oedema was induced by injecting 0.1mL of 1% carrageenin solution (Sigma Aldrich, USA) into the sub-plantar surface of the right hind paw of the rats.

The increase in paw was determined with a digital vernier caliper (China) for the period of 3 hours after the sub-plantar injection of 0.1mL of the 1% carrageenin solution. The mean paw circumference and percentage inhibition of oedema was calculated, using the formula for calculating inhibition (%) described by Perez et al.[20].

\[
\text{Inhibition} \% = \frac{\text{Mean no. of writing control} - \text{Mean no. of writing test}}{\text{Mean no. of writing control}} \times 100
\]

Data obtained from this study were expressed as mean ± SEM. Statistical analysis was performed using analysis of variance (ANOVA, Graph pad prism). P values < 0.05 were considered statistically significant.

**Table 1. Anti-inflammatory effect of aqueous leaf extract of Mangifera indica Linn on carrageenin-induced oedema in rats**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>Inhibition of paw (mm)</th>
<th>% inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>-</td>
<td>5.62±0.29*</td>
<td>23.6</td>
</tr>
<tr>
<td><em>M. indica</em></td>
<td>50</td>
<td>4.29±0.27*</td>
<td>26.3</td>
</tr>
<tr>
<td><em>M. indica</em></td>
<td>100</td>
<td>4.14±0.40*</td>
<td>26.3</td>
</tr>
<tr>
<td><em>M. indica</em></td>
<td>200</td>
<td>3.30±0.23*</td>
<td>41.2</td>
</tr>
<tr>
<td>Acetylsalicylate acid</td>
<td>50</td>
<td>4.36±0.11</td>
<td>22.4</td>
</tr>
<tr>
<td>Acetylsalicylate acid</td>
<td>100</td>
<td>3.05±0.20</td>
<td>45.7</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for five animals in each group. *p < 0.05 (ANOVA) when compared to acetylsalicylic acid 50 or 100 mg/kg control, respectively.

**Discussion**

From clear indications, the results obtained in this study shown that the inhibitory effects produced by the aqueous extract of MI are concurrently similar to the inhibitory effects of acetylsalicylic acid at 50 and 100mg/kg doses. These findings indicated that for each increase in standard dosage of MI, the acute inflammation induced in rat paws by carrageenin was drastically inhibited, this simply suggest dose-dependent effects. In most of the literature where MI was tested for anti-inflammatory effects, it been observed that higher doses shown more anti-inflammatory effects that the lower doses despite the fact that both are often within the
speculated ED50 for *Mangifera indica* in laboratory studies. We also compared specifically 100 and 200 mg/kg extract with the acetylsalicylic acid control 50 or 100 mg/kg and we discovered that, from our findings, MI could be producing anti-inflammatory responses as the dosage increases.

Previous studies have shown the ability of some non-steroidal anti-inflammatory drugs (NSAIDs) and phytochemicals to inhibit cellular components of inflammation due to the activation of leukocytes. The inhibitory effect of these compounds on the cellular phases of inflammation was attributed to the suppression of migration of leukocyte to the site of injury. This effect may have resulted from the reduction in the release of chemoattractants or in the ability of white blood cells to respond to these chemical substances. Therefore, the ability of MI to reduce the acute inflammation induced by carrageenin, suggest that it possesses phytochemical active compounds that could inhibit the migration of white blood cells to the site of inflammation by majorly by preventing leukocyte adherence, which is therate limiting step to the triggering actions of mediators of acute inflammation. It is well established in the literature that redness, loss of function and oedema formation are some of the cardinal signs of inflammation. Also, the activation of phospholipase C is a central component of the signal transduction in numerous cells, including leukocytes migration from human peripheral blood and its adherence. However, the role of PLC in the inflammatory process in vivo has been described to be the rate limiting steps for the formation of the arachidonate and activation of leukotrienes pathways in inflammatory reactions. It is believed that damaged to phospholipid layers of the cell membrane will automatically cause hydrolysis and in turns further synthesized the inflammatory mediators, thus, these potent mediators that are synthesized due to arachidonic metabolism are the major chemicals which trigger molecular pathways that give rise to the formation of the cardinal signs noticed in acute and chronic inflammation. Hence, in view of this, the capacity of MI to further reduce oedema formation and redness of the blood vessels, including loss of function, as this was noted in the saline treated group, strongly indicates the existence of anti-inflammatory phytochemicals which is mostly mangiferin to have altered the progression of carrageenin induced acute inflammation in rats. Farber et al. and Keck et al. explained that cytokines are the main mediators involved in the progression of systemic inflammatory responses. In particular, TNFα, IL-1b, IL-6 and IL-8 normally appear early in circulating blood in a classical sequence. However, the effects and the generation of these cytokines cannot be properly understood without the contribution of other mediators which hand in hand drive the progress and the severity of inflammatory reactions. In particular oxygen-derived free radicals have now been known to be major culprits in inflammatory diseases and stress. These toxic mediators in cohort with derived free radicals act in promoting the generation of cytokines by modulating the nuclear factors that regulate their synthesis, priming the endothelial cells by the induction of adhesion molecules involved in the recruitment of inflammatory cells, and finally producing additional tissue damage through their direct effect on different bio molecules. The broad spectrum of actions makes the oxygen free radicals crucial actors in the triggering event of systemic inflammatory response and the subsequent amplification of the proinflammatory cascade followed by the systemic response. In view of this, it is rightly expected that various mechanisms by which MI produce anti-inflammatory response should involve scavenging of free radicals and elevating antioxidant molecules, thereby preventing increase production of cytokines which are the major mediators involve in the progression of acute inflammation.

Non steroidal anti-inflammatory drugs (NSAIDs) have been approved for clinical use. However, their use has been limited by serious adverse effects such as gastric erosion or peptic ulcers, and fluid retention in the renal system. Studies have shown that MI extract have also been reportedly used as a medicinal plant, like other herbal extracts. However, its use as anti-inflammatory plant in folkloric practices is likely to be associated with peptic ulceration, gastric erosion or fluid retention in renal systems, which are noticeable in acetylsalicylic acid and some other NSAIDs treatments. Also, many different studies have suggested that macrophages are a potent source of carrageenin metabolites generated via arachidonic acid pathways. Therefore, MI extract would have inhibited the release of prostaglandin E2 (PGE2), which is a potent mediator of inflammatory disorders, particularly in chronic inflammation, and would have prevented the stimulation of macrophages and other probable major mechanisms of inflammatory actions.

**Conclusions**

Several reports in literature have provided information that *Mangifera indica* possess many medicinal properties, and in most cases possible mechanisms of its actions have been explored. Also, the fact that, the bioactive compounds such as mangiferin, polyphenols and carotenoids found in this plant have been identified to modulates severe pathways involve in inflammatory diseases, making it possible for *Mangifera indica* to produce pharmacological activities like antioxidant, immunomodulatory, anti-allergic, anti-inflammatory, antitumor, antidiabetic, lipolytic, monoamineoxidase-inhibiting, antimicrobial and antiparasitic. Similarly, investigatory efforts provided
standard aqueous extract of *mangifera indica* in Cuba under the brand name of vimang® which is available as anti-diabetic, antioxidant, anti-inflammatory and analgesic. However, vimang® is probably in used in Cuba only. And, there is no other information available to show if any other compound or product derived from *mangifera indica* is on clinical trials. Thus, there is need to further investigate possible ways by which formulations such as vimang® could be developed and further therapeutically studies should be done using vimang® in certain conditions in human volunteer, then most especially should be tested for toxicological studies to validate its general usage. Further emphases should also be made towards investigating its pharmacokinetics parameters in human volunteer, and the need to further investigate other means to translate other lead optimization derived in this plant for successful drug that could be accepted for the treatment, or as combination therapy against inflammatory ailments in hospitals and in pharmaceutical settings.

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**Conflicting interests**

The authors have declared that no competing interests exist.

**References**


