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Phytochemical screening and anti-inflammatory activity of *Moringa oleifera* pods-an *in vivo* design

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Abstract

Back ground: The practice of herbal medicine dates back to the very earliest period of known human history. There are evidences that herbs have been used in the treatment of diseases and for revitalizing the body system though most of the plants do not possess effective documentation supporting their activity.

Objective: In the present research the methanolic extract of *Moringa oleifera* pods was evaluated for its phytochemical constituents and physicochemical analysis along with its anti-inflammatory activity.

Method: The pharmacognostic study was performed by following standard protocols and *in vivo* anti-inflammatory activity was evaluated by carrageenan induced oedema model with the help of Zeitin's constant loaded lever apparatus.

Results: The physicochemical analysis showed the total ash value, water soluble extractive value and acid insoluble ash value was 9.0% w/w, 2.0% w/w, 1.5% w/w respectively. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, phenols, tannins, flavonoids, saponins and glycosides. The anti-inflammatory activity showed at the doses of 200 and 400 mg/kg b.w and Indomethacin at 10mg/kg produced dose dependent significant reduction in carrageenan induced rat maximal paw oedema.

Conclusion: The methanolic extract of *Moringa oleifera* pod has potential in terms of anti-inflammatory properties. These data's would help in the development of pod profile for the plant, and can be used in herbal medicines as it has fewer side effects as compared to those synthetic drugs.

Keywords: *Moringa oleifera*, pods, anti-inflammatory activity, indomethacin, paw edema, carrageenan, zeitlin's constant loaded lever

Introduction

Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness". There are many different systems of traditional medicine, and the philosophy and practices of each are influenced by the prevailing conditions, environment, and geographic area within which it first evolved (WHO 2005), however, a common philosophy is a holistic approach to life, equilibrium of the mind, body, and the environment, and an emphasis on health rather than on disease. Generally, the focus is on the overall condition of the individual, rather than on the particular ailment or disease from which the patient is suffering, and the use of herbs is a core part of all systems of traditional medicine. The pharmacological treatment of disease began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose [1]. The pharmacological treatment of disease began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose. Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds [2].

About 200 years ago, the first pharmacologically active pure compound, morphine, was produced from opium extracted from seeds pods of the poppy *Papaver somniferum*. This discovery showed that drugs from plants can be purified and administered in precise dosages regardless of the source or age of the material. This approach was enhanced by the discovery

of penicillin. With this continued trend, products from plants and natural sources (such as fungi and marine microorganisms) or analogs inspired by them have contributed greatly to the commercial drug preparations today. Examples include antibiotics (e.g. penicillin, erythromycin); the cardiac stimulant digoxin from fox-glove (*Digitalis purpurea*); salicylic acid, a precursor of aspirin, derived from willow bark (*Salix spp.*); reserpine, an antipsychotic and antihypertensive drug from *Rauwolfia spp.*; and anti malarials such as quinine from *Cinchona* bark and lipid-lowering agents (e.g. lovastatin) from a fungus. Also, more than 60% of cancer therapeutics on the market or in testing is based on natural products. Of 177 drugs approved worldwide for treatment of cancer, more than 70% are based on natural products or mimetics, many of which are improved with combinatorial chemistry. Cancer therapeutics from plants include paclitaxel, isolated from the Pacific yew tree; camptothecin, derived from the Chinese “happy tree” *Camptotheca acuminata* and used to prepare irinotecan and topotecan; and combretastatin, derived from the South African bush willow [3, 4].

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. *Moringa oleifera* is the fast-growing, drought-resistant tree and it is widely cultivated in tropical and subtropical area [5].

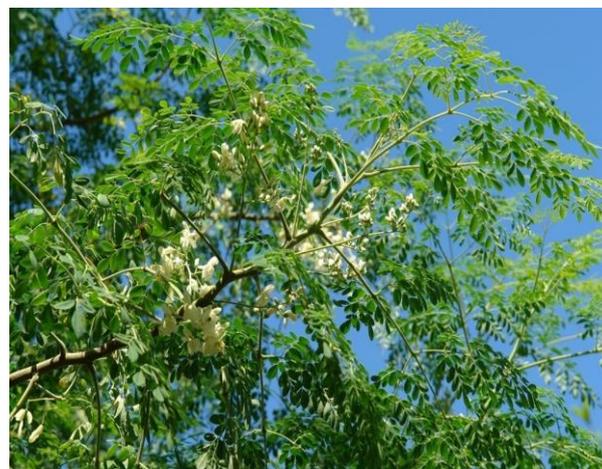


Fig 1: *Moringa oleifera* plant



Fig 2: *Moringa oleifera* pods

Botanical classification

Table 1: Scientific classification of *Moringa Oleifera*

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	<i>Moringa</i>
Species	<i>M. oleifera</i>
Bionominal Name	<i>Moringa oleifera</i> Lam.

In the present study, pod extract of *Moringa oleifera* was studied for its phytopharmacological investigation (Phytochemical screening and *in vivo* anti-inflammatory activity) followed by the standard protocols.

Materials and Methods

Collection of plant materials

The *Moringa oleifera* pods were collected from local market in Visakhapatnam, Andhra Pradesh, India. The freshly collected plant materials were washed under running tap water, and then distilled water, shade dried and pulverized in electric grinder. The powdered material was stored in air tight container for further successive extraction.

Extraction process

The powdered material was subjected to extract with methanol solvent for about three complete cycles. The obtained residue was vacuum filtered, concentrated by distillation, dried completely and stored in desiccator for further use.

Plant material	Solvent	No. of cycles	Weight of the extract
Dried pods 1.0 kg	Methanol	3	46.8 g

Physicochemical analysis of *Moringa oleifera* pods

Physicochemical parameters

The ash values and extractives values were performed according to the official methods described in the Indian Pharmacopoeia and WHO guidelines on quality control methods for medicinal plant materials.

Determination of total ash

About 2 to 3 grams (accurately weighed) pod extract was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450 °C) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to the air dried powder. The procedure was repeated five times to get constant weight.

Determination of acid insoluble ash

The total ash was boiled for 5 minutes with 10% w/v dilute hydrochloric acid and filtered through an ash less filter paper (Whatman no. 41). The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed.

Determination of alcohol soluble extractive

5 grams of the pod extract was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowing it to stand for 18 hrs. It was filtered rapidly taking precautions against loss of alcohol and 25 ml of the filtrate was evaporated to dryness in a tared bottomed shallow dish at 105 °C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried powder.

Determination of water soluble extractive

About 5 grams of the pod extract was added to 50 ml of water at 80 °C and to it 2 grams of keiselguhr was added and filtered. 5 ml of the filtrate was transferred to a tared evaporating dish, the solvent was evaporated on a water bath, drying was continued for half an hour, finally it was dried in a hot air oven for 2 hours and weighed. The percentage of water soluble extractive was calculated with reference to air dried drug.

Fluorescence analysis

Fluorescence analysis of the pod extract was observed in UV light (Long wavelength – 365 nm and Short wave length – 245 nm). The drug powder was treated with various reagents like 1M hydrochloric acid and alkaline solutions like 1N sodium hydroxide, Ferric chloride, iodine and 1M sulphuric acid etc. They were subjected to fluorescence analysis in UV-light.

Preliminary phytochemical screening ^[6, 7]

The different chemical tests were performed for establishing profile of the pod extract for its chemical composition; the following chemical tests for various phytoconstituents in the methanol extract was carried out as described below.

(A) Test for alkaloids

i) Dragendorff's test: In a test tube containing 1ml of extract, add few drops of dragendorff's reagent. Appearance of orange colour indicates the presence of alkaloids.

ii) Wagner's test: To the extract, add 2 ml of wagner's reagent. Formation of a reddish brown precipitate indicates the presence of alkaloids.

iii) Mayer's test: To the extract, add 2 ml of mayer's reagent, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's test: To the extract, add 2 ml of hager's reagent; the formation of yellow precipitate indicates the presence of alkaloids.

(B) Test for amino acids

i) Ninhydrin test: Add two drops of ninhydrin solution to the extract; no formation of characteristic purple colour indicates the absence of amino acids.

(C) Test for anthocyanins

i) To the extract, add 10% sodium hydroxide. No formation of blue colour shows the absence of anthocyanins.

ii) To the extract, add conc. sulphuric acid. No formation of yellowish orange colour confirms the absence of anthocyanins.

(D) Test for carbohydrates

i) Molisch's test: To the extract, add 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

ii) Fehling's test: To the extract, add equal quantities of fehling's solution A and B on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

iii) Benedict's test: To 5 ml of benedict's reagent, add the extract and boiled for two minutes and cool. Formation of red precipitate showed the presence of carbohydrates.

(E) Test for coumarins

i) To 1 ml of extract, add 1 ml of 10% sodium hydroxide. No formation of yellow colour indicates the absence of coumarins.

(F) Test for fixed oils and fats

i) Spot test: A small quantity of extract is press between two filter papers. No oil stains on the paper indicates the absence of fixed oils and fats.

(G) Test for flavanones

i) To the extract, add 10% sodium hydroxide and the colour changes from yellow to orange, which indicates the presence of flavanones.

ii) To the extract, add conc. sulphuric acid and the colour changes from orange to crimson red, which indicates the presence of flavanones.

(H) Test for flavones

i) Shinoda test: To the extract, add a few magnesium turnings and 2 drops of concentrated hydrochloric acid, formation of red colour showed the presence of flavones.

ii) To the extract, add 10% sodium hydroxide or ammonia; formation of dark yellow colour indicates the presence of flavones.

(I) Test for glycosides: For detection of glycosides about 50mg of extract is hydrolyse with concentrated HCl for 2hrs on a water bath and filter. The hydrolyzed is subject to following tests

i) Borntrager's test: To 2ml of hydrolysate, add 3 ml of chloroform and shake well. To the separated chloroform layer, add 1ml of 10% ammonia solution and observe for colour. No formation of pink colour indicates the absence of anthraquinone glycosides.

ii) Keller killiani test: About 50mg of the extract is dissolve in 2 ml of glacial acetic acid and add 2 drops of 5% ferric chloride solution and mix, to this add 1 ml of H₂SO₄, no reddish brown colour appears at the junction of two liquid layers indicating the absence of steroidal glycosides.

(J) Test for phenols

i) Ferric chloride test: To the extract, add few drops of 10 % aqueous ferric chloride. Appearance of blue/green colour indicates the presence of phenols.

(K) Test for proteins

i) Biuret test: To the extract, add 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution. No formation of violet colour indicates the absence of proteins.

ii) Xanthoprotein test: To the extract, add 1 ml of concentrated nitric acid. A white precipitate will form; it is then boil and cool. Then add 20% sodium hydroxide or ammonia. No orange colour indicates the absence of aromatic amino acids.

iii) Tannic Acid test: To the extract, add 10% tannic acid. No formation of white precipitate indicates the absence of proteins.

(L) Test for quinones

i) To 1 ml of the extract add 1 ml of concentrated sulphuric acid. No formation of red colour shows the absence of quinones.

(M) Test for saponins

i) To 1 ml of the extract, add 5 ml of water and the tube is shake vigorously. Copious lather formation indicates the presence of saponins.

(N) Test for steroids

i) Liebermann burchard Test: To 1ml of extract, add 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid. The solution become red, then blue and finally bluish green indicates the presence of steroids.

(O) Test for tannins

i) To few mg of extract, add ferric chloride, formation of a dark blue or greenish black colour showed the presence of tannins.

ii) The extract was mix with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

(P) Test for terpenoids

i) Salkowski test: To 1 ml of extract, add tin (one bit) and thionyl chloride. No formation of pink colour indicates the absence of terpenoids.

ii) Hirshonn reaction: The extract is heat with trichloroacetic acid; no formation of red to purple colour indicates the absence of terpenoids.

(Q) Test for volatile oils

i) To the extract, add alc. solution of sudan III reagent. No

formation of red colour indicates the absence of volatile oils.

ii) Alkaline test: To the extract, add a drop of tincture alkaline. No formation of red colour indicates the absence of volatile oils.

Anti-inflammatory design

The complexity of inflammatory process and the diversities of the drugs that have been found effective in modifying the process have resulted in the development of numerous methods for detecting anti-inflammatory substances. In the present investigation, the anti-inflammatory activity of the methanolic extract of pods of *Moringa oleifera* was tested. The method that followed was carrageenan induced rat paw oedema model.

Apparatus available for measurement of oedema (Paw thickness/volume)

1. Zeitlin's constant loaded lever
2. Plethysmograph

Animals (175- 225g)	: Albino Wistar rats of either sex
Carrageenan	: 1% suspension in saline
Teat drugs	: Methanolic extract of pods of <i>Moringa oleifera</i> (At the doses of 200, 400mg/kg b.w)
Standard drug	: Indomethacin (10mg/kg b.w)
Drug vehicle	: Sodium carboxymethyl cellulose in water
Instrument	: Zeitlin's apparatus was used to measure the paw thickness

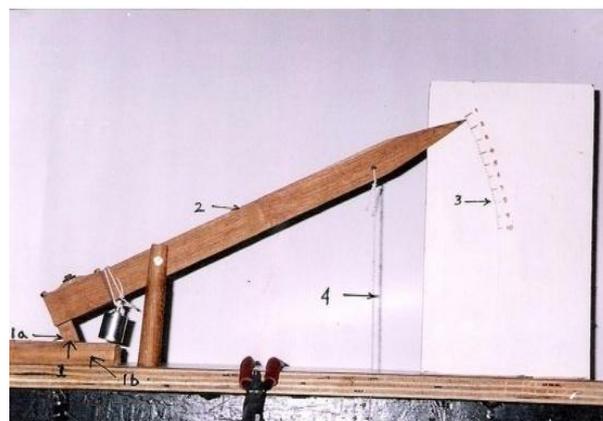


Fig 3: Zeitlin's constant loaded lever (Paw thickness measuring devices)

1. Place, where the paws use to be kept to measure the thickness.
2. Constant load lever.
3. Graduated scale numbered between 1-10 and divided by 0.5cms.
4. Thread to pull down the lever with right leg in order to facilitate to keep the paw in between pointer 1a and basement 1b.

Experimental procedure

Inflammation was induced in the right hind paw of each rat by sub plantar injection of 1% carrageenan suspension (0.1 ml). The left hind paw of the rat was injected 0.1 ml of saline. Animals were divided into different groups (each contains 4 rats) as follows:

Group A received drug vehicle 1% sodium carboxymethyl cellulose.

Group B received standard drug Indomethacin at the dose 10mg/kg.

Group C and D received methanolic extract of *Moringa oleifera* at the doses 200 and 400 mg/kg body weight respectively.

Two hours after administration of doses, each rat was injected with saline subcutaneously into sub-plantar tissue of the left hind paw. The paw thickness of each rat was measured using Zeitlin's apparatus before carrageenan injection and every hour up to 6hrs after carrageenan injection. The percentage inhibition of paw oedema was calculated by using the following formula.

$$\% \text{ Increase in paw thickness} = \frac{Y_t - Y_0}{Y_0} \times 100$$

Where,

Y_t = Paw thickness at time (1, 2, 3, 4, 5 and 6th) after injection.

Y_0 = Paw thickness at 0 hour (before injection).

Effect of crude extract of *Moringa oleifera* 200, 400 mg/kg along with Indomethacin (10 mg/kg) on the total paw oedema in Carrageenan induced rats.

Statistical analysis

All experimental values were expressed as the mean ± the standard deviation. The results were tabulated and the statistical significance (significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) of the results was carried out by using unpaired student T-test.

Results and Discussion

The pods of *Moringa oleifera* was assayed for preliminary phytochemical screening, physicochemical analysis and anti-inflammatory activity of methanol extract with indomethacin and the results were represented in following figures and tables.

Physicochemical analysis of *Moringa oleifera* pods

The ash values, extractives values and fluorescence analysis were performed and the results were illustrated in Table 2 and 3, and the preliminary phytochemical screening of pods has been summarized in Table 4.

Table 2: Physicochemical parameters

Parameters	Extractive value results (% w/w)
Total ash	9.0
Acid insoluble	1.5
Alcohol soluble	-
Water soluble	2.0

Table 3: Fluorescence analysis

Reagent	Short wave	Long wave
Sulfuric acid	Green	Olive Green
Hydrochloric acid	Green	Purple
Iodine	Green	Olive Green
Ferric chloride	Green	Dark Brown
Sodium hydroxide	Green	Green

Preliminary Phytochemical Screening

Our observation revealed that in the preliminary

phytochemical screening was found that the methanolic pod extract of *Moringa oleifera* contain alkaloids, steroids, saponins, carbohydrates, phenols etc. The preliminary phytochemical screening results were shown in Table 4.

Table 4: Preliminary phytochemical screening of *Moringa oleifera* pods

Constituents	Methanolic extract
Alkaloids	+
Amino acids	-
Anthocyanins	-
Carbohydrates	+
Coumarins	-
Fixed oils and fats	-
Flavonones	+
Flavones	+
Glycosides	-
Phenols	+
Proteins	-
Quinones	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	-
Volatile oils	-

(+) Present, (-) Absent

Carrageenan-induced paw edema is a most-valuable model to investigate anti-inflammatory effect of natural and chemical products. Sub-plantar injection of 1% carrageenan (0.1 ml) produced marked, sustained and time related increase and decrease in the rat hind paw oedema of the control group. Paw swelling or oedema was reached peak level at 4 hour after the injection of carrageenan and gradually decreased in the following hours. The methanolic extract of *Moringa oleifera* pods at the doses of 200 and 400 mg/kg b.w and indomethacin at dose 10 mg/kg produced dose dependent significant reduction in carrageenan induced rat maximal paw oedema. The results suggested that the pods of *Moringa oleifera* possessing anti-inflammatory activity, but to a lower extent. The preliminary phytochemical examination suggested that the pods having alkaloids, sterols, saponins and flavonoids etc.

It is therefore assuming that since the plant possessing sterols. The anti-inflammatory activity of the plant may be due to the presence of the above said category of compounds. It is therefore worth study further to isolate the pure molecules responsible for anti-inflammatory activity.

Table 5: Percentage inhibition of carrageenan induced paw oedema in rats

Group	Treatment	% inhibition of maximal paw oedema during 6 th hour
Group A	Drug vehicle	0.0±0.99
Group B	Standard (Indomethacin)	64.75±0.02
Group C	Dose 200 mg/kg	42.55±0.02
Group D	Dose 400 mg/ kg	56.86±0.02

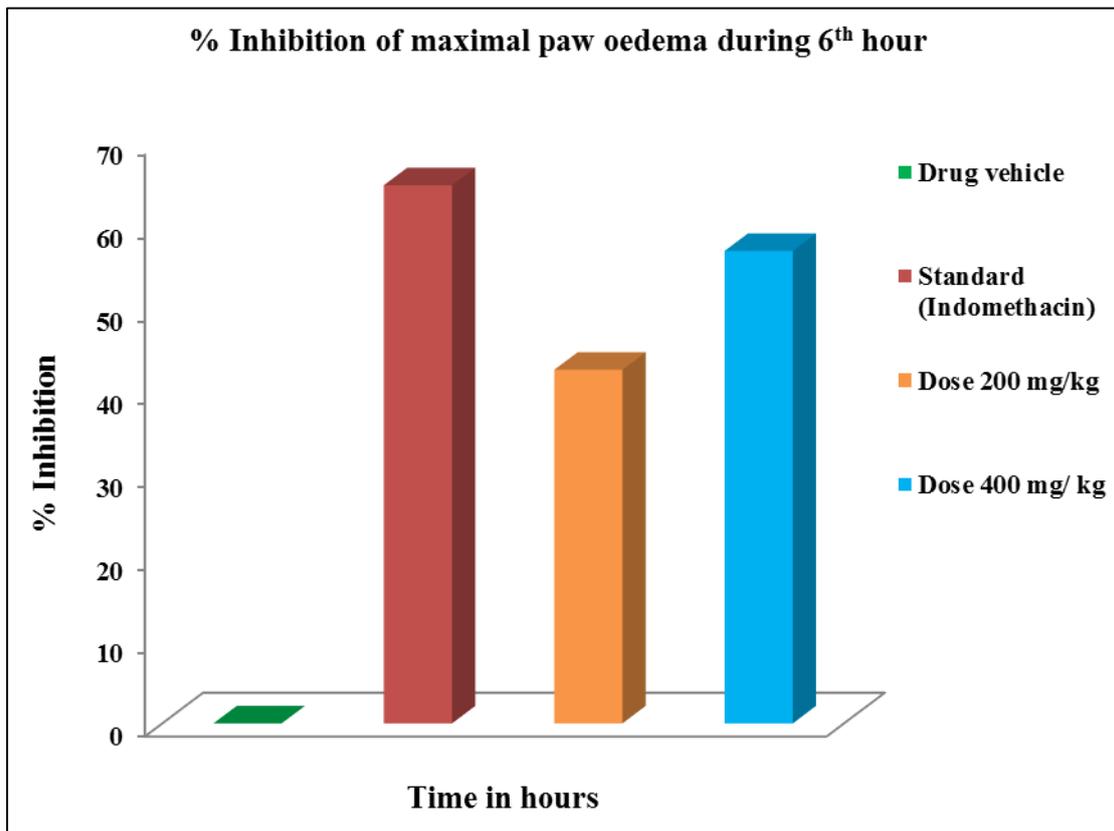


Fig 4: Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with the methanolic extract of *Moringa oleifera*

Conclusion and Future Prospects

The results showed that the methanolic extract of *Moringa oleifera* pod has potential anti-inflammatory property. Further studies are planned to explore the phytochemical profile using HPLC/HPTLC. The bioactivity studies on different fractions of pod extract have to be performed to identify the probable active compound (s). Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind.

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Conflict Of Interest

Author declares that there is no conflict of interest to disclose.

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