IJPSR (2017), Volume 8, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 December, 2016; received in revised form, 10 February, 2017; accepted, 17 February, 2017; published 01 July, 2017

RELATIVE EXPLORATION OF *IN-VITRO* ANTI- INFLAMMATORY ACTIVITIES OF DIFFERENT *ARTOCARPUS* SEED EXTRACTS

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Keywords:

Artocarpus seed oil, Membrane stabilization assay, Red blood cells

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ABSTRACT: The study was undertaken to investigate and compare *in vitro* anti inflammatory activities of five varieties of hexane and methanol seed extracts each, specifically *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus hirsitus, Artocarpus inciscus* and *Artocarpus integer* of *Artocarpus* species belonging to the family Moraceae by means of most prominent membrane stability assay. Results revealed that, the percentage of membrane stability exhibited by the test samples were concentration dependent and compared constructively with that of standard Diclofenac. Amongst two extracts methanolic seed extracts had highest percentage of inhibition and in both extracts Artocarpus integer had uppermost membrane stabilization capacity. The statistical analysis with student 't' test showed significant difference for tested factors in between hexane and methanolic seed extract groups. The study disclosed that *Artocarpus* seed extracts could be seen as a potential natural source of membrane stabilizer and was capable of providing an alternative remedy for the management and source of membrane stabilizer and further studies were suggested to isolate the active principles responsible for the bustle.

INTRODUCTION: Inflammatory diseases are currently treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) [1]. Unfortunately, both of these widely prescribed drug classes have significant negative side effects. Natural product based anti-inflammatory agents with a transcriptional mode of action, good efficacy and lower risk of side effects offer promising treatment and prevention of inflammation related conditions [2, 3]. Tissue degeneration is mainly mediated by reactive oxygen and nitrogen species and proteases produce from infiltrated inflammatory cells [4, 5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(7).2975-81

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (7). 2975-81

The vitality of cells depends upon the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin [6, 7]. An injury to RBC membrane will further render the cell, more susceptible to secondary damage through free radical induced lipid peroxidation [6, 7]. Compounds with membrane stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators [8]. Essential oils have ability to scavenge free radicals and anti-inflammatory activities. For example, chamomile essential oil has been used for centuries as an anti-inflammatory and also for alleviating the symptoms associated with eczema, dermatitis and other pronounced irritation [9]. However, there are other examples of essential oils (eucalyptus, rosemary, lavender, millefolia) along with other

plants (pine, clove and myrrh) that have been used as mixed formulations as anti-inflammatory agents [10]. Carrageenan-induced mouse paw oedema is frequently used to determine the anti-inflammatory activity of diverse bioactive compounds such as plant extracts and essential oils [11]. The anti-inflammatory activity of essential oils may be attributed not only to their antioxidant activities but also to their interactions with signaling cascades involving cytokines and regulatory transcription factors and on the expression of pro-inflammatory genes [12]. A large number of essential oils contain various bioactive compounds exhibiting health beneficial properties, anti-oxidative, antimicrobial and anti-inflammatory effects and their preventive and therapeutic use increases. Numerous natural products have been already tested in various animal models for the development of new anti-inflammatory therapeutics [13]. Thus the key objective of this current cram was to resolve and compare *in vitro* anti inflammatory activities of five varieties of hexane and methanol seed extracts each, explicitly *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus hirsitus, Artocarpus inciscus and Artocarpus integer* of *Artocarpus* species belonging to the family Moraceae.

MATERIALS AND METHODS:

Chemicals: Chemicals and reagents used in the study were purchased from Merck. All additional chemicals used were analytical grade. Altogether the experiments were performed at room temperature unless otherwise stated.

Collection of seeds: Five varieties of jackfruit seeds were collected from Visakhapatnam nearby areas including Simhachalam and Kaviti. The five varieties are *Artocarpus heterophyllus (A. heterophyllus), Artocarpus integrifolia (A. integrifolia), Artocarpus hirsitus (A. hirsitus), Artocarpus inciscus (A. inciscus) and Artocarpus integer (A. integer)*. The fruits were cut and the seeds removed from the perianth of fruits. The seeds were then sliced with knife and dried. The dried seeds were grinded to fine powder.

Extraction of oil by Soxhalation: The *Artocarpus heterophyllus, Artocarpus integrifolia, Artocarpus inscicus, Artocarpus hirsitus* and *Artocarpus integer* seed oils were extracted using soxhlet extraction method with analytical grade hexane and methanol as refluxing solvents. At the completion of the extraction process, the oils were recovered from the mixture by distillation and stored in dessicator until required to exploit [14].

Membrane stabilization Assay: The human red blood cell (HRBC) membrane stabilization method has been used to study the anti-inflammatory activity [15]. Blood was collected from the healthy volunteers and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % sodium chloride in distilled water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85 %, pH 7.2) and a suspension was made with isosaline (10 % v/v).

The assay mixture contained 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36 %), 0.5 ml of HRBC suspension and 1 ml of various concentrations of the test seed extracts. Diclofenac sodium was used as reference drug. In the control solution instead of hyposaline, 2 ml of distilled water was added. The mixtures were incubated at 37 ^{0}C for 30 min and centrifuged. The absorbance of the supernatant solution was read at 560 nm. The percentage of haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula.

$$\text{Percentage of membrane stabilization} = 100 - \frac{\text{OD of drug treated sample}}{\text{OD of control}} \times 100$$

Statistical analysis: The results of *in vitro* studies were given as Mean±Standard deviation (SD) of three replicates and analysed with Student's t-test (Paired Samples Test) to know the significance between verified parameters among hexane and methanolic seed extracts. 'P' value less than 0.05 was considered as significant difference in the analysis. All the statistical analysis was resolved using SPSS software.

RESULTS AND DISCUSSION: Stabilization of the RBCs membrane was studied to establish the mechanism of anti-inflammatory action of five varieties of hexane and methanol *Artocarpus* seed extracts. The seed extracts were effective in inhibiting the hypotonic solution induced

haemolysis at different concentrations. These extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents include bactericidal enzymes and proteases, which upon extracellular release cause further tissue inflammation and damage [16]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation [7]. Tested seed extracts repressed the haemolysis of RBCs to varying degree as displayed in **Table 1** along with associated statistical counters.

All five verities of hexane seed extracts showed high RBC membrane stabilization, in another words percentage of inhibition. The outcomes of the analysis mentioned below were at 50 mg/ml. Maximum percentage of inhibition was experimental in *Artocarpus integer* with 23.7 ±0.32, next to this *Artocarpus heterophyllus* showed 22±0.3. Sensible tricks were practical in *Artocarpus hirsitus* and *Artocarpus inscicus* through 20.8±0.26 each. Minimum activity was perceived in *Artocarpus integrifolia* via 20.6±0.4. Subsequently, with methanolic seed extracts, uppermost action was perceived in *Artocarpus integer* with 24.1±0.36, judicious activities were observed in *Artocarpus heterophyllus* with 23.8±0.15 and *Artocarpus hirsitus* by 23.2±0.4. Lastly minimum activities were detected with *Artocarpus integrifolia* and in *Artocarpus inscicus* through 22.8±0.25 and 22.7±0.2 proceedingly. Standard drug Diclofenac Sodium showed maximum membrane stabilization with 41±0.26 at 1mg/ml. Among two extracts methanolic seed extracts had highest percentage of inhibition and in addition *Artocarpus integer* had uppermost membrane stabilization. The percentage of inhibition for both seed extracts were less than that of the standard may be due to hydrophobicity. The statistical analysis of *in vitro* (n=3) studies of *Artocarpus* seed extracts with student 't' test showed significant difference, except few, for tested factors in between hexane and methanolic seed extracts groups.

Earlier studies revealed that the ethanolic extract of *Operculina turpethum* has showed significant activity at various concentrations and its effect was compared with the standard drug Diclofenac [17]. Gambhire *et al.,* (2009) [18] reported that methanol extract of *Murraya koenigi* leaves produces significant anti-inflammatory activities in dose dependent manner in membrane stabilization. Umapathy *et al.,* (2010) [19] confirmed that aqueous extract of *Albuca setosa* possess membrane stabilization properties. Habibur Rahman *et al.,* (2012) [20] accounted the erythrocyte membrane stabilization effect by *Eucalyptus globulus* oil. Ravi kiran and Raghava Rao (2014) [13] reported membrane stabilization assets of *Ceiba pentandra* seed oil. It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro*, while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules [21, 22]. Akinwunmi and Oyedapo (2015) [23] demonstrated that, flavonoid rich fraction of *Monodora myristica* had momentous *in vitro* anti-inflammatory potentials by stabilizing red blood cell membrane. The test samples therefore could be regarded as a natural source of membrane stabilizer and was capable of providing an alternative remedy for the management and source of membrane stabilizer.

TABLE: 1. *IN VITRO* ANTI-INFLAMMATORY ACTIVITIES (PERCENTAGE OF INHIBITION) OF *ARTOCARPUS* SEED EXTRACTS BY HRBC METHOD

Seed Varieties	Hexane Extract 25 mg/ml	Methanolic Extract 25 mg/ml	Hexane Extract 50 mg/ml	Methanolic Extract 50 mg/ml	Hexane Extract 100 mg/ml	Methanolic Extract 100 mg/ml	1mg/ml
A. heterophyllus	13.6±0.36	14.2±0.38	22±0.3	23.8±0.15	16.6±0.31	18.7±0.26	
A.integrifolia	14±0.25	14.5±0.42	20.6±0.4	22.8±0.25	16.8±0.26	18.1±0.21	
A.hircitus	12.5±0.35	13.6±0.21	20.8±0.26	23.2±0.4	17.2±0.2	18.5±0.35	
A.inciscus	12.6±0.42	14.2±0.31	20.8±0.25	22.7±0.2	16.9±0.31	17.8±0.21	
A.integer	14.1±0.44	14.8±0.23	23.7±0.32	24.1±0.36	17.9±0.15	18.9±0.25	
Standard (Diclofenac Sodium)							41±0.26

Paired Samples Test

***A. heterophyllus* 25 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-.56667	.41633	.24037	-1.60090	.46756	-2.357	2	.142

***A. heterophyllus* 50 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-1.76667	.37859	.21858	-2.70715	-.82619	-8.082	2	.015

***A. heterophyllus* 100 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-2.06667	.40415	.23333	-3.07062	-1.06271	-8.857	2	.013

Paired Samples Test

***A. integrifolia* 25 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-.43333	.63509	.36667	-2.01097	1.14431	-1.182	2	.359

***A. integrifolia* 50 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-2.13333	.15275	.08819	-2.51279	-1.75388	-24.190	2	.002

***A. integrifolia* 100 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-1.26667	.32146	.18559	-2.06521	-.46813	-6.825	2	.021

Paired Samples Test

***A. hircitus* 25 mg/ml**	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-1.10000	.43589	.25166	-2.18281	-.01719	-4.371	2	.049

***A. hircitus* 50 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-2.36667	.66583	.38442	4.02069	-.71265	-6.156	2	.025

***A. hircitus* 100 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-1.33333	.28868	.16667	-2.05044	-.61622	-8.000	2	.015

Paired Samples Test

***A. inciscus* 25 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-1.60000	.69282	.40000	-3.32106	.12106	-4.000	2	.057

***A. inciscus* 50 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-1.93333	.25166	.14530	-2.55849	-1.30817	-13.306	2	.006

***A. inciscus* 100 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-.90000	.50000	.28868	-2.14207	.34207	-3.118	2	.089

Paired Samples Test

***A. integer* 25 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-.66667	.66583	.38442	-2.32069	.98735	-1.734	2	.225

***A. integer* 50 mg/ml**	**Paired Differences**							
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
				Lower	**Upper**			
Hexane seed oil - Methanol seed oil	-.43333	.30551	.17638	-1.19225	.32558	-2.457	2	.133

A. integer 100 mg/ml	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Hexane seed oil - Methanol seed oil	-1.06667	.11547	.06667	-1.35351	-.77982	-16.000	2	.004

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Diclofenac Sodium	268.408	2	.000	41.00000	40.3428	41.6572

CONCLUSION: The study revealed that all varieties of *Artocarpus* seed extracts could be seen as potential natural sources of membrane stabilizers and were capable of providing an alternative remedy for the management and source of membrane preservatives. On the other hand, the most common used non steroidal anti-inflammatory drugs can cause gastric erosions, exacerbate asthma, and cause kidney and liver damages. Therefore, natural products have attracted interest as potential therapeutic agents for handling inflammation.

ACKNOWLEDGEMENT: There is no acknowledgement.

CONFLICT OF INTEREST: No conflict of interest.

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How to cite this article:
Sirisha N, Archana B, Brahmaji S and Rao TR: Relative exploration of *in-vitro* anti- inflammatory activities of different *Artocarpu*s seed extracts. Int J Pharm Sci Res 2017: 8(7): 2975-81.doi: 10.13040/IJPSR.0975-8232.8(7). 2975-81.