EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF VYOSHADI GUGGULU AND NAGARADI QWATHA

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ABSTRACT

The present study was undertaken to evaluate the Anti-Inflammatory activity of Vyoshadi Guggulu (VG) and Nagaradi Qwatha (NQ) in albino rats. The Anti-Inflammatory action in experimental models was studied by Formaldehyde Induced Pedal Oedema Method. Forty eight adult Wistar rats were divided into eight groups of six each and maintained under uncontrolled conditions. Group I was taken as control and Group II treated with the standard drug Ibuprofen 100mg /kg. Groups III and IV were administered with Vyoshadi Guggulu 270 mg/kg and Nagaradi Qwatha 8.1ml/kg respectively. Groups V was administered with combination of Vyoshadi Guggulu 270 mg/kg and Nagaradi Qwatha 8.1ml/kg. Groups VI and VII were administered with 540mg /kg of Vyoshadi Guggulu and 16.2ml/kg of Nagaradi Qwatha respectively. Group VIII was administered with combination of both with Vyoshadi Guggulu at dose of 540mg /kg and Nagaradi Qwatha 16.2ml/kg. It is observed that the both drugs individually and in combination in single doses shows considerable Anti-Inflammatory action in animal models The reduction in the inflammation in injected paw of the experimental animals significant (P<0.001) and indicates that the trial drugs contain active anti-inflammatory principles acting both centrally and peripherally

Keywords: *Vyoshadi Guggulu, Nagaradi Qwatha, Formaldehyde Induced Pedal Oedema Method, Anti-Inflammatory Activity*

INTRODUCTION

Inflammation is a universal host defense process involving a complex network of cell-cell, cell-mediator and tissue interactions. It occurs in response to a variety of harmful stimuli viz. physical, chemical, traumatic, antigen challenge, infectious agents and ionizing radiations etc. Apart from *exogenous factors* (physical, chemical, mechanical, nutritional and biological etc.) *endogenous factors* (immunological reactions, neurological and genetic disorders) also contribute to inflammatory response.

Inflammation most commonly occurs when microbial invasion or tissue injury overcomes the body's non-specific defense mechanisms. Subsequent to infection, immune system gets activated, communication and coordination occurs between different classes as well as actions of immune cell to produce inflammation is tightly regulated by the body and is the starting point of the body's self-repair process initiated by body's defense system to thwart pathologic assaults but occasionally it runs amok, leading to physiological chaos and death[1].

The inflammation could be acute, sub-acute or chronic in nature. The acute inflammation is short lasting whereas chronic inflammation may persists for weeks, months or years. There are 3 principle components of an inflammatory response a. increased blood flow, b. increased capillary permeability and c. Increased migration of leucocytes into the affected area. Active hyperaemia, exudation and accumulation of neutrophils and macrophages are observed at the inflammatory site in an inflammatory response. The cardinal features of inflammation are Erythema *(rubor)*,Swelling *(tumor)*,Heat *(calor)*,Pain *(dolor)* and *Loss* of function *(function laesa)*[2]

The inflammatory diseases cover a broad spectrum of conditions including auto immune diseases (e.g. Rheumatoid Arthritis), Osteo-Arthritis, Inflammatory Bowel Diseases, Multiple Sclerosis, Asthma, Chronic Obstructive Pulmonary Disease, Allergic Rhinitis, Infectious Diseases, various types of cancers and cardiovascular diseases etc. Although inflammation is the unifying factor, treatment approach required for each of the inflammatory disease is often unique. Each patient population has distinct therapeutic needs that are inadequately served by current prevent and treatment strategies.

Research in the last few decades has shown the inflammation is regulated by a large number of pro-and anti-inflammatory mediators such as histamine, prostaglandins (PGE_2 and prostacyclins), leukotrienes (LTB_4), serotonin, bradykinin, cytokines (IL-1, IL6, IL-8, IL-11, TNF-α), reactive oxygen species, growth factors, lysosomal contents of neutrophils, adepokines (leptin, adiponectin, resistin), etc.[3] The extent of involvement of these mediators varies depending on the nature of inflammatory dis-

eases. In the recent years there has been increased focus on the component III of inflammatory response, the leucocyte migration[4]. Adhesive interactions involved in leucocyte extravasation are regulated by cytokines and are crucial for leucocyte localization at sites of inflammation chemokines. The first steps of leucocyte recruitment include rolling on the vessel wall mediated by selectins and glycoproteins bearing the sailyl lewis moiety[5].

Adhesion assays evaluate the binding the thrombin activated human platelets to neutrophils. These adhesion molecules appear to play a major role in the development persistence of inflammatory diseases by promoting infiltration of inflammatory cells (neutrophils) in to the site of inflammation. Adhesion between the activated platelets and neutrophils is mediated by P-selectin. Cytokines have been shown to play central roles in inflammatory diseases such as Rheumatoid arthritis, Psoriasis and septic Shock, and inhibition of their action or activation is a proven or promising approach to modulation of this diseases.[6]

Until a few years ago, inflammatory disorders were treated primarily with relatively non-selective anti – inflammatory drugs such as cortico-steroids and various non-steroidal anti-inflammatory drugs, however, nowadays specific mediator antagonists alone or in combination and gene therapy are also being tried. Efforts to develop new safer and more effective anti-inflammatory drugs are based on the improved understanding of the role of key mediators identified as the key culprits in this malady.

Present day anti-inflammatory drug discovery is based on preliminary in-vitro observations in a number of standard anti-inflammatory assays, in which the test com-

pound produces unusual potent antagonism of inflammatory such as arachodonic acid pathways and some cytokine cascades. The effective candidate drug in in-vitro tests is later tested in whole animal models of acute, sub-acute and chronic inflammation.

In-vivo methods predict the therapeutic effectiveness of a test, which has shown potential in-vitro. It is recommended to concomitantly use several in-vitro methods, which together can mimic a broad spectrum of acute, sub-acute and chronic inflammatory events such as redness, heat, plasma exudation, oedema, pain, leucocyte migration, tissue proliferation and partial necrosis. Whole animal like guinea pig, rat, mouse, rabbits or dogs may be used for the purpose.

The prominent *In-vivo models* of inflammation are formaldehyde or carrageenan induced oedema, pleural exudation and cotton pellet implantation. The methods of early pleural exudation and formaldehyde or carrageenan induced oedema assess the efficacy of the compound against transudative and exudative phases of inflammatory reaction, respectively. On the other, the methods of induced arthritis and cotton pellet implantation are used to study the efficacy of drugs against pro-proliferative phases of inflammations.[7]

There are many studies available on single drugs about their pharmacological effects. *Vyoshadi Guggulu* and *Nagaradi Qwatha* are the formulations with minimal in number easily available herbal ingredients, with no information regarding their pharmacological activity, attracts pharmacological importance. These trial drugs are of different forms with difference in their proportions, claimed to be effective in the treatment of *Amavata*. In this study, it is planned to evaluate the Anti-inflammatory activity of both *Vyoshadi Guggulu* and **Nagaradi Qwatha** by *Formaldehyde Induced Pedal Oedema* in animal models.

MATERIALS AND METHODS

Drugs:

- *Vyoshadi Guggulu (VG)*[8] a poly Herbal formulation containing *Trikatu*, *Triphala*, *Trimada* and *Guggulu* as the main ingredient with a quantity equal to the quantity of all other ingredients **(Table 1)**, is explained by *Vagbhata* as a *shamanaushadhi* in the treatment of *Amavata* as these ingredients possesses both *Amapachaka* and *Vatanulomana* actions. The drug was specially prepared at *Rajashree Ayurveda pharmacy – Udupi* for this study.

- *Nagaradi Qwatha (NQ)*[9] is an herbal formulation containing the extracts of the medicinal plants viz. *Nagara*, *Harithaki* and *Amritha* **(Table 2)**. These constituents are possessing the *deepana, pachana* and *vatanulomana* qualities. The trial drug is prepared in *S.D.M.Ayurveda Pharmacy – Udupi.*

Determination of drug dose [10]

The rat dose was calculated from the human dose of 3 g/day with the *Conversion Factor 0.018*. Hence the calculated dose of *Vyoshadi Guggulu* for the rat of 200 g body weight is 54 mg (i.e. 270mg /kg body weight). The dosage form of the pill was prepared as a suspension in distilled water and used for all the experimental purposes

The rat dose was calculated from the human dose of 90 ml/ day with the *Conversion Factor 0.018*. Hence dose of *Nagaradi Qwatha* for the rat of 200 g body weight is calculated as 1.6 ml (i.e.8.1ml/kg body weight) and the liquid form of the Qwatha is used for this experimental purpose.

Selection of animals, caring and handling:

A total of 48 healthy Wistar rats (150–200 g), of either sex, bred locally in the animal house of *S.D.M.Centre for Research in Ayurveda and Allied Sciences, Udupi,* were selected for the study. They were housed in normal uncontrolled conditions individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment. All animals were fed with sterile commercial pelleted rat feed supplied by *VRK Nutritional Solutions (Sangli* - India) and had free access to water. Animals were kept under fasting for overnight and weighed before the experiment. The study was commenced after obtaining approval of Institutional Animal Ethics Committee *(IAEC approval letter No. SDMCAU/ACA–49/ EC – A / 10-11 Dt. 09*[th] *July 2010*.)

Study design:

The rats were randomly allocated into eight groups of six rats each for testing Anti-inflammatory activity of both *Vyoshadi Guggulu* and **Nagaradi Qwatha**by *Formaldehyde Induced Pedal Oedema.* The trial / reference standard drugs were administered orally with help of intra gastric tube for five days and initial left hind paw volumes were recorded with the help of Plethysmograph by immersing the left hind paw up to the tibio-tarsal articulation.

- *Group* **I** - served as ***Negative Control*** and received normal tap water.
- *Group* **II** - administered **Ibuprofen** (*Cipla ltd, Mumbai)* 100mg/kg as **standard drug**
- *Group* **III** - administered with **Vyoshadi Guggulu 270mg/kg**
- *Group* **IV** - received **Nagaradi Qwatha 8.1 ml/kg**
- *Group* **V** - administered with the combination of **Vyoshadi Guggulu 270mg/kg & Nagaradi Qwatha 8.1 ml /kg.**
- *Group* **VI** - administered with **Vyoshadi Guggulu 540mg/kg**
- *Group* **VII** - administered with **Nagaradi Qwatha 16.2 ml/kg**
- *Group* **VIII** - administered with the combination of **Vyoshadi Guggulu 540 mg /kg & Nagaradi Qwatha** at **16.2 ml /kg**

Instruments & Chemicals:

1. Monopan Balance
2. Mortar and pestle
3. Weighing Scale
4. 2% Formaldehyde solution (*Lobort Pre-Chempvt. Ltd-Surat*)
5. Digital Plethysmometer (*Orchid Scientifics-Nashik*)
6. Digital Conductivity tester- DIST-4 (*Hanna Instruments*)
7. Intra-gastric tube

Procedure [11]

The procedure of Brownlee (1950) as described by Nataraj (1985) was employed to screen the anti-inflammatory activity of *Vyoshadi Guggulu* and *Nagaradi Qwatha*against formaldehyde induced hind paw oedema in rats. On the 5th day, after one hour of administration of trial and standard drugs, the animals of different groups were individually injected subcutaneously with 0.05 ml of 2% formaldehyde solution into the sub plantar area of the hind paw using insulin syringe. Paw volumes were again measured at + 45 minutes, + 90 minutes, + 180 minutes and + 24 hours after formaldehyde injection, with the help of Digital Plethysmometer.

Evaluation

The mean paw oedema for each treated group was determined and compared with that obtained for the control group. *Inhibition Percentage of inflammation* (**I%**), was derived, using the formula

$$I = \frac{\{N_0 - N_1\}}{N_0} \times 100$$

Where N_1 = the mean paw size for each group after formaldehyde treatment, and N_0 = the mean paw size obtained for each group before formaldehyde injection.

Statistical Analysis

The results were analyzed for statistical significance using **one way ANOVA**. A P-value **<0.050** was considered significant. Computer statistical package **SIGMASTAT (Version 3.5)** was used for analysis.

RESULTS

In the formaldehyde induced paw oedema method, both *Vyoshadi Guggulu* (VG-SD) and *Nagaradi Qwatha* (NQ-SD) 270 mg/kg and 8.1ml/kg and their combination (VG&NQ – SD) *270 mg/kg and 8.1ml/kg* caused significant increase in the anti-inflammatory activity. (**Table 1**)The percentage of increase in the anti-inflammatory activity was dose-dependent and differed significantly among the groups of rats (*P<0.001*) receiving different dose levels of Vyoshadi Guggulu and Nagaradi Qwatha at different time intervals.(**Fig. 1**).

The percentage of increase in the inhibition of inflammation caused by the Vyoshadi Guggulu in single doses (*270mg/kg*) was significantly detectable at 45 minutes(71%) and at 90 minutes (78%). Nagaradi Qwatha (*8.1ml/kg*) also showed a significant increase in the inhibition of inflammation with 59% and 58% at early and late intervals of the study. The combination of Vyoshadi Guggulu and Nagaradi Qwatha in single doses (*270mg/kg + 8.1ml/kg*) showed a significant change in the percentage (77%) of inhibition of inflammation.

The Double Dose of Vyoshadi Guggulu (540 mg /kg) has showed highly significant increase in the anti-inflammatory activity (78%) at 45 minutes, (76%) at 90 minutes, (88%) at180 minutes and (81%) at 24 hours. Nagaradi Qwatha in double dose (16.2 ml / kg) also showed highly significant increase of anti-inflammatory activity at all intervals of the study i.e. (68%) at 45 minutes, (59%) at 90 minutes, (53%) at180 minutes and (80%) at 24 hours.

Standard drug (Ibuprofen100mg/kg) showed a significant change on comparison with trial drugs in their single doses but not greater than in their double doses. In all intervals of the study, there is significant (P<0.001) increase in anti-inflammatory activity by Vyoshadi Guggulu and Nagaradi Qwatha when compared to the control group.

The combination of Vyoshadi Guggulu and Nagaradi Qwatha in single dose (270 mg/kg and 8.1ml/kg) and at double dose (540 mg /kg &16.2 ml /kg) respectively showed their peak of activity in late phase by 77% and 86%.

DISCUSSION AND CONCLUSION

Numerous experimental methods for evaluation of anti-inflammatory drugs have been developed over the last few years. These methods help not only understanding the pathogeneses of inflammation but also explore the anti-inflammatory mechanisms as well as to identify the suitability of drugs for specific inflammatory diseases.

Formaldehyde induced paw oedema test, is planned to evaluate the anti-inflammatory activity Vyoshadi Guggulu, Nagaradi Qwatha with individual and combined forms with different doses. The Formaldehyde test

is a very useful method for not only assessing ant nociceptive drugs but also helping in the elucidation of the action mechanism. This test is sensitive for various classes of analgesic drugs have two distinct phases, reflecting different types of pain. The early phase (initial pain) reflects a direct effect of formaldehyde on nociceptors (neurogenic pain) whereas the late phase reflects tissue injury or inflammatory pain [12]

Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin.[13] The mediators, including histamine, 5-HT, the kinins and their complements, have become the recent focus of attention as the metabolites of arachodonic acid (AA). Alone or in appropriate combination, AA products of COX pathway are capable of producing the characteristic signs of inflammation: vasodilatation, hyperemia, pain, oedema, and cellular filtration. The COX products, particularly prostaglandin E2 (PGE2), contribute to increased blood flow through a vasodilatation action, but the lipoxygenase (LOX) pathway is necessary for vascular leakage and oedema consequently on cellular infiltration.[14,15]

The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The later phase of oedema is due to the release of prostaglandins, protease, and lysosome[16,17]This leads to a dilation of the arterioles and veinules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravasated, and oedema forms[18]. The centrally acting drugs such as narcotics inhibits both phases equally, while peripherally acting drugs only inhibits the second phase It is also well known that the formaldehyde

model may involve sensorial C-fibers in early phase and a combined process generated by peripheral inflammatory tissue and functional changes in the dorsal horn in late phase[19]

The reduction in the inflammation in injected paw of the experimental animals significant (P<0.001) and indicates that the trial drugs contain active anti-inflammatory principles acting both centrally and peripherally This probably indicates that **Vyoshadi Guggulu** and **Nagaradi Qwatha** exerts its anti-inflammatory activity through both peripheral inhibitory actions (inflammatory pain) and central activity relates to antagonistic action of the nociceptors (neurogenic pain)

On the basis of these findings, it may be inferred that **Vyoshadi Guggulu** and **Nagaradi Qwatha** has anti-inflammatory activity.At present, there are no reports on investigation to identify the active components present in **Vyoshadi Guggulu** and **Nagaradi Qwatha**; further investigations are anticipated to identify the active components the trial drugs.

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Table 1 Ingredients of *Vyoshadi Guggulu*

Sl.No	Name	Botanical Name	Part Used	Quantity
1.	*Shunti*	Zingiber officinaleRosc.	Rhizome	**1 Part**
2.	*Maricha*	Piper nigrum Linn.	Fruit	**1 Part**
3.	*Pippali*	Piper longum Linn.	Fruit	**1 Part**
4.	*Haritaki*	Terminalia chebula Retz.	Fruit pulp	**1 Part**
5.	*Vibhitaka*	Terminalia belericaRoxb.	Fruit pulp	**1 Part**
6.	*Amalaki*	Emblica officinalisGaertn.	Fruit pulp	**1 Part**
7.	*Chitraka*	Plumbago zeylanica Linn.	Root	**1 Part**
8.	*Musta*	Cyperus rotundus Linn.	Rhizome	**1 Part**
9.	*Vidanga*	Embelia ribesBurm.	Fruit	**1 Part**
10.	**Guggulu**	**Commiphora mukul Engl.**	**Gum oleo-resin**	**9 Parts**

Table 2 Ingredients of Nagaradi Qwatha

Sl.No	Name	Botanical name & Family	Part Used	Quantity
1.	*Nagara*	Zingiber officinaleRosc.	Rhizome	**2 parts**
2.	*Haritaki*	Terminalia chebula Retz.	Fruit pulp	**4 parts**
3.	*Amrita*	**Tenospora cardifolia (willd.) Miers**	**Stem**	**6 parts**

Table3. Anti-Inflammatory activity of *Vyoshadi Guggulu* and *Nagaradi Qwatha* by**Formaldehyde Induced Oedema** in Albino Rats

GROUP , DRUG & DOSAGE	Formaldehyde induced Paw Oedema (Mean ± SEM)				
	Basal	*45 minutes*	*90 minutes*	*180 minutes*	*24 Hours*
Control	0.727 ± 0.040	-	-	-	-
Standard Ibuprofen 100mg /kg	0.703 ± 0.019	1.093±0.034	1.157± 0.045	1.050±0.030	1.140±0.019
VG-SD 270mg /kg	0.725 ±0.034	1.048±0.040	0.968±0.088	0.865±0.066	1.103±0.10**
NQ–SD 8.1ml/kg	0.767 ± 0.044	1.048±0.068*	0.975±0.111	0.840±0.040	1.037±0.075*
VG&NQ-SD 270mg/kg + 8.1ml/kg	0.703 ± 0.026	0.963±0.059	0.858±0.051	0.768±0.037	1.033±0.116*
VG-DD 540mg /kg	0.713 ± 0.023	1.057±0.035**	1.035±0.044**	1.130±0.080**	1.082±0.045**
NQ–DD 16.2 ml/kg	0.708 ± 0.041	0.992±0.049**	0.915±0.023**	0.877±0.026*	1.068±0.026**
VG&NQ-DD 540mg/kg+16.2ml/kg	0.693 ± 0.022	1.030±0.032**	0.973±0.043**	0.932±0.032**	1.075±0.273**

Result expressed as **mean ± SEM** from six observations; ***P<0.050; ** P<0.010***
VG=*Vyoshadi Guggulu*; **NQ**=*Nagaradi Qwatha*; **SD**= *Single Dose*; **DD**= *Double Dose*
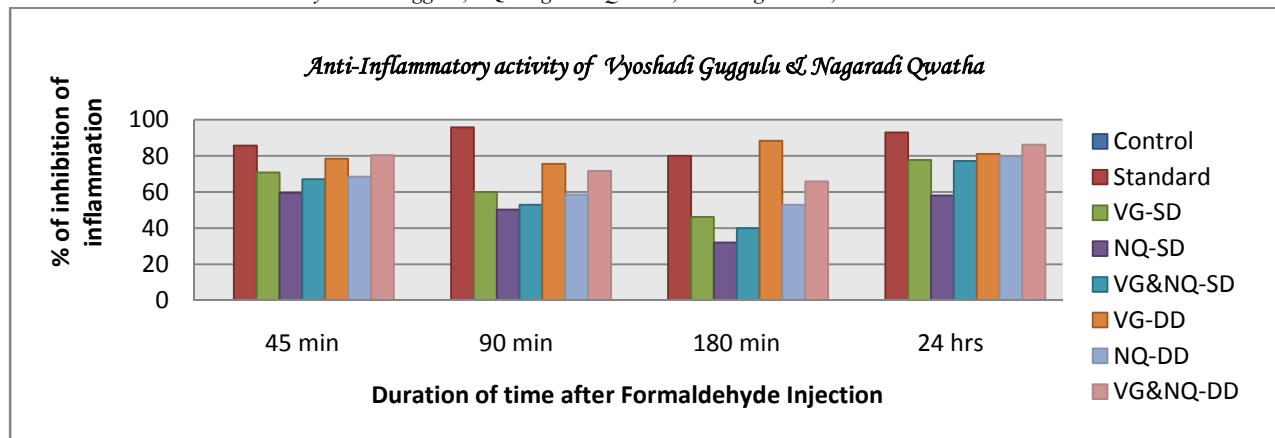

Fig. 1   Anti-Inflammatory activity of ***Vyoshadi Guggulu*** and ***Nagaradi Qwatha****by*
Formaldehyde Induced Oedema in Albino Rats

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