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COMPARATIVE IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF *GOMUTRA SADHITA TRIPHALA KWATHA* AND *GOMUTRA SADHITA TRIPHALA ARKA*

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ABSTRACT

Inflammation in Ayurveda can be correlated with Shotha. There are formulations in Ayurveda which are indicated in Shotha. Some of the formulations remained untouched. Here one formulation *Gomutra Sadhita Triphala kwatha* in Ayurvedic texts is explained in the Shotha conditions. But due to some lacunas in *Kwatha kalpana* the dosage form was changed into *Arka*. Then both *kalpanas* were taken for the in-vitro anti-inflammatory study. Protein denaturation is documented cause of inflammation. So change in protein denaturation suggests changes in inflammation. If protein denaturation is inhibited it is told that inflammation can also be inhibited. Protein denaturation inhibition was used to assess anti-inflammatory efficacy in vitro. Bovine serum albumin was used in the protein denaturation inhibition study at different concentrations. After the study it was found that both *Kwatha* and *Arka* have anti-inflammatory properties as percentage of inhibition was found to be good at different concentrations. *Gomutra Sadhita Triphala kwatha* was having high percentage of inhibition than the *Gomutra Sadhita Triphala arka*.

Keywoerds: *Kwatha*, *Arka*, Anti-Inflammatory, In-Vitro Study

INTRODUCTION

Inflammation is a defence mechanism that enables the body to protect itself against infection, burn, toxic chemical allergens or any other harmful stimuli. Heat, redness, discomfort, swelling, and disrupted physiological activities are all signs of inflammation in response to injury, sickness, or destruction. It's a complicated process that's usually linked to pain, and it includes things like increased vascular permeability and protein denaturation. Protein denaturation is a well-known cause of inflammation. The confrontation of a protein is solely dependent on weak valence forces. Hence it can be disrupted easily by a variety of physical and chemical agents. The process is known as denaturation. Protein molecules play a crucial role in the functioning of living systems. Most importantly, the majority of proteins have a very specialized role to play. Protein structure is what dictates this specificity and the three-dimensional structure is particularly important. The protein loses its functionality when this unique three-dimensional structure is broken, and it is considered to have experienced denaturation. The interactions that determine the tertiary structure of proteins, such as hydrogen bonding, are not as strong as covalent chemical bonds. Because these interactions are rather weak, they can be disrupted with relatively modest stresses1. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are the most often used drugs to treat inflammatory diseases. The greatest disadvantage in the presently available potent synthetic anti-inflammatory drug lies in their toxicity and reappearance of symptoms after discontinuation.2 Inflammation in Ayurveda is known by different names in different contexts namely, *shotha, shopha*, *utsedha* and *samhata*. While modern medicine regards inflammation as a symptom or rather as the body's natural healing response to wounds. Ayurveda treats the concept of inflammation as symptom of disease, an independent disease and complication of diseases. The natural products today symbolizes safer in contrast to the synthetic drug that are regarded as unsafe to human environment. In Ayurvedic texts there are many *shothahara* (anti-inflammatory) formulations available. One such drug *Gomutra Sadhita Triphala Kwatha* is mentioned in *Bhaishajya ratnavali*.3 But due to the less shelf life i.e.24 hours4, high dose of aministration5 and also due to high administration dose *Kwatha* is not palatable. So, the modification of *Kwatha* is done into *Arka* as it is having less administration dose and more shelf life6.

So, study is planned to prepare *Kwatha* as well as *Arka* and subjected to in-vitro anti-inflammatory activity by Protein Denaturation Inhibition Method.

MATERIALS & METHODS

Collection of Raw Materials:

Haritaki, *bibhitaki* and *Amalaki* were collected from Aimil Pharmaceuticals Pvt. Ltd. Nalagarh (H.P.) The *Gomutra* for the study was collected from Gaushala in Goraj, Waghodia, and Vadodara Gujarat.

Preparation of *Kwatha* and *Arka*:

Kwatha and *Arka* for in-vitro study was prepared in the GMP certified Parul Ayurved Pharmacy. The final products received are taken for the in-vitro study.

Table No 1: Raw Drug Quantity for *Kwatha*

S. No.	Drugs	Latin Name	Part used	Quantity
1.	*Haritaki*	Terminalia chebula Retz.	Dried fruit pericarp	1part
2.	*Bibhitaki*	Terminalia bellerica Roxb.	Dried fruit pericarp	1part
3.	*Amalaki*	Emblica officinalis Gaertn.	Dried fruit	1part
4.	*Gomutra*	Cow's urine	-	16parts

Table No 2: Raw drug Quantity for *Arka*

S. No.	Drugs	Latin Name	Part used	Quantity
1.	*Haritaki*	Terminalia chebula Retz.	Dried fruit pericarp	1part
2.	*Bibhitaki*	Terminalia bellerica Roxb.	Dried fruit pericarp	1part
3.	*Amalaki*	Emblica officinalis Gaertn.	Dried fruit	1part
4.	*Gomutra*	Cow's urine	-	10parts

IN-VITRO PROTEIN DENATURATION INHIBITION METHOD

Compounds that prevent protein denaturation in vitro could be employed as anti-inflammatory drugs. The anti-inflammatory effect of a sample was performed by using serum albumin. The bovine serum albumin assay aims to eliminate the usage of live specimens in the drug development process as much as feasible. When Bovine serum albumin is heated, it undergoes denaturation (heat induced protein denaturation) and express antigens associated with type 3 hypersensitivity reactions, which can be related to the inflammation.

Materials required:

- Bovine serum Albumin
- Hydrochloric acid-1N
- Phosphate buffer
- Distilled Water

Procedure:

Different concentrations of sample (0.78-50µL) were taken directly from the sample and were used for the study. The test control consists of 0.45mL of Bovine serum albumin and 0.05mL of distilled water. The test solutions consist of 0.45mL of Bovine serum albumin and 0.05mL of different concentrations of sample. Product control consists of 0.45mL of distilled water and different concentrations of sample. All the above solutions were

adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37^{o} C for 20minutes and the temperature was increased to 57^{o} C for 3 minutes. After cooling, 2.5ml of phosphate buffer was added to the solutions. The absorbance was measured using UV-VISIBLE Spectrophotometer at 660 nm (Cary 60 UV-visible, Agilent Technologists)

- **TEST SOLUTION(0.5 ML) :** 0.05ml of test solution +0.45 ml of Bovine serum albumin
- **Test Solution1:** 0.05ml of *Gomutra Sadhita Triphala kwatha* and 0.45ml of Bovine serum albumin
- **Test solution2:** 0.05ml of *Gomutra Sadhita Triphala arka* and 0.45ml of Bovine serum albumin
- **TEST CONTROL SOLUTION (0.5ML):** 0.05ml of distilled water +0.45ml of Bovine serum albumin
- **PRODUCT CONTROL SOLUTION (0.5ML):** 0.05ml of test solution and 0.45ml of distilled water.
- **Product control solution1-** 0.05ml of test solution 1 and 0.45ml of distilled water.
- **Product control solution2-**0.05ml of test solution 2 and 0.45ml of distilled water.

CALCULATION

The percentage inhibition of protein denaturation can calculated as

% inhibition= 100- [((optical density of test solution- optical density of product control) ÷ optical density of test control)) ×100]

- The optical density (OD) was measured by spectrophotometry.

Interpretation

The anti-inflammatory efficacy of a drug can be expressed in terms of half maximal inhibition concentration (IC50). It is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganisms) by half. The values are typically expressed as molar concentration. It is commonly used as a measure of antagonist drug potency. According to the FDA, IC50 represents the concentration of a drug that is required for 50% inhibition in-vitro. IC50 can be used to compare the potency of two antagonists. The lower the IC50 value, the stronger the inhibitor is. The IC50 values of the investigational products were calculated using ED50 Plus V1.0 software and were tabulated.

RESULT:

Table No 3: Sample: GTA (OD of test control= 0.1708)

Concentrations (µl/3ml)	OD of test solution	OD of Product control	% of inhibition
0.78	0.1567	0.0212	20.66745
1.56	0.1573	0.0376	29.91803
3.125	0.1579	0.0574	41.15925
6.25	0.1688	0.1208	71.86696
12.5	0.1633	0.1229	76.3466
25	0.1633	0.1266	79.80094
50	0.1563	0.1335	86.65105

Table No 4: Sample: GTK (OD of test control= 0.1708)

Concentrations (µl/3ml)	OD of test solution	OD of Product control	% of inhibition
0.78	0.1582	0.0291	24.41452
1.56	0.1565	0.0453	34.89461
3.125	0.1534	0.0602	45.43326
6.25	0.1544	0.1267	83.78220
12.5	0.1540	0.1271	84.25059
25	0.1476	0.1308	90.16393
50	0.1443	0.1326	93.14988

Table No 5: IC_{50} Value calculated

Investigational Product	IC_{50}
Gomutra Sadhita Triphala Arka	11.81 µl/3ml
Gomutra Sadhita Triphala Kwatha	7.56 µl/3ml

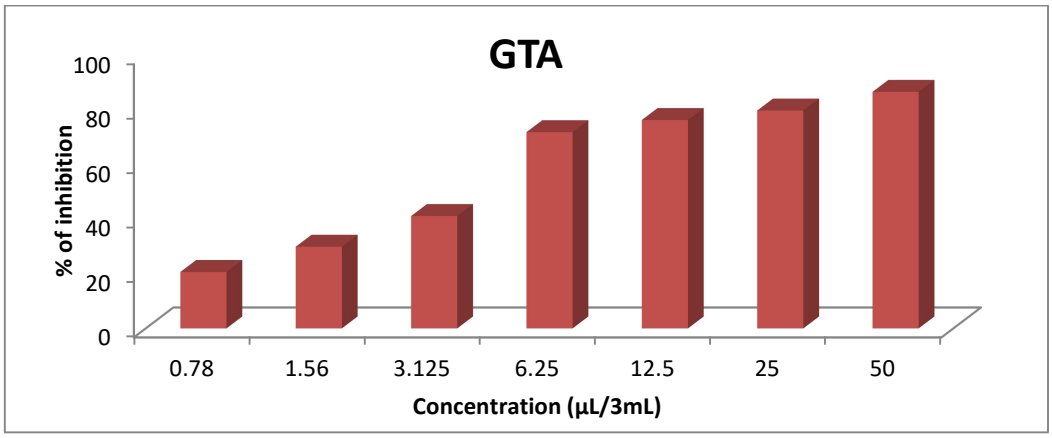

Figure no. 1

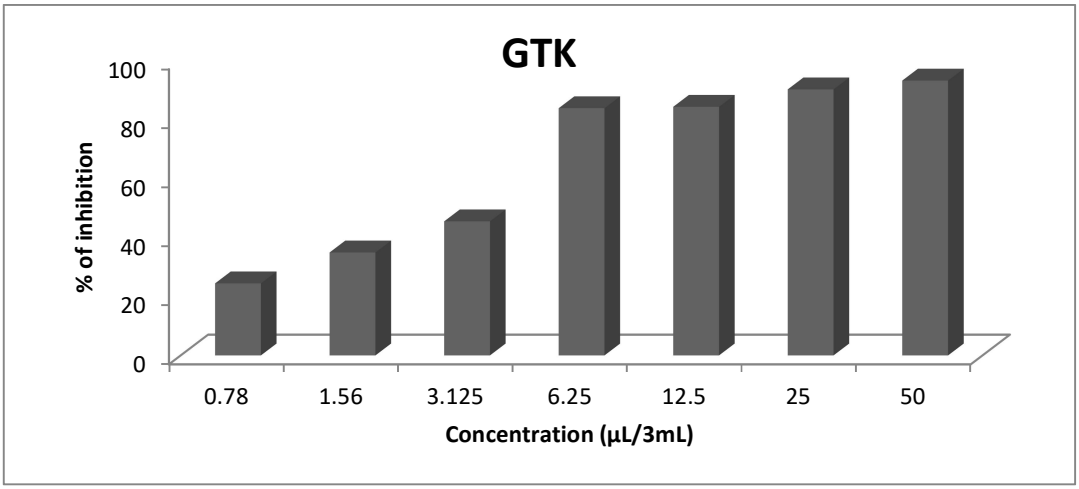

Figure no. 2

DISCUSSION

Denaturation of proteins has an unpredictable mechanism which includes modification in electrostatic hydrogen, hydrophobic and disulphide bonding.[7] Denaturation of protein causes the production of auto antigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation. Hence, by inhibition of protein denaturation inflammatory activity can be inhibited.[8] In the present study, two different formulations i.e. *Kwatha* and *Arka* are prepared from the same drugs and comparative study was done. All the drugs in the formulations are proved to be anti-inflammatory. But to some lacunas in *Kwatha Kalpana* dose modification was done into *Arka*. These drugs are having the volatile principles in them and their individual *Arka* are also mentioned in the texts so the modification was done into *Arka* form. *Gomutra* used in the study was freshly collected and brought to the pharmacy in the morning. This *Gomutra* was strained using the double layered cloth to remove the impurities. Then this filtered *Gomutra* was taken for testing some analytical parameters like pH, refractive index, specific gravity. Next morning *Gomutra* was again collected from the same place and same cow and bought in the pharmacy. Again filtered and run for the same analytical tests. The values of analytical parameters were nearly similar and this *Gomutra* was then taken for the preparation of the *Kwatha* and *Arka*. In *Kwatha Kalpana*, the water is mentioned as Drava dravya (liquid media) but here instead of water *Gomutra* was taken as Drava dravya (liquid media). In *Arka Kalpana*, there are differences in opinion on the amount of water to be taken. So here method as directed in AFI[9] was followed i.e. 10parts of Drava (liquid) to be taken. Here also *Gomutra* was taken as Drava dravya instead of water. Denaturation of proteins is the well documented cause of inflammation. The compounds that inhibit the denaturation of protein may be used as anti-inflammatory agents. The anti-inflammatory effect of drug can be expressed in terms of half maximal inhibition concentration (IC50) by calculating the percentage of inhibition. It is said lower the IC50 value, stronger the inhibitor. From the above calculations it is seen that both the drugs are having the anti-inflammatory properties. *Arka* shows maximum of 86% of inhibition whereas *Kwatha* shows 93% of inhibition. The IC50 value of *Kwatha* is also less than the *Arka*. This may be due to the reason that *Arka* is distillation of drugs so it contains only the volatile principles. But in *Kwatha* apart from volatile principles other active

ingredients may be present in the liquid that may be having the anti-inflammatory properties.

CONCLUSION

From the above study it is concluded that both the drugs are having the anti-inflammatory properties. The IC50 value of *Kwatha* is 7.56 µl/3ml and for *Arka* is 11.81 µl/3ml. as *Kwatha* is having the lower value of inhibition concentration so it is stronger inhibitor than the *Arka*. But *Arka* is also having the good percentage of inhibition and it can also be used in the treatment of inflammation. And further studies can be conducted with the synthetic drugs for comparing their anti-inflammatory activity.

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