INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

# Evaluation of anti inflammatory activity of *Argentum nitricum, Staphysagria and Ignatia amara* in experimental animal model.

**Humera Anser*[1] and Rahila Najam[2].**

[1.]Department of pharmacology.Jinnah University for women. Pakistan.

[2.] Department of pharmacology. University of Karachi. Pakistan.

**ABSTRACT**

Inflammation is a transient natural reply of the tissues to detrimental spur such as any damage, exogenous and endogenous antigens, proposed to clean up or remove the stimulus and healing the injured tissue eventually and results in rejuvenation and recurring to homeostasis. Many drugs are used to treat all types of inflammatory conditions for example Non steroidal anti-inflammatory drugs (NSAIDs), steroids, and immunosuppressant drugs but their uses are associated with serious harmful effects. Due to population preference to alternative treatments there is an increase desire to do preclinical studies for the evaluation of pharmacological action or their clinical efficacy. Various homeopathic remedies are used to relief inflammation, in this study three remedies *Argentum nitricum, Ignatia Amara* and *Staphysagria* were selected.

**Material and method:** 1% w/v acetic acid solution was used that was injected intradermally into the plantar side of the right hind paw of rat. Rats were divided into five groups, each consisting of 10 animals. Group one served as control, group two served as standard reference, group three received *Argentums nitricum*, group four received *Staphysagria*, group five received *Ignatia amara*.

**Result:** The effect of *Staphysagria>Ignatia amara>Argentum nitricum* as anti inflammatory agent is significant in the written order when compared with the reference drug that is ibuprofen at 2 hour i.e. the % inhibitions were 28.83%, 23.76%, 20.42% respectively.

**Conclusion**: All these remedies are promising agents with anti inflammatory activity in the animal model, used in this study. Among them *Staphysagria* showed maximum inhibition to inflammatory process.

**Key words:** Prostaglandins, *Argentum nitricum, Staphysagria, Ignatia amara.*

## 1. INTRODUCTION

The basic ideology of homeopathy is the "law of resemblance", according to which the patient can be treated by administering remedies which, when administered to healthy peoples, induces symptoms that are similar to those which are present in the patients with that specific disease. Due to population preference to alternative treatments there is an increase desire to do preclinical studies for the evaluation of pharmacological action or their clinical efficacy[1]. Homeopathy is a system of alternative medicine which is considered as a false science[2].This system include treatments that targets the underlie cause of the disease. This system uses minimal concentration of substance that is capable of producing effect[3].

Inflammation can be defined as a favorable and useful reaction of the body against any harmful injury. Inflammation is a transient natural reply of the tissues to detrimental spur such as any damage, exogenous and endogenous antigens, proposed to clean up or remove the stimulus and healing the injured tissue eventually and results in rejuvenation and recurring to homeostasis[4]. Although inflammation is a positive defense mechanism of the

body, several disorders are caused by inflammation that persist for long period of time, for example pain, Diabetes mellitus, allergies, atherosclerosis, obesity and cancer. Furthermore, inflammation is the leading cause that may lead to chronic diseases.[5,6]

Many drugs are used to treat all types of inflammatory conditions for example Non steroidal anti-inflammatory drugs (NSAIDs), steroids, and immunosuppressant drugs[7] but their uses are associated with serious harmful effects like ulcer formation[8], angioedema, liver failure, headache, anemia due to hemolysis, hyperglycemia, bones fracture, problems that may results from immune suppression[9].Taking into consideration all the possible adverse effects of these drugs limit their use for long-term. Complementary and alternative medicines are generally thought to be safe and are constantly being used for their anti-inflammatory potential[10].

Various homeopathic remedies are used to relief inflammation for example *Arnica Montana*, *Atropa belladonna*, *Hamamelis virginiana*[11]. *Argentum nitricum* is very effective for the inflammation of eyes[12]. Conjunctiva turns pink or red in color, eye lids became swollen and become sticky in the morning. This remedy is also indicated in corneal ulceration and opacity usually found in patients suffering from gonorrhea[13].

The use of *Argentum nitricum* has been proven in the history as disinfectant and bactericide since the Alexander invaded India. It is distributed widely in all tissues of human body in the range of 0.002-0.008 µg/g w/w[14]. It is also used to treat cutaneous warts[15]. It is used as an antimicrobial agent and effective against infections caused by E.coli[16]. In dentistry the dentist use swabs soaked in *Argentum nitricum* for the treatment of mouth ulcer and throat ulceration[17].*Argentum nitricum* is also useful in asthenopia (eye strain), in ulceration of uterine cervix also useful in nephralgia from kidney congestion or due to renal calculi passage through the ureter[18].

*Ignatia Amara* (strychnos Ignatia) is a large climbing shrub growing in Philippine island and in china. It contain strychnine chemically it is similar to nux vomica but differ in symptom. It is a spinal remedy, and prescribed to those who suffer from melancholia, headaches. Ignatia amara causes increased excitability and useful in spasm, in labor spasm, in convulsions, in dyspepsia, in proctalgia, in hemorrhoids after labor, in sore throat, in stomach cramps[19].

*Staphysagria* (Delphinium staphysagria) belongs to family Ranunculaceae. Whole plant parts i.e. leaves, stem and roots contain plant alkaloid delphinine, which is structurally similar to aconitine that is an allosteric modulator of voltage gated sodium channel. Its use in post coital cystitis has been documented[21]. *Staphysagria* is also useful in joint pain that usually appears due to tenderness, injured, immobile limbs. *Staphysagria* is also recommended by homeopaths for the treatments of Styes, teething problem, Blepharitis, Psorisis, hemmorhoids.[22]

Figure I.[20]

**Staphysagria**

## 2.MATERIAL AND METHOD

### 2.1 Animal selection:

Adult albino rats (both sexes), weighing 150 – 200 g were used for anti-inflammatory activity. The animals were housed two animals in one cage in a room maintained at 25 ± 2°C, temperature and relative humidity of 55-65%and were maintained under a 12 hours light / dark cycle i.e. light on from 08.00 a.m to 08.00 p.m in the animal house of the Department of Pharmacology, University of Karachi. The animals were given standard diet and had free access to clean and purified drinking water but the animals were deprived of food overnight, before the experiment. 10 animals were used in each group.

Animals were handled as per specification provided in Helsinki Resolution 1964 and the study was approved by our Board of Advanced Studies and Research vide Resolution No.10(50).

### 2.2 Animals model:

The anti-inflammatory activity was studied using acetic acid induced paw edema. For making the model of inflammation 1% w/v acetic acid solution was used that was injected intradermally into the plantar side of the right hind paw of rat. Rats were divided into five groups, each consisting of 10 animals. Group one served as control, group two served as standard reference, group three received *Argentums nitricum*, group four received *Staphysagria*, group five received *Ignatia amara*. This model is used to check the anti-inflammatory

activity of NSAIDs[23]. Acetic acid induces the activation of arachidonic acid cascade, ultimately resulting to the formation of inflammatory mediators (Thromboxane, prostaglandins) that in turn increases vascular permeability leading to edema and pain. Ibuprofen is used as a standard drug, a potent NSAID and COX1 and COX2 inhibitor.

**2.3 Remedies:**

This study was designed to discover the effects of homeopathic remedies on acute inflammation model via induction of paw edema in the rat. In this study three remedies *Argentum nitricum, Staphysagria, Ignatia amara* of 30 C potency were selected. The standard drug used was ibuprofen. All drugs were administered orally, 0.1ml of 1% w/v solution of acetic acid was injected subcutaneously in the sub plantar region of hind paw of rats.

The potency selected because literature survey showed that these remedies range from 30-200 C and even 30 C potency had shown effects in treatment of purulent ophthalmia, in acute inflammation of conjunctiva, in corneal ulceration and in peptic ulcer as well as this potency has a benefit of once daily dosing of the respective remedies[12,24,25.]

**2.4. Dosing**:

The anti inflammatory activity was studied using acetic acid induced paw edema test. The test remedies i.e. *Argentums nitricum, Staphysagria, and Ignatia amara*, water and ibuprofen were administered orally to the rats. Homeopathic remedies were diluted and these homeopathic remedies were administered 30 minutes prior to induction of paw edema. 0.1 ml of diluted homeopathic remedies were administered orally by using feeding syringes to the rats. The dose of ibuprofen administered was according to the body weight for anti-inflammatory activity as a reference drug in the dose of 400 mg, it was also administered orally to the animals prior to paw edema induction.

The paw edema was recorded using plethysmometer (Ugo basile, Italy) at different time intervals i.e. paw size before administration of acetic acid (time0=baseline), paw edema immediately after administration of acetic acid, then after 1,2,3,4 hours respectively for each rat.

**2.5. Determination of paw edema by plethysmometer**.

The paw edema is measured by using plethysmometer (Ugo basile Italy). The paw was immersed in the measuring tube, the displacement of water occurred into the second tube, this water displacement is sensed by platinum electrodes. The Control Unit of Plethysmometer detects the changes in conductance and transmits an output signal to the digital display indicating the volume displacement measured (0.01 ml resolution). The activity was measured as % inhibition calculated by using formula mentioned below[26].

The percentage inhibition of inflammation was calculated from the expression:

$$\% \text{ inhibition} = D0 - Dt/D0 \times 100.$$

where D0 was the average inflammation (hind paw edema) of the control group at a given time; and Dt was the average inflammation of the drug treated (that is, reference drug for example Ibuprofen or test drug for example *Argentum nitricum)* rats at the same time[27,28].

**2.6. Statistical analysis:**

All the data is presented as Mean±S.D. (n=10). Statistical analysis was performed using One Way Analysis Of Variance (ANOVA) followed by post hoc test Multiple comparisons. All the analysis were performed using Social Sciences version 20 (SPSS,Inc.,Chicago,IL,USA).

**3. RESULTS**

The anti-inflammatory effect of *Argentum Nitricum, Staphysagria, Ignatia Amara*, acetic acid induced paw edema in rats is given in table 1 and shown in graph 1. The results indicated as percentage inhibition of edema in rats paw.

All test remedies that is *Argentum Nitricum, Staphysagria, Ignatia Amara* given to the rats according to the animal weight have the varying degree of anti inflammatory effect as compared to the control and reference (standard) drug Ibuprofen.

The results showed that *Argentum nitricum,* showed percent inhibition of edema which were 2.83% at 1 hour, 20.42% at 2 hour, 9.92% at 3 hours, 10.27% at 4 hour as compared to reference drug.

*Staphysagria* showed percent inhibition of edema 10.77% at 1 hour, 28.83% at 2 hour, 9.92% at 3 hour, 8.27% at 4 hour as compared to reference drug.

.*Ignatia Amara* showed percent inhibition that is 21.64% at 1 hour, 23.76% at 2 hour where as at 3 and 4 hours the results were insignificant as compared to reference drug.

The reference drug Ibuprofen showed highly significant results at similar time intervals with percentage inhibition of edema 30.09% at 1 hour, 37.05% at 2 hour, 16.17% at 3 hour, 15.33% at 4 hour.

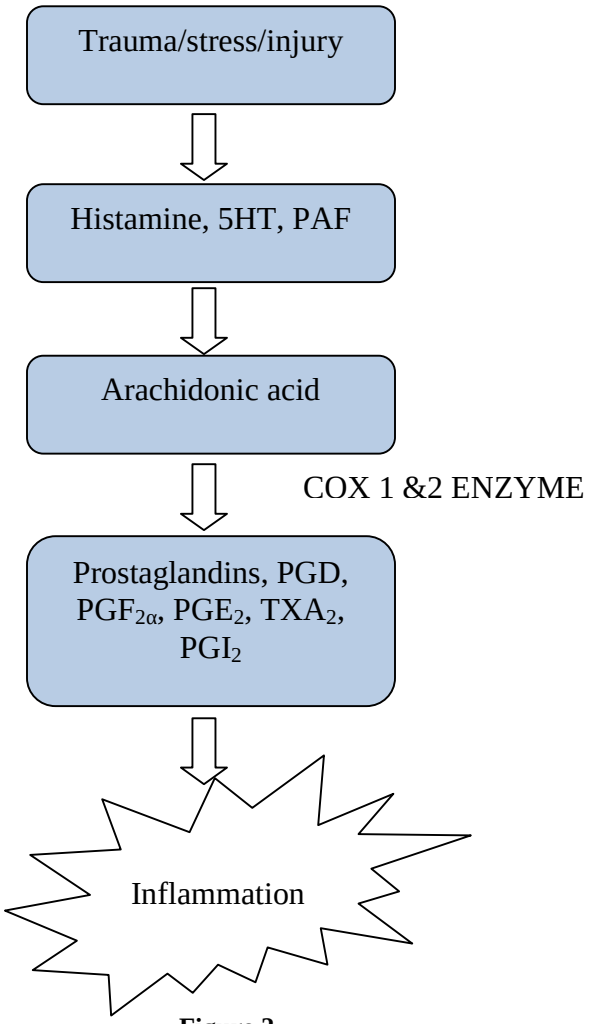
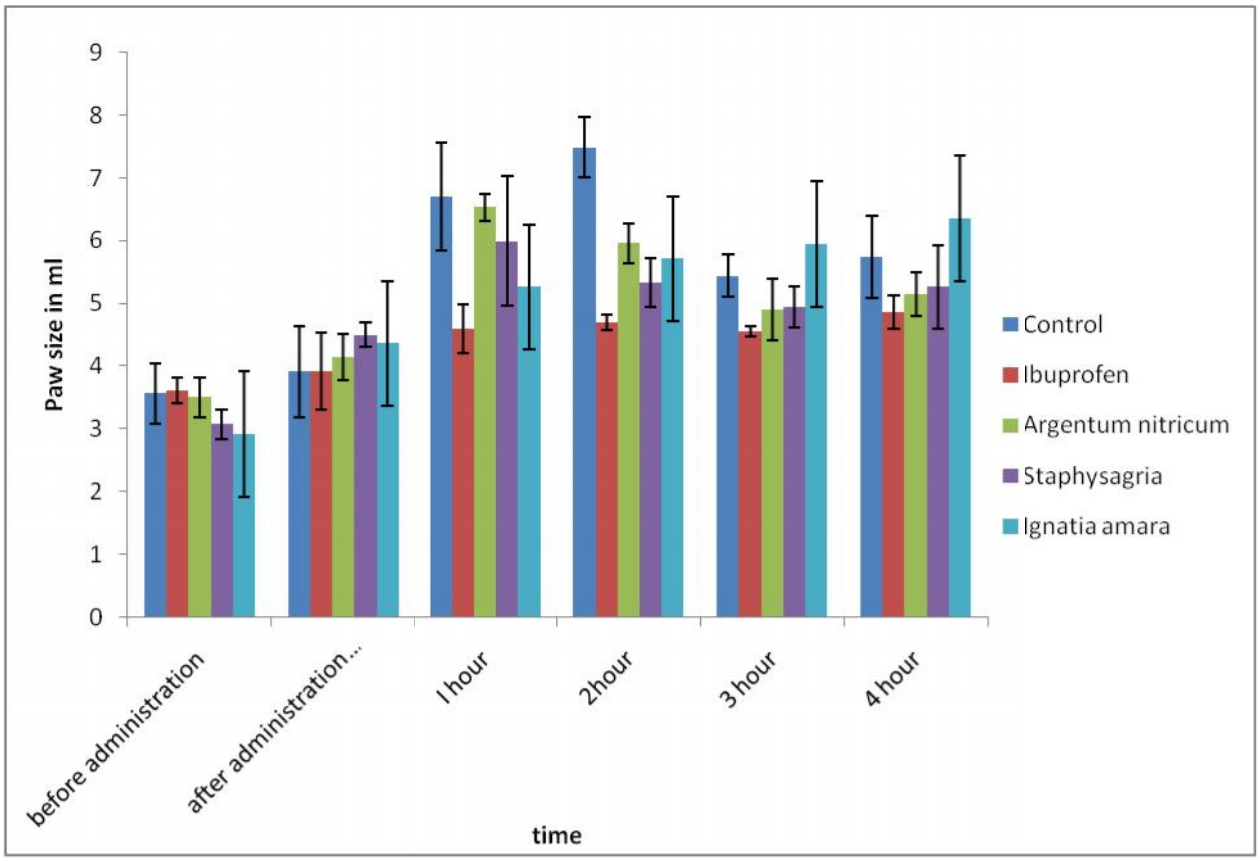



**Figure 2**
Inflammatory cascade

**Table 1**
**Anti inflammatory activity of drugs *Argentum nitricum, Staphysagria, Ignatia amara* and Ibuprofen in comparison with control.**

| Drugs Groups | Before acetic acid treatment (displacement in ml) (mean±SD) | After acetic acid administration (displacement in ml) (mean±SD) | Results shown as percent inhibition | Percent inhibition of edema | | | |
|---|---|---|---|---|---|---|---|
| | | 0 hour | | 1hr | 2hr | 3hr | 4hr |
| Control | 3.5600±0.488 | 3.911±0.736 | | - | - | - | - |
| *Argentum nitricum* | 3.5030±0.327 | 4.1485±.377 | | 2.83%*** | 20.42%*** | 9.92%(IS) | 10.27%(IS) |
| *Staphysagria* | 3.073±0.24 | 4.5±0.19 | | 10.77%*** | 28.83%* | 9.19%(IS) | 8.18%(IS) |
| *Ignatia amara* | 2.926±0.33 | 4.36±0.355 | | 21.64%(IS) | 23.76%*** | -9.19*** | -10.80*** |
| Ibuprofen | 3.61±0.205 | 3.9270±.623 | | 30.09%*** | 37.05%*** | 16.17%*** | 15.33%** |

*p≤0.05 significant,
**p≤0.01 moderately significant,
***p≤0.001 highly significant,
IS = Insignificant.




**GRAPH 1**
**Antiinflammatory activity of *Argentum nitricum, Saphysagria, Ignatia amara* and ibuprofen in comparison with control.**

Post hoc Tukey & LSD test were applied which showed that Ibuprofen compared with control showed highly significant results ($p \leq 0.001$) while *Argentum nitricum* ($p \leq 0.001$) and *Staphysagria* ($p = 0.001$) also showed highly significant results. *Ignatia amara* showed insignificant results when compared with standard (Ibuprofen) at 1 hour.

Post hoc Tukey & LSD test were applied which showed that Ibuprofen, *Argentum nitricum*, *Ignatia amara* showed highly significant results ($p \leq 0.001$) while *Staphysagria* showed significant results that is ($p \leq 0.05$) when compared with standard drug at 2 hour.

Post hoc Tukey & LSD test were applied which showed that Ibuprofen showed highly significant results ($p \leq 0.001$) and *Ignatia amara* showed highly significant increase in paw size ($p \leq 0.001$) at 3 hours. *Argentum nitricum and Staphysagria* showed insignificant results at 3 hour when compared with standard drug.

Post hoc Tukey & LSD test were applied which showed that Ibuprofen showed moderate significant results when compared with control ( $p \leq 0.01$) while *Argentum nitricum*, and *Staphysagria* showed insignificant results but *Ignatia Amara* showed highly significant increase in paw size ($p \leq 0.001$) when compared with standard drug.

The effect of *Staphysagria>Ignatia amara>Argentum nitricum* as inflammatory agent is significant in the written order when compared with the reference drug that is ibuprofen at 2 hour i.e. the % inhibitions were 28.83%, 23.76%, 20.42% respectively.

The maximum effect caused by Staphysagria was 28.83% inhibition of the acetic acid induced rats paw edema at 2 hour when compared with Ibuprofen.

## 4. DISCUSSION

This study was designed to determine the anti inflammatory activity of homeopathic remedies

(*Argentum nitricum, Staphysagria, Ignatia amara)* on acetic acid induced inflammation in the rat paw and their effects were compared with reference drug Ibuprofen.

Acetic acid induces inflammation in the body tissues with the release of inflammatory mediators in two phases. Histamine, 5HT, PAF are released in early phase (time duration 90-180 min) while in late phase Prostaglandins, kinins, proteases, lysosomes are released.[29,30] The free radicals synthesis, PGs synthesis ($PGE_2$, $PGF_{2\alpha}$), TNF-α, IL1, IL2 induces inflammation and nociception through nociceptors stimulation.[31]They may also stimulate iNOS and COX 2[32,33] that ultimately increases the oxido-nitrosative stress in inflamed rat paw.

Most of the NSAIDs are effective in late phase of inflammation where they suppress the COX 2 expression and in turn decreases the PGs synthesis.[34,35] All test remedies showed very significant anti inflammatory activity in the early phase of inflammation (i.e.1st and 2nd hour). In the late phase the anti inflammatory efficacy was found insignificant (Table 1, Graph 1). *Argentum nitricum, Staphysagria and Ignatia amara* showed maximum % Inhibition after 2 hour of inflammation induction in comparison with control but the anti inflammatory activity was found less than as compared to Ibuprofen.

All of these three test remedies have potential to decrease the release of inflammatory mediators at an early phase than as compared to late phase where NSAIDs works better to suppress COX 2 expression as well as PGs synthesis.

## 5. CONCLUSION

All these remedies are promising agents with anti inflammatory activity in the animal model, used in this study. Among them *Staphysagria* showed maximum inhibition to inflammatory process. The mechanism of these drug is proposed on the basis of knowledge of inflammatory cascade. Hence further studies at molecular level are required for a better understanding of exact mechanism and site of action of these remedies.

## 6. REFERENCES


1. Conforti A, Bellavite P, Bertani S, Chiarotti F, Menniti-Ippolito F, Raschetti R. Rat models of acute inflammation: a randomized controlled study on the effects of homeopathic remedies. BMC Complement Altern Med, 2007; 17:7:1.
2. Raimo Tuolema Science, Protoscience, and Pseudoscience. Joseph C. Pitt ,Marcello Pera(ED) . Rational Changes in Science. 1987; Part I: 83-101. DOI 10.1007/978-94-009-3779-6_4 Print ISBN 978-94-010-8181-8.
3. Hahnemann CFS: Organon of medicine. Edited by Joseph. 1994. Reves from the 5th and 6th edition(1842).Haifa: Homeopress Ltd.
4. Egger G, "In search of a germ theory equivalent for chronic disease," Preventing Chronic Disease.2012; 9: 1–7.
5. Mizuno Y., Jacob R. F., and Preston Mason R., "Inflammation and the development of atherosclerosis—effects of lipid-lowering therapy," Journal of Atherosclerosis and Thrombosis, 2011; 18( 5): 351–358.
6. CIHR Canadian Institutes of Health Research, "Inflammation in chronic disease initiative," 2012; http://www.cihr-irsc.gc.ca/e/44070.html.
7. Su S, Wang T, Duan JA, Zhou W, Hua YQ, Tang YP, Yu L, Qian DW. "Anti-inflammatory and analgesic activity of different extracts ofCommiphora myrrha" .Journal of Ethnopharmacology, 2011; 134( 2): 251–258.
8. Laine L., Smith R., Min K., Chen C., and Dubois R. W., "Systematic review: the lower gastrointestinal adverse effects of non-steroidal anti-inflammatory drugs," Alimentary Pharmacology and Therapeutics, 2006; 24( 5): 751–767.
9. Rodrigo L., De Francisco R., Pérez-Pariente J. M. et al., "Nimesulide-induced severe hemolytic anemia and acute liver failure leading to liver transplantation," Scandinavian Journal of Gastroenterology, 2002; 37( 11): 1341–1343.
10. Tag H., Das A. K., and Loyi H., "Anti-inflammatory plants used by the Khamti tribe of Lohit district in eastern Arunachal Pradesh, India," Natural Product Radiance, 2007; 6(4): 334–340.
11. Lussignoli S, Bertani S, Metelmann H, Bellavite P, Conforti A: Effect of Traumeel S, a homeopathic formulation, on blood-induced inflammation in rats. Complement Ther Med 1999; 7: 225-230
12. Schaller, Ulrich C. and Klauss, Volker. "Is Credé's prophylaxis for ophthalmia neonatorum still valid?". Bulletin of the World Health Organization,2001; 79(3): 262–266. doi:10.1590/S0042-96862001000300017.

13. Rawat PS. A homeopathic treatise on homeopathic help, Research and allied work –Part III, Homeopathy Ezine, 2011.
14. Ajit kulkarni. Argentum-The precious noble metal in homeopathy.Similima.com Everything on homeopathy , 2012. Retriwed from http://www.similima.com
15. Sterling, J. C.; Handfield-Jones, S.; Hudson, P. M.; British Association of Dermatologists (2001)."Guidelines for the management of cutaneous warts". British Journal of Dermatology, 2001; 144 (1):4 – 11.doi:10.1046/j.13652133.2001.04066.x. PMID 11167676. edition.
16. Zhao, G; Stevens, SE. "Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion". Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine, 1998; 11(1): 27 -32. doi:10.1023/A:1009253223055. PMID 9450315.
17. Manish Bhatia. Argentum Nitricum. Hpathy Ezine, January, 2010 retriewed from http://hpathy.com/e-books/lectures-on-homeopathic-materia-medica/argentum-nitricum/
18. Farrington E.A .n.d. homeopathic material medica by farrington.argentum nitricum retrievwd from http://www.vithoulkas.com/en/books-study/online-materia-medica/2864-argentum-nitricum.html
19. William Boericke. Homeopathic material medica by William Boericke, M.D. presented by Medi –T. Ignatia Amara St Ignatius Bean, 1927; 9th edition. Retrived from http://www.homeoint.org/books/boericmm/preface.htm
20. Figure 1 (Retrieved from http://homeopathyplus.com.au/wp-content/uploads/2013/02/379241_56434334 0262112_1203455128_n.jpg)
21. Ustianowski P A. A clinical trial of staphysagria in post coital cystitis.Homeopathica, 1999. retrieved from http://homeopathy.ac.nz/full-text-articles/a-clinical-trial-of-staphysagria-in-postcoital-cystitis/.
22. Adolph Lippe.M.D. Text book of MATERIA MEDICA,1886; Publishers:AJ Tafel Constantine Hering.1877. Retrieved from http://homeopathyplus.com.au/staphysagria)
23. Otterness IG, Bliven ML: Laboratory models for testing NSAIDs. In Non Steroidal Anti Inflammatory Drugs. Edited by Lombardino JG.Groto, Connecticut: John Wiley and Sons 1985;112-251.
24. Dr Bhasker Bandu Jhafnav, AT Homeopathy home. B, Jain Publishers, 1997; ISBN: 81-8056-286-7. Book code B-3959.
25. Ratja scheutt. Francisco X. Eizayaga and the Layer Approach to Homeopathy. ;.Retrieved from http://hpathy.com/homeopathy-papers/francisco-x-eizayaga-and-the-layer-approach-to-homeopathy/
26. Tanko, Y , Mohammed, A. ,Okasha, M. A., Umar, A. H and Magaji, R. A.l., anti-nociceptive and anti-inflammatory activities of ethanol extract of syzygium aromaticum flower bud in wistar rats and mice . Afr. J. Trad. CAM, 2008; 5 (2): 209 - 212 ISSN 0189-6016.
27. Gupta M, Mazunder UK, Sambath Kumbar R, Gomath P, Rajeshwar Y, Kakoti BB, Tamil Selven V. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhina racemosa stem bark in animal models. J. Ethnopharmacol, 2005; 98: 267-273.
28. Sawadogo WR, Boly R, Lompo M, Some N. Anti-inflammatory, analgesic and antipyretic activities of Dicliptera verticillata. Int. J. Pharmacol, 2006; 2: 435-438.
29. Olajide OA,Makinde MJ, Awe SO, Effects of aqueous extracts of Bridelia ferruginea stem bark on caragennan induced edema and granuloma tissue formation in rats and mice.J.Ethnopharmacol,1999; 66: 113-117.
30. SalveniniD,Wang ZQ, Bourdon DM, Stern MK, Curree MG, Manning PT. Evidence of peroxynitrite involvement in the carragennan induced rat paw edema.Eur.J. Pharmacol,1996; 303: 217-224.
31. Choi E.M.(2007) Anti nociceptive and anti inflammatory activity of pine (pinnus densiflora)pollen extract. Phytother Res, 2007; 21: 471-475.
32. Pavlick KP,Laroux FS, Wolf RE,Gray L,Hiffmann J, Grishan MB, Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease Free.Radic.Biol.Med 2002; 33:311-322.
33. Moncada S, Higgs A. The I-arginine-nitric oxide pathway. The New England J.Med,1993; 329: 2002-12.
34. Panthong a,Kanjana Pothi D, Taesotikul T,Phankummoon A, Panthong K, Reutrakul

V.Anti inflammatory activity of methanolic extracts from Ventilago harmandiana Pierre.J.Ethnopharmacol, 2004; 91: 237-242.

35. Calixto JB,Medeiros R, Fernandes ES, Ferreira J,Calibrini DA, Campos MM, Kinnin B1 receptors: Key G protein coupled receptor and their role in inflammatory and pain ful process. Br.J.Pharmacol.2004; 143: 803-818.