Anti-inflammatory Activity of Neuropathic Pain Reducing Herbal Medicine Based on Edema Inhibition of CARR-induced Sprague Dawley Paws

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Abstract. Neuropathic pain is nerve pain whose treatment still has side effects when used for the long term. Regulation and modulation of the immune system is needed to reduce the inflammatory reaction that triggers the pain. Increasing neural tension may induced with nerve inflammation which producing compounds responsible for signalling pain, fever and heat. Ginger (Zingiber officinale Roscoe), cloves (Syzygium aromaticum L.) and nutmeg (Myristica fragrans Houtt) have been known to contain phenolic compounds that are efficacious as anti-inflammatory agents. This study aims to prove the anti-inflammatory activity of neuropathic pain reducing herbs consisting of a combination of the three ingredients. Result from herbs formulation and reflux extraction then analysed for anti-inflammatory activity using in vivo & Winter method with Sprague-Dawley white male rats (Rattus norvegicus) and quantitative phenol content. Anti-inflammatory analysis done by 6 groups of rats, which is normal control, negative control, positive control, dosage I (given herbal extract 1.125 mL/200 g BW), dosage II (given herbal extract 2.25 mL/200 g BW) and dosage III (given herbal extract 4.5 mL/200 g BW). Anti-inflammatory of 10 g herbs extract in 250 mL solvent then observed through the inhibition of oedema formation on rats’ paw along the time. Dosage III group has the higher inhibition percentage as in 75.76%, which almost equivalent as normal control group (82.28%).

1. Introduction
Neuropathic pain was affected 7–10 % of the general population. Neuropathic pain usually divided according to the cause of nerve injury. This illness mostly caused either by nerve system structure located in the vertebral area or genetic. Neuropathic pain is a condition in which part of the nerve system became tense mostly caused by over sensitive stimulants which emitted pain sensation. Neural tension pain sufferers generally feel electrocuted and stabbing pain sensation causing depression, anxiety, until sleep disturbances [1][2].

Stimulants such as infection, pressure, and viruses are damaging the nerve which can stimulate inflammatory response by immune cells [3]. Neuropathic pain is usually caused as an effect of other diseases such as cancer, diabetes mellitus, spinal degenerative, and diseases caused by viral infections [4]. The inflammatory response involves several pathways such as prostaglandin and interleukin synthesis, protein receptor adhesive response, and platelet-activation factors. The mentioned stimulants will activate the phospholipids hydrolysis process of membranes into arachidonic acid. The
arachidonic acid then will be synthesized by COX enzymes into prostaglandins PGE₂, PGH₂. The presence of these nociceptor stimulation can cause excruciating pain [5][6].

Until lately, efforts to deal with neuropathic pain that are commonly carried out are the administration of antidepressant drugs such as amitriptyline in the first line and anticonvulsant drugs such as gabapentin and carbamazepine as a second line [7]. Both types of pharmaceutical drugs can control several types of seizures also treat nerve pain but have some side effects, especially for the long-term use. Surgery can also give permanent medication. However, surgery requires a large amount of risk and has the possibility that the pain will reappear in a certain time.

Empirically, stated herbal medicine cured neuropathic pain which consisted of ginger (Zingiber officinale Roscoe), cloves (Syzygium aromaticum L.), and nutmeg (Myristica fragrans Houtt) were practically used. These herbal ingredients have known to contain beneficial phenolic compounds from previous scientific journals. Phenolic compounds are plants secondary metabolism which are beneficial for human. The phenolic compounds in Ginger, Nutmeg, and clove, specifically quercetin can conduct an anti-inflammatory response by inhibiting COX enzymes, TNF-α and IL-1β which will reduce the inflammation [8].

Therefore, this study had the aim to investigate the anti-inflammatory activities of neuropathic pain reducing herbal medicine consist of multi herbs aqueous extract from nutmeg, clove, and ginger. The selection of plants considered in empirical traditional uses.

2. Materials and Method

2.1. Herbal Medicine Extraction
Herbal samples for the whole research such red ginger (Zingiber officinale var rubrum) rhizomes procured from Depok Indonesia traditional market, dried clove buds (Syzygium aromaticum L.) and nutmeg seed (Myristica fragrans Houtt) were obtained from local vendors in Maluku Indonesia. All herbs materials were fully washed and dried at room temperature, and then Clove and Nutmeg continues dried in an oven at a temperature of 50 °C for 15 minutes. The ginger rhizome already cleaned was peeled and sliced, while the clove and nutmegs crushed to a coarse powder using a ceramic mortar and pestle. The prepared 10 grams multi herbs (Clove, Nutmeg and Ginger) were extracted using a reflux apparatus with 250 mL distilled water at 80 °C for 90 minutes. Extracts were filtered using a strainer followed by five times used Axiva qualitative filter paper Grade-1. Liquid extract of the herbs was stored in an airtight glass bottles and stored at temperature 5 °C.

2.2. Experimental Animals
36 Sprague-Dawley white male rats (±200 g) were acclimatized for 7 days at QLab, Universitas Pancasila, Jakarta, Indonesia with 12 h light and dark cycle. All animals were provided with pellet diet and water ad libitum.

2.3. Animal Treatment
Animals were divided into six groups, each consisting six males. Group I was the normal control group, administered with water orally without any treatment; Group II was positive control group which orally administered with Diclofenac sodium 2%; Group III was negative control group which orally administered with Na CMC 0.5%; Group IV, V and VI were orally administered herbal extract at 1.125, 2.25, 4.5 mL/200 g b.wt.

2.4. Edema Weight Determination
After treated, the animals were injected by carrageenan (CARR) 1% as inflammation induction on the paw except for Group I. The animal’s paws were weighed with plastometer 1 hour before the injection as initial normal weight. The animals were then weighed in 1-hour interval for 5 hours observation. Injection period were observed as hour 0, and the volume differences were gradually taken.
2.5. Edema Inhibition Determination
The edema weights that were recorded within the observation period were counted for the edema weight reduction within time which represent the inflammation inhibition. The paws volume differences were calculated to the initial paws weight, and the area under curve were then taken to indicates the edema inhibition percentage of which the herbal medicine took responsibility.

3. Results and Discussion
The edema weight throughout the 5-hour observation period of CARR-induced rats’ paw can be used as an indicator of anti-inflammatory activity because of the weight differences overtime indicates the inflammatory inhibition. If the CARR-induced edema weight reduced over time, it means that the inflammation was inhibited by the oral medicine treatment. The greater the weight reduction, the better the anti-inflammatory activity of medicine took place.

Herbal medicine showed significant edema weight reduction over time. Shown in Figure 1 and Table 1 where they can reduce the edema back into initial normal weight.

![Figure 1. Effect of 5 hours CARR-induced rats’ paws after administration of herbal medicine on edema weight.](image)

**Table 1.** Edema volume of animal group within the observation time.

| Animal Group | Edema Volume (mL) |
|--------------|------------------|
|              | 0    | 1    | 2    | 3    | 4    | 5    |
| Normal       | 0.51  | 0.46  | 0.48  | 0.43  | 0.38  | 0.34  |
| Negative     | 0.84  | 1.23  | 1.29  | 1.03  | 1.04  | 1.03  |
| Positive     | 0.17  | 0.47  | 0.25  | 0.03  | 0.10  | 0.01  |
| Dosage I     | 0.73  | 0.65  | 0.92  | 0.96  | -0.01 | 0.03  |
| Dosage II    | 0.43  | 0.83  | 0.81  | 0.19  | 0.13  | 0.07  |
| Dosage III   | 0.49  | 0.52  | 0.50  | 0.20  | -0.10 | -0.17 |
The test results showed a decrease on edema weight in the group that was given the diclofenac sodium or herbal medicine dosage I, II and III. Decreased edema weight indicates inhibition of inflammation by the medication. This is occurred because of the medicine that has anti-inflammatory activity inhibits COX enzyme to synthesize phospholipids into pain-stimulating prostaglandin, therefore the inflammation response stopped, indicating with decreased edema weight. Phenolic acid in ginger (*Zingiber officinale* Roscoe), dried clove buds (*Syzygium aromaticum* L.) and nutmeg (*Myristica fragrans* Houtt) such as gingerol, shogaol, myristicin, eugenol, are known to clinically have the ability of anti-inflammatory response. Gingerol, shogaol, and other structurally related substances in ginger inhibit prostaglandin and leukotriene biosynthesis through suppression of 5-lipoxygenase or prostaglandin synthetase. Additionally, they can also inhibit synthesis of pro-inflammatory cytokines such as IL-1, TNF-α, and IL-8 [9]. The herbal medicine worked in synergic within its constituent ingredient to put anti-inflammatory activity to inhibit the prostaglandin synthesis pathway directly.

Based on test results, herbal medicine-administered rats showed anti-inflammatory activity more gradually compared to positive group in which gave immediate response, but as effective shown by the edema weight reduction over time. This happened because the herbal medicine needs longer time for it to work effectively after orally administered than pharmaceutical medicine. Dosage I, Dosage II and Dosage III were generally worked effectively 3 hours after CARR 1% induction, while the positive control group (Diclofenac sodium 2%) were able to suppress the inflammation immediately after the CARR induction.

The area under curves (Figure 2) for each animal group were calculated to determine the inflammation inhibition percentage (Table 2). The greater the area under curve, the least of inflammation inhibition took place. All of the area under curve were then deducted to the negative control group, to determine the effectiveness of medicines inflammation inhibition.

![Figure 2. Area under curve of edema weight after inflammation injection.](image-url)

| Animal Group | Edema Inhibition (%) | Rats Number | Avg | Rank |
|--------------|----------------------|-------------|-----|------|
| Normal       | -                    | -           | -   | -    |
| Negative     | -                    | -           | -   | -    |
| Positive     | 89.62                | 87.74       | 70.75| 71.70| 75.16| 98.43| 82.23| 1 |
| Dosage I     | 56.92                | 35.85       | 32.39| 50.94| 51.89| 43.71| 45.28| 4 |
| Dosage II    | 53.46                | 66.67       | 66.67| 44.34| 61.32| 56.92| 58.23| 3 |
| Dosage III   | 60.38                | 67.14       | 79.87| 82.70| 71.70| 92.77| 75.76| 2 |
According to Table 2, dosage III (4.5 mL/200 g b.wt. of herbal medicine) showed highest anti-inflammatory activity (75.76%) compared to other herbal medicine dosages. Anti-inflammatory activity of dosage III gave parity result towards positive control group (Diclofenac sodium 2%) which showed 82.23% of inflammation inhibition. The highest anti-inflammatory activity was from positive control group (82.23%), followed by dosage III (75.76%), dosage II (58.23%) and dosage I as the lowest (45.28%).

Active compounds were identified in Neuropathic Pain Reducer extract by using UPLC-QTOF-MS show very complicated chemical system containing many types of compounds that has pharmacology effect analgesics, anti-inflammatory, anti-depressants and anti-convulsions that used as treatments for neuropathic pain. [1]. This shows that phenolic compounds contained in the constituent herbal ingredients can stimulate the release of anti-inflammatory response that will reduce pain. That means, the herbal medicine can be an alternative medication to reduce neural tension pain caused by inflammation as well as pharmaceutical drugs.

4. Conclusion

Neural tension reducing herbal medicine proven to have anti-inflammatory activity based on the CARR-induced rats paws edema inhibition ability with 45.28% inhibition on dosage I (1.125 mL/200 g), 58.23% on dosage II (2.25 mL/200 g) and 75.76% on dosage III (4.5 mL/200 g) as the highest. Dosage III (4.5 mL/200 g) gave parity results with positive control group (Diclofenac sodium 2%) by 13.52% difference. Although herbal medicine needs longer time to work effectively, but it was proven to be able to substitute pharmaceutical anti-inflammatory drugs as neural tension reducing herbal medicine.

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