JOURNAL OF PHARMACOLOGY AND BIOMEDICINE
www.rbscience.co.in

E-ISSN: 2456-8244

Standardization of marketed preparation GH Pain Nil Powder for antiinflammatory and analgesic action

Basant Rathore*, Sandeep Jain

IPS College of Pharmacy, Gwalior, Madhya Pradesh

*Corresponding Author

Email ID – basant.rathore922@gmail.com

Abstract

The primary objective of the current investigation was to standardize the formulation GH Pain Nil Powder, marketed for management of joint pain according to the WHO guidelines for establishing scientific evidence for applicability of the formulation for human use. The procured pouches of GHPN were yellowish-brown in color, with characteristic odor and bitter taste. They were available as granular powder (Churna). GHPN exhibited 8.9% total ash with 2.65% acid insoluble ash and 5.1% water soluble ash. The water soluble and alcohol soluble extractives were 1.48% and 1.4% respectively suggesting the formulation to be suitable for human use. The results of preliminary qualitative phytochemical screening revealed that except proteins and amino acids all the classes of phytochemicals were present in GHPN. TLC analysis of GHPN was done using Allicin as the marker using ethylacetate: chloroform: water (5:3:1) as the solvent system. Allicin appeared at Rf value of 0.61 on the TLC plate as bluish-green spot. The quantitation of the Allicin was done by HPLC method and it was found that GHPN contained 4.12 µg Allicin per 5 g of GHPN. The anti-inflammatory activity was evaluated using carrageenan induced rat paw edema method and it was seen that GHPN was able to inhibit only 28.27% edema formation in rat paws. The analgesic action of GHPN was evaluated using tail flick method and the response time pain stimulus (thermal) was observed. The highest reaction time for GHPN was 6.26 ± 0.053 sec at 60 min post administration while it was 3.48 ± 0.079 sec and 7.23 ± 0.151 sec for saline and Ibuprofen respectively at the same time duration.

Keywords: Standardization, herbal, Allicin, anti-inflammatory, analgesic, extractives

Received 25/08/2021; Revised 06/09/2021; Accepted 07/09/2021

Scan QR Code to visit Website

Introduction

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Gautam et al., 2003). The World Health Organization (WHO) encourages, recommends and promotes traditional/ herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them (Wani, 2007). The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies (Vaidya and Devasagayam, 2007). About 88% of the world's inhabitants rely mainly on traditional medicine for their primary health care (Kochhar, 1981).

Standardization of herbal formulations is essential in order to assess the quality of drugs on the basis of the concentration of their active principles and thereby justify the acceptability of herbal formulations in modern system of medicine (Yadav and Dixit, 2008). Standardization of herbal drugs comprises of total information and controls to guarantee consistent composition of all herbals including analytical operations for identification, marker based estimation and assay of active principles. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organizations dealing with ayurvedic and herbal products (Zafar et al., 2005; Patra et al., 2010).

The primary objective of this work was to standardize an online promoted herbal powder detailing for quality and viability. Standardization of homegrown detailing implies the affirmation of its personality and assurance of its quality and purity.

Standardization of GH Pain Nil powder is not reported till date. Hence this study was conducted with the aim to standardize this formulation with respect to its physicochemical properties, organoleptic properties, and marker quantitation.

Material and Methods

GH Pain Nil powder was purchased from Swami Herbal Ayurveda, Agra. Allicin was used as the marker compound and was isolated as per reported procedure. All chemicals and reagents used were for AR grade and purchased from Oxford Fine Chemicals, Mumbai. Experimental animal were procured from local registered breeders.

Organoleptic Standardization of GHPN

The organoleptic properties evaluated for GHPN include taste, odor, color and texture. They were physically evaluated using sense organs.

Determination of Total Ash

2 g of GHPN was placed in a suitable tared crucible of silica previously ignited and weighed. The powder was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash

was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash (W.H.O, 1998).

Acid Insoluble Ash

The ash obtained as above was boiled for 5 min with 25 mL of dilute hydrochloric acid; the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated (Meena et al., 2010).

Water Soluble Ash

The ash was boiled for 5 min with 25 mL of water; the insoluble matter was collected in an ash less filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 45°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to air-dried drug was calculated (Meena et al., 2010).

Alcohol Soluble and water soluble extractive value

5 g of GHPN was macerated with 100ml of alcohol/ chloroform-water in a closed flask for twenty-four hours, shaking frequently during the first six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air dried drug and is represented as % value (Mukherjee, 2002).

Preliminary Phytochemical Screening of GHPN (Mehta et al., 2017)

The presence of phytochemicals was tested using the qualitative tests for alkaloids, glycosides, saponins, tannins and phenolics, flavonoids, protein, and sterols & terpenoids.

Extraction of marker compound from GHPN

As the formulation contained *Allium sativum* as one of its constituent and it is widely reported that Allium alleviates pain and reduces inflammation. Hence the main component of Allium responsible for its anti-inflammatory and analgesic properties, **Allicin**, was selected as the marker compound for the present investigation (Batiha et al., 2020).

Standar Allicin was extracted from garlic cloves as per the procedure reported by Mathialagan et al (2017). Briefly, 10 g garlic cloves were blended with 100 mL of distilled water for 1 min using a high speed overhead stirrer. The treated GHPN was transferred to a 100 mL flask and placed into the ultrasonic area of the ultrasonic bath cleaner. Sonication was performed at 25°C for duration of 90 min. After sonication the solution was separated from impurities by centrifugation at 3,000 g for 2 min and filtered to remove the undissolved GHPN and stored at 4°C.

TLC analysis of GHPN and Allicin standard

TLC- method was developed using Precoated TLC Plate (Silica gel 60 F_{254}) for the standardization of GHPN. Different solvents Toluene, Benzene, Ethyle Acetate, Acetic Acid, Formic Acid, Chloroform, Methanol, Water, Hydrochloric Acid were screened for development of mobile phase. The Visualizing agent such as UV chamber, Iodine, Folins reagent, Methanolic Ferric chloride and Mayer's reagent were used to identify the spots. The optimized mobile was ethyleacetate: chloroform: water (5:3:1).

Quantitative estimation of Allicin in GHPN

Allicin in the GHPN was quantified by a HPLC method which involved using a C18 column, UV detector and detection wavelength of 254 nm and flow rate of 0.75 mL/min for the mobile phase comprising of methanol-water (50/50) (Chong et al., 2015). The total duration of run was 10 min. Allicin from garlic cloves was used as standard in varying concentration.

Evaluation of analgesic and anti-inflammatory action

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Carageenan induced rat paw edema method

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of GHPN (Kemisetti and Manda, 2018).

Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. GHPN was administered in dose of 100 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard antinflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 3 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– GNPH was administered in dose of 100 mg/kg.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

% inhibition = C-T/ C x 100

C= Paw volume (mL) in vehicle treated group (control)

T= Paw volume (mL) in drug treated group

Tail flick method

The analgesic activity was evaluated using tail flick method (Fan et al., 2014).

Animals were divided into 3 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– GNPH was administered in dose of 100 mg/kg.

About 5 cm from the distal end, tail of each rat was immersed in warm water maintained at 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15 \text{ sec} - \text{reaction time for saline}} X\ 100$$

Results and Discussion

Physicochemical and organoleptic characters

The procured pouches of GHPN were evaluated for texture, color, taste and odor. The results are shown in table 1 and the appearance of the powder is depicted in figure 1.

Table 1 Organoleptic features of GHPN

Color	Odor	Taste	Texture
Yellowish-Brown	Characteristic	Bitter	Granular powder (churna)

Figure 1 Appearance of GHPN (A) Powder, (B) Sachet

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The results of water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, total ash, water soluble ash, acid insoluble ash are presented in table 2.

Table 2 Physicochemical properties of GHPN

Parameter	Weight of Sample (g)	Weight of ash/extractive (g)	% Value
Total Ash	2	0.178	8.9
Acid insoluble Ash	2	0.053	2.65
Water soluble Ash	2	0.102	5.1
Water soluble Extractives	5	0.074	1.48
Alcohol soluble Extractives	5	0.07	1.4

Total ash value of is an indication of the amount of minerals and earthy materials present in the formulations. GHPN exhibited 8.9% total ash with 2.65% acid insoluble ash and 5.1% water soluble ash. The water soluble and alcohol soluble extractives were 1.48% and 1.4% respectively suggesting the formulation to be suitable for human use.

Qualitative phytochemical screening

The powder of GHPN was subjected to various chemical tests for preliminary screening of the class of phytoconstituents present in them. The result is presented in table 3.

Table 3 Phytochemical screening of GHPN

Phytochemical Tested	Observation	Inference
Alkaloid	Cream precipitate formation in Mayer's Test	Present
Glycoside	Greenish color in acetic acid layer in Keller-Killiani Test	Present
Saponin	Frothing Formation	Present
Tannins	Yellow color precipitate in Alkaline Reagent Test	Present
Phenolics	Bluish green color in Ferric chloride Test	Present
Flavonoids	Red color formation in Zinc reduction Test	Present
Proteins and Amino acids	No color formation in Ninhydrin Test	Absent
Sterols	Green Color in Burchard Test	Present
Triterpenoids	Grey color in Salkowski Test	Present

As it can be seen from the results except proteins and amino acids all the classes of phytochemicals were present in GHPN. This is mainly due to the presence of wide variety of plant material (as indicated on the label) in the formulation.

Extraction of Allicin

The extraction of Allicin from garlic cloves was performed using ultrasonication extraction method reported by Mathialagan et al (2017). The yield of Allicin was found to be 1.13% of the total weight of clove taken. The color of Allicin was found to be light yellow and it was obtained as an oily liquid.

TLC Analysis of GHPN and Allicin

TLC analysis of GHPN was done using Allicin as the marker using ethyleacetate: chloroform: water (5:3:1) as the solvent system. Mayer's reagent was used as the developing agent for detecting Allicin and UV Chamber for used for spotting the other components of GNPH. Allicin appeared at Rf value of 0.61 on the TLC plate as bluish-green spot.

Quantitation of Allicin in GHPN

Allicin was eluted using HPLC method employing methanol-water (50:50) as the mobile phase. Allicin was eluted at retention time 3.953 min using the mobile phase. The HPLC chromatogram of GHPN exhibited peaks at 2.343, 2.547, 2.850, 3.953, 4.397, 5.793 and 7.703 min. The peak at 3.953 was attributed to the presence of Allicin in GHPN. The quantitation of the Allicin was done from the calibration curve of peak area obtained from standard Allicin and it was found that GHPN contained 4.12 μg Allicin per 5 g of GHPN. This suggests a very small amount of Allium sativum in the formulation.

Lower concentration of Allicin might result in poor anti-inflammatory and analgesic action in experimental models. Though the other herbs present may contribute towards the action of the formulation.

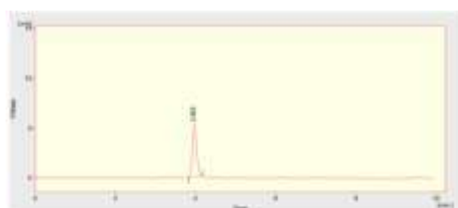

Figure 2a HPLC chromatogram of Allicin

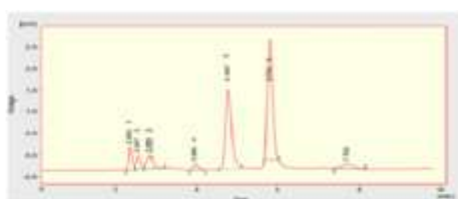

Figure 2b HPLC chromatogram of GHPN

Evaluation of analgesic and anti-inflammatory action of GHPN

Table 4 shows the effect of GHPN and standard drug as compared to the normal saline control at different hours in carageenan-induced rat paw edema model using vernier caliper. Ibuprofen at dose of 10 mg/Kg inhibited 77.5% edema after 6h of administration whereas GHPN was able to inhibit only 28.27% edema formation.

Table 4 Effect of GHPN on rat paw edema

Group	Change in Paw thickness (mm) [% inhibition of edema]			
	1h	2h	4h	6h
Normal Saline	1.46 ± 0.015	3.12 ± 0.012	3.71 ± 0.014	3.43 ± 0.015
Ibuprofen	0.48 ± 0.007 [67.12%]	0.93 ± 0.01 [70.19%]	0.96 ± 0.014 [74.12%]	0.77 ± 0.025 [77.55%]
GHPN	1.31 ± 0.027 [10.27%]	2.61 ± 0.025 [16.34%]	2.77 ± 0.029 [25.33%]	2.46 ± 0.035 [28.27%]

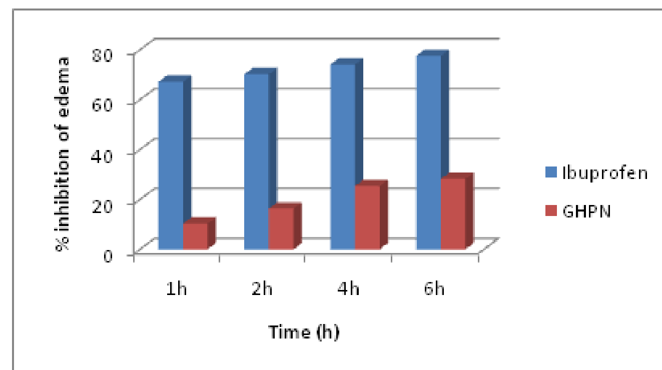

Figure 3 Comparison of anti-inflammatory effect of ibuprofen and GHPN

Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. As shown in the table, GHPN was not able to inhibit edema significantly in the early hours but was able to inhibit edema considerably at 6h. The anti-inflammatory effect of GHPN was very less as compared to Ibuprofen (figure 3).

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis (Seibert et al., 1994). Therefore, it can be inferred that the inhibitory effect of GHPN on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

The results of analgesic activity of GHPN by tail flick method are shown in table 5. Rats treated with normal saline (vehicle control) did not exhibit any significant difference in the response time on tail-flick throughout the 60 min duration of observation.

Table 5 Effect of GHPN on tail flick response

Group	Response Time in seconds				
	0 min	**15 min**	**30 min**	**45 min**	**60 min**
Vehicle Control	3.10 ± 0.061	3.41 ± 0.090	3.39 ± 0.096	4.06 ± 0.087	3.48 ± 0.079
Ibuprofen	4.03 ± 0.084	5.44 ± 0.062	6.08 ± 0.095	6.79 ± 0.142	7.23 ± 0.151
GHPN	3.91 ± 0.147	5.17 ± 0.177	5.79 ± 0.015	6.11 ± 0.165	6.26 ± 0.053

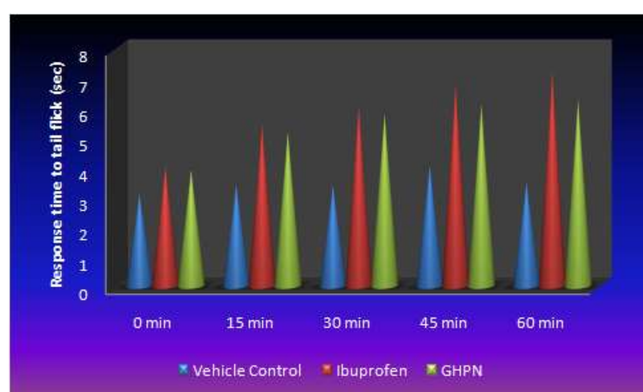

Figure 4 Comparison of analgesic effect of ibuprofen and GHPN

The duration of response time in Ibuprofen and GHPN was significantly higher as compared to the saline treated animals. The highest reaction time for GHPN was 6.26 ± 0.053 sec at 60 min post administration while it was 3.48 ± 0.079 sec and 7.23 ± 0.151 sec for saline and Ibuprofen respectively at the same time duration.

Conclusion

Plant materials are consumed throughout the developing and developed worlds as home based remedies, in over-the-counter drug products, and as raw material for the pharmaceutical industry, and they represent a sizeable proportion of the global drug market. Therefore, it is essential to establish internationally recognized guidelines for assessing their quality. The assurance of the safety and efficacy of a herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product.

Adverse events reported to the regulatory authorities in relation to the use of herbal products are often attributable to poor quality of source material and manufacturing and processing factors, among others. Correct identification of source plant species and the selection of appropriate parts for use in herbal medicines are basic and essential steps for ensuring safety, quality and efficacy of herbal medicines. Hence, the safety and quality of herbal medicines at every stage of the production process have become a major concern to health authorities, health care providers, the herbal industries and the public.

From the present investigation various standardization parameters such as physicochemical standards like total ash, acid insoluble ash, water & alcohol soluble extractive values, phytochemical analysis, and pharmacological evaluation were carried out, it can be concluded that the formulation GH

Pain Nil Powder contains good characteristics and it may be harmless for human use.

Acknowledgement

The authors are thankful to the management of IPS College of Pharmacy, Gwalior for providing necessary facilities to carry the research work.

References

Batiha GE-S, Beshbishy AM, Wasef LG, Elewa YHA, Al-Sagan AA, Abd El-Hack ME, Taha AE, Abd-Elhakim YM, Devkota HP. Chemical constituents and pharmacological activities of Garlic (Allium sativum L.): A review. Nutrients, 2020; 12(3): 872. doi:10.3390/nu12030872

Chong K, Zamora MP, Dileshni A, Tilakawardane Nancy EB, James AR, Liu Y. Investigation of allicin stability in aqueous garlic extract by high performance liquid chromatography method. J Scientific Res reports, 20156; 4: 590-598.

Fan S-H, Ali NA, Basri DF. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of Quercus infectoria (Olivier) in Rats. Evidence-Based Complementary and Alternative Medicine, 2014; http://dx.doi.org/10.1155/2014/976764

Gautam V, Raman RMV and Ashish K. Exporting Indian healthcare (Export potential of Ayurveda and Siddha products and services) Road beyond boundaries (The case of selected Indian healthcare systems). Export-Import Bank of India, Mumbai, 2003; 4–54.

Kemisetti D, Manda S. Synthesis and comparison of peg-ibuprofen and peg-ketoprofen prodrugs by *in vitro* and *in vivo* evaluation. J Drug Del Ther, 2018; 8(4): 145-154.

Kochhar SL. 1981. Tropical crops: A textbook of economy botany. London: Macmillan Pub Ltd.268–71.

Mathialagan R, Mansor N, Shamsuddin MR, Uemura Y, Majeed Z. Optimisation of Ultrasonic-Assisted Extraction (UAE) of Allicin from Garlic (Allium sativum L.). Chemical Engineering Transactions, 2017; 56: 1747-1752

Meena AK et al. Standardisation of Ayurvedic polyherbal formulation, Pancasama Churna. International Journal of Pharmacognosy and Phytochemical Research, 2010; 1: 11-14.

Mehta S, Singh RP, Saklani P. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. Int J Pharmacogn Phytochem Res, 2017; 9(4): 523-527.

Mukerjee PK. Quality control of herbal drugs, Business horizons Pharmaceutical publisher, New Delhi, 2002.

Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Nat Acad Sci, 1994; 91:12013–12017.

Vaidya ADB and Devasagayam TPA. Current status of herbal drugs in India: An overview. J Clin Biochem, 2007; 41(1):1–11.

Wani MS. Herbal medicine and its standardization. Pharmaceutical Reviews, 2007; 5(6).

WHO (1998). Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva.

Yadav NP and Dixit VK. Recent approaches in herbal drug standardization. Int J Integr Biol, 2008; 2:195-203.

Zafar R, Panwar R and SagarBhanu PS. Herbal drug standardization. The Indian Pharmacist, 2005; 4(36):21-25.