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Original Article

A Double-blind, Randomized Controlled Study of Triterpenoids based Standardized Gotu Kola Leaves Extract in the Patients with Tension Type Headache

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ABSTRACT

Introduction: Triterpenoid-based standardized *Centella asiatica* leaves extract as nasal solution (INDCA-NS) has shown potential in animal models of stress-related disorders. **Objective:** Efficacy and safety evaluation of INDCA-NS in patients with Tension-type headache (TTH). **Methods:** Ninety-one patients with TTH were randomized and administered INDCA-NS (46 patients) or PLACEBOCA-NS (45 patients) nasal spray (400 µg/day, 100 µg in 100 µL shot/ nostril, twice daily) for 28 days, with a 14-day follow-up. Efficacy (headache duration, frequency, and intensity; 21-item "Depression, Anxiety, and Stress Scale" (DASS-21); "Work Productivity and Activity Questionnaire" (WPAI: SHP); "World Health Organization Brief" (WHOQOL-BREF), cortisol and DHEA levels, rescue medications), and safety outcome measures (including adverse events) were monitored. **Results**: Subacute administration of INDCA-NS (but not PLACEBOCA-NS) significantly reduced the duration of headache and TTH's effect of TTH on WPAI:SHP (Q5) scores, namely productivity in working patients ($P < 0.05$, within the groups). The INDCA-NS showed significant difference ($P < 0.05$) in headache duration with and without covariate adjustment (vs. PLACEBOCA-NS). Both treatments found safe, well- tolerated, with no treatment-related adverse events, significantly reduced headache frequency, pain intensity, DASS-21 (stress domain), TTH's impact on daily activities (Q6 of WPAI:SHP), and improved WHOQOL scores ($P < 0.05$, within the group). The other outcome measures were not statistically significant within or between groups. **Conclusion**: Subacute INDCA-NS reduced headache duration and improved work productivity in TTH patients.

Keywords: *Centella asiatica* leaves; Chronic Stress; Pain; Work productivity.

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INTRODUCTION

Psychological stress in daily life is a major causative factor for headache disorders [1]. Among the 52% of the estimated global prevalence of active headache disorder, 26.0% is attributed to tension-type headache (TTH), 14.0% to migraine, and 3% to chronic daily headache [2]. Recently, the global prevalence of TTH has reached 3% of the global population, with 900 million new cases added annually [3].

TTH is the most common form of bilateral headache, that is attributed to psychological factors (stress and anxiety)[4]. It involves focal neurologic deficits or central sensitization towards sustained contraction of skeletal muscles in the head and neck with resultant headaches [5, 6]. Episodic TTH (frequent or infrequent) are most prevalent types of TTH with chronic TTH [7]. The pain associated with TTH is mild-to-moderate, non-pulsating, pressing, with photophobia or phonophobia, without nausea and vomiting, and unaffected by routine activity [7, 8]. TTH is known to impair quality of life, social events, and can cause work-related problems and a substantial financial impact [9].

Both peripheral and central mechanisms contribute to the pathophysiology of TTH-associated pain [10]. The most notable of these are the neurotransmitter serotonin (5-HT) receptors [11, 12]. Clinically, many 5-HT receptor agonists (e.g., the 5-HT1A receptor agonist, buspirone) have been used to treat various pain- and anxiety-related conditions [13, 14], and chronic TTH [15-17]. Sumatriptan, a 5-HT1A-like agonist, has been reported to have significant efficacy in reducing headache intensity in chronic TTH [18] but not in episodic TTH [19]. Additionally, psychosocial stress acts as a trigger point, leading to the accumulation of bradykinin and other biochemical mediators, resulting in peripheral nociceptor excitation and a lower pain threshold [20, 21].

The "International Headache Society" recommends a prophylactic schedule to improve patients' quality of life with reduced reliance on acute headache medications [22]. A prophylactic or preventive schedule was used to reduce the frequency and severity of TTH. For example, the American Academy of Family Physicians suggested ibuprofen or acetaminophen for preventive purposes. [23]. However, a recent report at tertiary headache centers revealed that 50% of patients with episodic TTH never tried preventive medication. [24]. Major prophylactic options include tricyclic and tetracyclic antidepressants to reduce the attacks frequency and severity [25, 26].

The pharmacological management options for acute attacks vary from pain relievers, such as aspirin or ibuprofen, combination analgesic medications with sedatives, and triptans [27]. However, treatment options of TTH is limited by the absence of sufficient efficacy and associated side effects [28]. For example, tricyclic antidepressants may cause constipation, drowsiness, and dry mouth, whereas overuse of pain-relievers can cause headaches and acidity [29, 30]. The role of non-pharmacological approaches (mindfulness, yoga, and tai chi) and complementary

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medicines and supplements (e.g., melatonin and vitamin D) are emerging as significant [31] better and safer options for headaches and TTH management.

Central sensitization is predominant in chronic TTH[32], whereas peripheral pain mechanisms are involved in the pathophysiology of infrequent and frequent episodes of TTH[33]. The major mechanism underlying episodic TTH is psychological stress[34, 35]. In addition, a significant relationship has been reported between the severity and duration of episodic TTH and stress levels[36]. Therefore, stress-relieving properties, in addition to anti-nociceptive efficacy, may be a crucial factor for exploring prophylaxis options for TTH, especially episodic TTH.

One potential option that can address the symptoms (i.e., episodic pain) as well as trigger (i.e., stress) of TTH are leaves of asiatic pennywort or Gotu kola (*Centella asiatica* L. Urban (CA), Family: Apiaceae) from India, Sri Lanka, and Madagascar [37]. The primary bioactive constituents of CA leaves are triterpenes, including asiaticoside, madecassoside, asiatic acid, and madasiatic acid [38, 39]. CA leaves crude extract oral supplementation have been reported to possess efficacy against mood and anxiety disorders[40, 41].

The triterpenoid-based standardized CA leaves extract (INDCA) has been reported to have a wide range of preclinical efficacy against central nervous system disorders such as depression, anxiety, and cognitive decline, with anti-nociceptive, anti-inflammatory, and wound-healing potential [41, 42]. Prophylactic anti-nociceptive efficacy against migraine-like pain in animals, probably through 5-HT1A/1 B agonist action was reported by oral administration of INDCA [43]. Subacute oral pretreatment with INDCA has been reported to cause a significant reduction in bradykinin-induced vocalization in rats [41, 43], probably by reducing the number of neurons, nociceptors, and the pain threshold in TTH [20, 21]. In addition, subacute oral administration of INDCA reduces chronic mild psychological stress and anxiety induced in laboratory rats [44], probably through "hypothalamic-pituitary-adrenal" (HPA) axis and reduction in serum corticosterone (a major stress marker)[45, 46].

Intranasal administration of INDCA nasal solution (INDCA-NS) has been reported significant prophylactic anti-nociceptive activity during animal studies against acute migraine [43], recurrent migraine [47], and chronic stress [48] in separate experiments. In addition, the robust safety profile and absence of anosmia (especially crucial for nasal formulations) during a 28-day repeated-dose toxicology study in rats [49] suggested INDCA-NS as safe prophylactic use for TTH management.

Nasal route has been found to be useful for the management of central nervous system disorders [50], including migraines [51, 52] in recent years. The nasal route has several advantages, including ease, non-invasiveness, better compliance, self-administration, avoidance of gastrointestinal tract discomfort, fewer side effects, and better bioavailability [53-55]. In addition, complementary medicines in the intranasal route, such as INDCA-NS, need to be explored in search of better and safer options against TTH.

Therefore, the present study aimed to investigate intranasal administration of INDCA-NS on headache, psychological symptoms, work productivity, daily activities, quality of life, stress-related hormones, and safety in patients with TTH.

MATERIALS AND METHODS

Design

The present randomized, placebo-controlled, parallel study used double-blind design and was conducted as per applicable ethical and regulatory guidelines, including "National Ethical Guidelines for Biomedical and Health Research involving Human patients" [56], and "Declaration of Helsinki" of 1975. The protocol was accepted by human ethics committees of institutes namely Parul Institute of Ayurveda and Research Centre Vadodara, India (SBS/IBPL/INDCA 10120) and JSS Ayurvedic Hospital and Medical College, Mysore, India (1/JSSAMC/Clinical Trial/2020-21). The study was initiated after registration with "Clinical Trial Registry of India" (No: CTRI/2020/10/028285).

Participants

The patients were screened for eligibility based on the following inclusion criteria: male or female patients, aged 18 years and above, who were experiencing episodic TTH (diagnosed with based on "International Classification of Headache Disorders 3rd edition" (ICHD-3) criteria and having mild to moderate stress i.e., score ≥15 to ≤ 25, based on the stress subdomain score from 21-items "Depression, Anxiety, Stress Scale" (DASS-21) [57, 58], and willing to provide written informed consent for procedures were recruited. The exclusion criteria were: Pregnant or breastfeeding women, menopausal women experiencing headaches, anyone with secondary headache to other causes, anyone with current or past serious illness, dependent on alcohol or substance abuse, using CNS-acting medicines that could cause serotonin syndrome, allergy, or sensitivity to any of the ingredients in the investigational product.

The sample size was calculated considering a type I error of 5% (α = 0.05) and type II error of 20% (β = 0.20, power = 80%) using a web-based system, GLIMMPS (v 2.2.8) [59], and input data for headache duration from relevant research publications [60]. Using input data, the mean headache duration of 6 h with a standard deviation of 4.8 h and required change in headache duration between the INDCA-NS and PLACEBOCA-NS groups of 2 h or more (equivalent to d= 0.42), the required sample size was 78. With 15% dropout rate possibility, a size of 90 patients was required as minimum.

Randomization and blinding

After baseline screening evaluations, 91 patients were randomized after signing informed consent in a 1:1 ratio by an independent statistician to receive either INDCA-NS or PLACEBOCA-NS for 28 days using a computer-generated randomization code by a person who was not involved in the assessments. The assignment of patients and investigators to treatment groups was concealed through blinding to promote impartiality and eliminate bias (double-blind design).

Interventions

The investigational products (IP) were 15 mL solutions of INDCA-NS or matching placebo (PLACEBOCA-NS) containing inert excipients, packed in a nasal spray, consisting of a high-density polyethylene bottle with a screw-on-pump to deliver a 100 µL spray shot / nostril with 100 µg per shot. INDCA-NS (INDCA extract powder present) and PLACEBOCA-NS (INDCA extract powder absent) were supplied by Indus Biotech Limited (Pune, India) and manufactured in nasal spray dosage forms in a licensed manufacturing facility at Source Natural Food and Herbal Supplement Ltd. (Hyderabad, India). The IP was stored in cool and dry locations and away from sunlight in a room with access control. INDCA-NS contained 68.76% total triterpenoids (sum of asiaticoside, madecassoside, asiatic acid, and madecassic acid), characterized as per a previously reported procedure [61].

The doses of the investigational products were calculated based on efficacy studies of anti-nociceptive and anti-stress activities in animal models of migraine [47] and chronic mild unpredictable stress [48]. Intranasal administration of INDCA-NS has shown significant efficacy at doses of 5 µg (against stress and pain) [47] and 15 µg (against serum cortisol levels, a stress biomarker) twice per day in rats [48]. Therefore, 10 µg/per nostril, twice a day, was considered an effective preclinical dose in rats. The surface area of the human nasal cavity (175 cm^2) [62] is approximately

22 times larger than that of rats (8 cm^2) [63]. The daily human equivalent dose of INDCA-NS was calculated to be approximately 400 µg (22 × 10 µg =440), which was derived as 100 µg/nostril/twice a day/person. Each participant received two shots of nasal spray (one in the morning and one in the evening) with a volume of 100 µL per shot of the IP (INDCA-NS or PLACEBOCA-NS) during the treatment period of 28 days.

Procedure

The patients attended a baseline visit on D0 and documented baseline assessments such as demographic information, medication history, medical history, especially headache, current medications, vital parameters (temperatures, pressures and rates), biochemical parameters (hematology and serum biochemistry), and the DASS 21 questionnaire to determine eligibility based on inclusion and exclusion criteria.

The recruited patients were then provided with numbered nasal spray bottles and instructions for administering the investigational product for the next 28 days. They were instructed to avoid antihistamines, record their headache severity, medications, and any illnesses or adverse events (AEs) or serious AEs (SAEs) experienced in the participant diary and visit the site on D14 (D14) and D28 of the treatment, along with remaining medication and diary. The patients completed the following assessments at baseline (screening and enrolment Visit on D0, D7, D14, D28 and post-treatment follow-up D42.

Outcome measures

The duration of headache was primary efficacy measure with secondary efficacy measures, included the number of headache days as defined earlier [64], frequency of headache attacks, and frequency and amount of rescue medication consumed by patients, were documented in the diary dispensed to the patients, and the total was calculated on each visit. Other secondary outcome measures, were headache pain intensities (using a 10-point visual analog scale, VAS) [65]; scores of DASS-21 [57, 58]; "Work Productivity and Activity Impairment: Specific Health Problem" (WPAI: SHP) Questionnaire, "World Health Organization - Quality of Life" (WHOQOL-BREF) [66] at baseline (D0), D14, and end of treatment (D28) and 14-days of post-treatment follow-up on D42.

Blood samples were collected from the participants by a trained phlebotomist in the morning and sent to the central laboratory (Dr. Lal Path Labs, New Delhi. India) for serum cortisol and dehydroepiandrosterone (DHEA) measurements (baseline and D28) and safety outcome measures, such as hematology, clinical biochemistry, and urinalysis (at baseline, D28 and D42). Serum cortisol and DHEA levels were measured using chemiluminescence and immunosorbent methods, respectively .

Safety outcome measures other than laboratory examinations included vital signs were recorded at baseline and 28-days. The number of dropouts, AEs or SAEs (if any), with description, intensity, duration, outcome, action taken, and opinion about causal relationships were recorded. All patient-related original documents, such as informed consent forms and laboratory reports, were collected and maintained at the site. The study-related data were captured with the allocated code to patients (without identifying information) in the source document and then in "case report forms" (CRF). Raw CRF data were compiled and verified for accuracy and then archived at the respective sites. At the end of treatment period, nasal spray bottles were collected from each participant, and the remaining volume was noted for compliance assessment. Percentage compliance was calculated for each participant based on the amount of treatment solution remaining versus the dispended amount in ml. Patients were recorded in the CRF as compliant or non-compliant based on 80% medication consumption.

Statistical Analysis

All data obtained from all recruited patients i.e. Intent-to-treat (ITT) population were considered for statistical analysis. Demographic data (age, weight, height), duration of headache (min), total of each domain of DASS-21, final total of DASS-21, serum concentrations of cortisol and DHEA, vital signs, and safety outcome measures were represented as mean ± standard deviation (SD) and analyzed between groups (t-test for independent samples) and within groups (paired t-test). Data on the number of headache days, frequency of headache episodes, and intensity scores on VAS, WPAI: SHP scores, and WHOQOL scores are represented as median (range as represented as minimum - maximum) and analyzed (Mann-Whitney U test, between the groups) and Wilcoxon signed-rank test with Bonferroni correction, within the groups. Data on medication compliance (number of patients complied and not compliant) and frequency of patients (with and without headache episodes) at each visit were analyzed using the chi-square test with continuity correction. Rescue medication data were analyzed using Fisher's exact test. Statistical analysis was performed using SPSS software (version 26.0). significance was set at $P < 0.05$.

RESULTS

Demographics

Initially, 92 potential patients were screened for eligibility, of which 91 (60 females and 31 males) met the criteria and were randomized and allocated either INDCA-NS (n = 46) or PLACEBOCA-NS (n = 45) (Figure 1, Table 1). All 91 patients (31 males and 60 females) completed the study, and no patient withdrew consent, was lost to follow-up, discontinued IP, or dropped out of the study. At screening, the homogenous distribution with respect to age, sex, height, and weight of patients in INDCA-NS and PLACEBOCA-NS groups, were found between the groups without statistical differences (Table 1).

Effects on primary outcome measures – headache duration

Data on the duration of headaches (min) are presented in Table 2. The mean headache duration at the baseline, D7, D14, D28, and D42 was not significantly different between the groups. However, the mean headache duration in the INDCA-NS group D7 and D14 showed a significant decrease (vs. baseline), whereas the PLACEBOCA-NS group did not show significant differences within the group comparisons. The within-group differences in values on D42 (v/s D28) in any of the treatment groups (INDCA-NS or PLACEBOCA-NS) were not statistically significant.

The relative duration of headaches (changes from baseline values) with and without adjustments for covariates (age and sex) was significantly different between the group on D7, D14, D28 but not on D42. However, relative changes were not statistically significant within the groups with or without covariate adjustments (age and sex).

Effects on headache episodes (numbers and frequency)

Data on the number of headache days, frequency of headache episodes, and pain intensity (VAS score) is reported in Table 3. For any of three parameters, between-group comparisons (INDCA-NS vs. PLACEBOCA-NS) did not show statistical significance on baseline D7, D14, D28, or D42, except for VAS score (at baseline). In addition, within the group comparisons, statistically significant reduction in median headache days, frequency of headaches, and pain intensity (VAS score) on Day7, D14, D28was found (vs. D0) for both treatments (INDCA-NS or PLACEBOCA-NS). However, the median values of all three parameters of both treatment groups on D42 did not show a statistical difference (vs. corresponding values on D28. Moreover, no

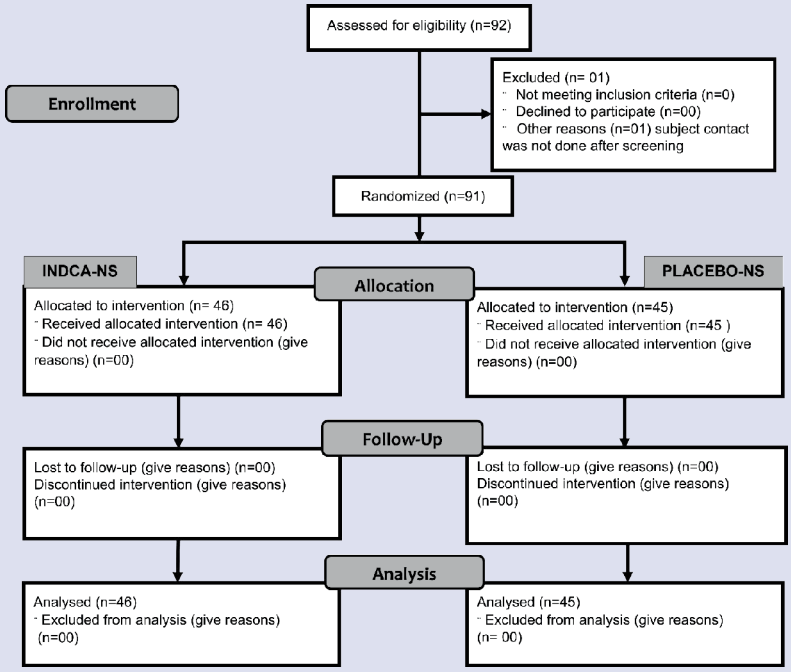

Figure 1: CONSORT flowchart.

Table 1: Summary of demographic characteristics.

Parameter	INDCA-NS (N=46)	PLACEBOCA-NS (N=45)	P-value
Gender	Number (%)		
Female	30 (65.20%)	30 (66.70%)	0.884*
Male	16 (34.80%)	15 (33.30%)	
	Mean ± standard deviation		
Age (years)	32.96 ± 9.89	34.93 ± 12.22	0.398@
Height (cm)	161.5 ± 10.69	162.12 ± 10.38	0.783@
Weight (kg)	62.86 ± 11.74	61.76 ± 10.29	0.636@

N = number of patients.
@ Between the group (t-test for independent samples)
* Between the group (Chi-square test)

Table 2: Effects on headache duration (min).

Visit	INDCA-NS		PLACEBOCA-NS		P-value*
	N	Duration (min)	N	Duration (min)	
Absolute Values (Mean ± Standard Deviation; Median)					
Baseline	46	242.39 ± 184.68; 160	45	178.44 ± 114.89; 120	0.251
D7	46	114.11 ± 120.38#; 84	45	169.80 ± 169.92; 120	0.102
D14	46	110.26 ± 120.07#; 72	45	138.91 ± 177.05; 90	0.640
D28	46	94.78 ± 102.79#; 83	45	119.20 ± 141.17#; 75	0.718
Day 42	46	121.24 ± 182.44; 60	45	103.89 ± 116.90; 75	0.929
Relative Values - Change in duration; (Mean ± Standard Deviation; Median)					
D7 (vs. Baseline)	46	128.28 ± 196.31; 100	45	8.64 ± 186.26; 30.0	0.004
D14 (vs. Baseline)	46	132.13 ± 208.19; 102.50	45	39.53 ± 209.86; 60.0	0.037
D28 (vs. Baseline)	46	147.61± 163.82; 98.50	45	59.24 ± 161.54; 73.0	0.011
D42 (vs. D28)	46	-26.46 ± 144.79; 0.0	45	15.31 ± 113.19; 0.0	0.106
Relative Values with covariate (age and sex) adjustment (Mean ± Standard Error)					
D7 (vs Baseline)	46	129.91 ± 28.32	45	6.981 ± 28.64	0.003
D14 (vs Baseline)	46	132.023± 31.11	45	39.639 ± 31.46	0.040
D28 (vs Baseline)	46	147.32 ± 24.24	45	59.542 ± 24.51	0.013
D42 (vs D28)	46	-58.06 ± 26.98	46	-2.527 ± 26.98	0.152

N = number of patients. D- Day
* Between the groups - Mann-Whitney U test,
# Significant Within the group's comparisons (D7, D14 or D28 v/s baseline) - Wilcoxon signed rank test with Bonferroni correction
Within the group's comparison (D42 vs D28) with Wilcoxon signed rank test with Bonferroni correction found no significant differences

statistical difference was found in frequency of patients with headache episodes as (v/s patients without headache episodes) between (INDCA-NS v/s PLACEBOCA-NS) or within (D7, D14, or D28 v/s Baseline) the groups comparisons.

Effect on scores of DASS-21 questionnaires

The scores for each of the three domains and the final total scores on the DASS-21 questionnaire are presented in Table 4. No significant differences between the groups were present in any of the domains or final total scores. However, within-group comparisons of stress domain scores in the INDCA-NS group showed a significant reduction (vs. baseline) on D14 and D28, whereas the PLACEBOCA-NS group showed a significant reduction only on D28. The INDCA-NS group showed a significant reduction in stress scores even after withdrawal of treatment, whereas the PLACEBOCA-NS group did not show such a reduction (D42 v/s D28).

Effects on scores of WPAI: SHP 2.0

The median scores for WPAI: SHP questionnaire are presented in Table 5. The 13 patients in each treatment group (INDCA-NS and PLACEBOCA-NS) were working professionals and responded to Q2–Q5. The scores of Q1–Q4 did not show a significant change between the groups on any of the days. A statistically significant reduction in Q5 on D14 and D28 (v/s baseline, within the group) was found in the INDCA-

Table 3: Effects on number of headache days, frequency of headache episodes and pain intensity (VAS score).

Visit	INDCA-NS		PLACEBOCA-NS		P-value*
	N	Median (Min - Max)	N	Median (Min – Max)	
Number of headache days					
Baseline	46	4 (2 -10)	45	4 (2-8)	0.918
D7	46	2# (0-6)	45	2# (0-7)	0.845
D14	46	1# (0-4)	45	1# (0-8)	0.706
D28	46	2# (0-7)	45	2# (0-12)	0.760
D42	46	2 (0-7)	45	2 (0-12)	0.760
Occurrence (frequency) of headache episodes					
Baseline	46	4 (2-20)	45	4 (2-7)	0.906
D7	46	2# (0-8)	45	2# (0-12)	0.999
D14	46	1# (0-4)	45	1# (0-12)	0.566
D28	46	2# (0-7)	45	2# (0-12)	0.800
D 42	46	1 (0-8)	45	1 (0-7)	0.941
Pain intensity score based on VAS					
Baseline	46	6 (4-8)	45	6 (4-8)	0.042
D14	46	4# (0-6)	44	4# (0-7)	0.110
D28	46	3.5# (0-4)	44	3# (0-7)	0.591
D42	46	2 (0-6)	45	3 (0-7)	0.462

N = number of patients. D-day
* Between the groups - Mann-Whitney U test
# Significant - Within the group comparisons (D7, D14 or D28 v/s baseline) - Wilcoxon signed rank test with Bonferroni correction
Within the group comparisons (Day 42 vs D28) were made with Wilcoxon signed rank test with Bonferroni correction and showed no statistical significance.

Table 4: Effect on scores of DASS-21 questionnaires.

Domain	Visit	INDCA-NS		PLACEBOCA-NS		P-value*
		N	Score (Mean ± SD)	N	Score (Mean ± SD)	
Depression	Baseline	46	14.30 ± 9.29	45	14.36 ± 9.31	0.979
	D14	46	13.26 ± 9.37	45	14.00 ± 9.59	0.711
	D28	46	13.96 ± 10.83	45	13.20 ± 9.53	0.725
	D42	46	13.22 ± 10.53	45	13.91 ± 11.55	0.765
Anxiety	Baseline	46	13.87 ± 8.57	45	15.11 ± 8.82	0.498
	D14	46	13.52 ± 8.78	45	14.27 ± 8.77	0.686
	D28	46	14.09 ± 10.69	45	14.00 ± 9.19	0.967
	D42	46	13.65 ± 10.6	45	14.80 ± 11.23	0.617
Stress	Baseline	46	20.61 ± 2.56	45	21.16 ± 2.75	0.328
	D14	46	19.57 ± 6.14#	45	19.60 ± 6.36	0.979
	D28	46	18.26 ± 8.29#	45	17.02 ± 6.96#	0.443
	D42	46	16.61 ± 8.21$	45	16.62 ± 9.47	0.994
Final Total	Baseline	46	48.78 ± 18.60	45	50.62 ± 19.63	0.647
	D14	46	46.35 ± 22.87	45	47.87 ± 23.22	0.754
	D28	46	46.30 ± 28.67	45	44.22 ± 24.42	0.710
	D42	46	43.48 ± 28.18	45	45.33 ± 31.19	0.767

N = number of patients., SD – Standard deviation D- day
DASS-21 - The Depression, Anxiety and Stress Scale - 21 Items
* Between the group - t-test for independent samples
# Significant within the group (Baseline vs D14 or D28) – Paired t-test
$ Significant within the group (D42 vs D28)- Paired t-test

Table 5: Effects on scores of WPAI: SHP 2.0.

Question	Visit	INDCA-NS		PLACEBOCA-NS		P-value*
		N	Median (Min-Max)	N	Median (Min-Max)	
Q2: How many hours did you miss from work because of your health problems?	Baseline	13	4 (2-10)	13	3 (2-8)	0.287
	D14	12	4 (0-9)	13	2 (0-8)	0.295
	D28	12	2 (0-8)	10	3 (0-8)	0.974
	Day 42	12	1 (0-12)	12	0 (0-8)	0.843
Q3: How many hours did you miss from work because of any other reason?	Baseline	13	2 (0-14)	13	2 (0-8)	0.687
	D14	12	2 (0-8)	11	2 (0-8)	0.999
	D28	12	2 (0-24)	11	2 (0-8)	0.525
	Day 42	12	1.5 (0-9)	12	2 (0-10)	0.590
Q4: How many hours did you work?	Baseline	13	36 (26-70)	13	34 (4-42)	0.169
	D14	12	34 (30-70)	13	34 (20-42)	0.936
	D28	12	36 (20-70)	11	34 (21-42)	0.880
	Day 42	12	34.5 (24-70)	13	30 (22-47)	0.295
Q5: How much did your health problems affect your productivity while you were working?	Baseline	13	5 (3-6)	13	6 (0-8)	0.225
	D14	12	4# (1-7)	13	3 (0-8)	0.237
	D28	13	4# (0-5)	11	3 (1-7)	0.575
	Day 42	11	3 (0-7)	12	2 (0-10)	0.827
Q6: How much did your health problems affect your ability to do your regular daily activities, other than work at a job?	Baseline	46	6 (3-8)	45	6 (1-8)	0.201
	D14	45	4# (1-6)	44	4# (0-7)	0.303
	D28	43	4# (0-6)	41	4# (0-7)	0.707
	Day 42	46	3 (0-8)	44	3 (0-7)	0.829

N = number of patients.
WPAI-SHP- Work Productivity and Activity Impairment Questionnaire: Specific Health Problem
Between the groups - Mann-Whitney U test,
Significant - Within the group's comparisons (D7, D14 or D28 v/s baseline) - Wilcoxon signed rank test
Within the group comparisons (D42 vs D28) were made with Wilcoxon signed rank test with Bonferroni correction and showed no statistical significance.

Table 6: Effects of treatment on WHOQOL scores in different domains.

Domain	Visit	INDCA-NS (N=46)		PLACEBOCA-NS (N = 45)		P-value*
		N	Median (Min - Max)	N	Median (Min - Max)	
Physical	Baseline	46	47 (31-69)	45	50 (31-69)	0.531
	D14	46	56# (38-75)	45	50# (31-81)	0.178
	D28	46	56# (31-75)	45	56# (31-75)	0.800
	Day 42	46	56 (38-69)	45	56 (31-81)	0.025
Psychological	Baseline	46	56 (38-69)	45	56 (38-69)	0.516
	D14	46	56# (44-69)	45	56# (31-69)	0.890
	D28	46	56# (44-75)	45	56# (31-75)	0.578
	Day 42	46	56 (31-69)	45	56 (38-81)	0.302
Social	Baseline	46	44 (0-94)	45	50 (0-81)	0.942
	D14	46	63# (31-100)	45	56# (44-100)	0.293
	D28	46	56# (31-94)	45	56# (19-81)	0.894
	Day 42	46	56 (19-100)	45	63 (25-94)	0.433
Environmental	Baseline	46	50 (31-75)	45	50 (38-75)	0.360
	D14	46	50# (44-81)	45	50# (31-75)	0.792
	D28	46	50# (38-69)	45	50# (25-75)	0.848
	Day 42	46	50 (31-81)	45	50 (38-81)	0.078

N = number of patients.
WHOQOL - Quality of life assessment developed by the WHOQOL
* Between the group - Mann-Whitney U test
# Significant – Within the group (D14 or D28 v/s baseline) - Wilcoxon signed rank test with Bonferroni correction
Within the group comparisons (Day 42 vs D28) were made with Wilcoxon signed rank test with Bonferroni correction and showed no statistical significance.

NS group and not PLACEBOCA-NS group. The response to Q6 was based on activity impairment and was responded by all patients. The analysis of responses to Q6 showed a statistically significant reduction in median scores on D14 and D28 (within the groups) for both treatments but did not show statistical significance between the groups on any day of the study.

Effects and individual domain score of WHOQOL questionnaire

The median scores of the individual domains (physical, psychological, social, and environmental) of the WHOQOL are tabulated in Table 6. The median scores differences were not significant between the

groups on any day of the study. Within-group comparisons showed a statistically significant enhancement in the quality of scores in all domains for both treatments (INDCA-NS and PLACEBOCA-NS groups). However, the improvement in domain scores for all three domains was not significant on D42, (v/s D28).

Effect on serum concentrations of cortisol and DHEA

As shown in Table 7, The mean cortisol or DHEA concentrations at baseline or on D28 did not show a statistical difference between the groups (INDCA-NS vs. PLACEBOCA-NS). The mean concentrations of both hormones decreased (not significant) after 28-days of INDCA-NS treatment (vs. baseline), whereas the mean concentrations of both hormones increased (not significant) after 28-days of treatment in the PLACEBOCA-NS group. The differences in serum cortisol or DHEA levels were not significant between or within groups.

Effect on vital signs

The between-group comparison of vital sign data, namely BP, body temperature, pulse rate, and respiratory rate (Table 8), did not show statistically significant differences at baseline, D14, D28 or D42, except for incidental significances in systolic BP (at baseline) and diastolic BP (at D28). All readings were within normal physiological reference ranges.

Hematology and biochemistry

All hematological and biochemical parameters (liver function, kidney function, and electrolytes) (Table 9) were within normal physiological reference ranges. No statistically significant differences were found at baseline or D28 in any of the parameters, except incidental differences in urea (at baseline and D28).

Safety outcomes, Compliance, Rescue mediation and adverse events

All 91 patients (100%) recruited completed the study with no dropouts or were lost to follow-up. Both treatments were well tolerated, and there was no incidence of SAE. Three incidences of AEs (viral fever) of mild-to-moderate intensity were reported (viral fever; 2 in INDCA-NS and one in PLACEBOCA-NS), though number of AEs was not significantly different between the groups (Fisher's exact test). No AEs were related to the treatment, and no patients required treatment for AEs or discontinued the study. Compliance with treatment was more than 80% for all visits, with no statistical difference found between the groups (INDCA_NS and PLACENBOCA-NS).

One patient in the INDCA-NS group (recorded at D7) and two patients in the PLACEBOCA-NS group (recorded 2 on D14 and one recorded on D28) required rescue medication (not significant, between the

Table 7: Effect on serum concentrations of cortisol and dehydroepiandrosterone (DHEA).

Parameter	Visit	INDCA-NS		PLACEBOCA-NS		P-value*
		N	Concentration Mean ± SD	N	Concentration Mean ± SD	
Serum cortisol (µg/dL)	Baseline	44	95.56 ± 50.17	42	89.81 ± 63.76	0.642
	D28	31	82.59 ± 40.31	31	90.13 ± 32.06	0.419
Serum DHEA (ng/mL)	Baseline	44	1.96 ± 1.55	42	2.02 ± 1.66	0.848
	D28	33	1.83 ± 1.22	32	2.24 ± 1.40	0.212

N = number of patients, SD - Standard deviation
* Between the group - Mann-Whitney U test
# Within the group (D28 v/s baseline) were made with Wilcoxon signed rank test with Bonferroni correction and showed no statistical significance

Table 8: Effect on vital signs.

Parameter	Visit	INDCA-NS		PLACEBOCA-NS		P-value*
		N	Mean ± SD	N	Mean ± SD	
BP systolic (mmHg)	Baseline	46	120.35 ± 4.57	45	118.11 ± 5.79	0.044
	D14	46	119.09 ± 4.34	45	118.04 ±5.82	0.335
	D28	46	119.59 ± 5.33	45	118.44 ± 5.88	0.334
	Day 42	46	118.22 ± 5.97	45	117.82 ± 5.12	0.736
BP diastolic (mmHg)	Baseline	46	79.13 ± 4.44	45	77.98 ± 6.21	0.31
	D14	46	77.67 ± 4.52	45	78.36 ± 4.66	0.48
	D28	46	79.74 ± 3.89	45	77.91 ± 3.59	0.022
	Day 42	46	78.52 ± 3.58	45	77.98 ± 3.47	0.464
Body Temperature (°C)	Baseline	46	37.33 ± 0.58	45	37.3 ± 0.64	0.774
	D14	46	37.33 ± 0.57	45	37.07 ± 1.52	0.271
	D28	46	37.31 ± 0.54	45	37.41 ± 0.58	0.425
	Day 42	46	37.34 ± 0.56	45	37.35 ± 0.56	0.934
Pulse rate (/min)	Baseline	46	75.41 ± 2.85	45	74.7 ± 2.68	0.228
	D14	46	74.89 ± 3.06	45	73.33 ± 9.06	0.273
	D28	46	75.35 ± 3.12	45	74.87 ± 3.42	0.485
	Day 42	46	75.09 ± 2.94	45	74.84 ± 3.07	0.701
Respiratory rate (/min)	Baseline	46	17.57 ± 1.19	45	17.82 ± 1.65	0.404
	D14	46	17.74 ± 1.24	45	17.69 ±1.2	0.845
	D28	46	17.54 ± 1.38	45	17.91 ± 1.16	0.173
	Day 42	46	75.09 ± 2.94	45	74.84 ± 3.07	0.701

N = number of patients, SD- Standard deviation
* Between the group - t-test for independent samples

Table 9: Effect of treatment on hematology and biochemistry.

Parameters	Day	INDCA-NS		PLACEBOCA-NS		P-value*
		N	Mean ± SD	N	Mean ± SD	
Hematology						
Hemoglobin (g/dL)	Baseline	45	12.91 ± 1.89	43	12.92 ± 2.13	0.986
	D28	33	12.93 ± 1.57	31	13.48 ± 1.86	0.206
PCV (%)	Baseline	45	40.44 ± 5.41	43	40.37 ± 5.92	0.952
	D28	33	39.65 ± 4.96	31	42.32 ± 6.30	0.064
RBC (million/mm^3)	Baseline	45	4.72 ± 0.57	43	4.63 ± 0.52	0.443
	D28	33	4.67 ± 0.58	31	4.75 ± 0.49	0.591
MCV (fL)	Baseline	45	85.29 ± 10.91	43	86.95 ± 10.30	0.465
	D28	33	86.61 ± 0.13	31	89.08 ± 0.83	0.304
MCH (pg)	Baseline	44	27.51 ± 3.83	43	27.76 ± 3.93	0.759
	D28	33	29.23 ± 9.73	31	28.19 ± 3.06	0.572
MCHC (g/dL)	Baseline	44	32.11 ± 1.60	43	31.90 ± 1.88	0.582
	D28	33	32.02 ± 0.91	31	31.51 ±1.67	0.137
RDW (%)	Baseline	44	15.51 ± 1.83	43	15.49 ± 2.18	0.965
	D28	33	15.54 ± 1.68	31	15.00 ± 1.29	0.153
TLC (x 1000/mm^3)	Baseline	42	6.87 ± 1.49	40	7.11 ± 2.09	0.548
	D28	33	7.21 ± 1.74	31	7.01 ± 1.74	0.640
DLC - Neutrophils (%)	Baseline	42	59.76 ± 7.84	40	60.79 ± 9.63	0.596
	D28	33	60.22 ± 7.29	31	61.51 ± 10.40	0.565
DLC - Lymphocytes (%)	Baseline	42	30.70 ± 5.81	40	30.28 ± 10.50	0.819
	D28	33	30.46 ± 6.38	31	27.55 ± 8.52	0.126
DLC - Monocytes (%)	Baseline	42	6.25 ± 2.88	40	6.62 ± 2.79	0.554
	D28	33	5.68 ± 2.14	31	6.02 ± 2.48	0.560
DLC - Eosinophils (%)	Baseline	42	2.49 ± 2.08	40	2.86 ± 2.13	0.426
	D28	33	2.93 ± 2.50	31	3.54 ± 5.88	0.583
DLC - Basophils (%)	Baseline	42	0.74 ± 0.33	40	0.60 ± 0.39	0.092
	D28	33	1.18 ± 2.30	31	0.96 ±1.35	0.649
ALC – Neutrophils (x 1000/mm^3)	Baseline	42	4.16 ± 1.12	40	4.44 ± 1.71	0.405
	D28	33	4.39 ± 1.36	31	4.41± 1.49	0.953
ALC – Lymphocytes (x 1000/mm^3)	Baseline	42	2.09 ± 0.51	40	2.04 ± 0.57	0.644
	D28	33	2.13 ± 0.52	31	1.92 ± 0.49	0.110
ALC – Monocytes (x 1000/mm^3)	Baseline	42	0.42 ± 0.20	40	0.44 ± 0.17	0.632
	D28	33	0.41 ± 0.13	31	0.43 ± 0.17	0.698
ALC - Eosinophils (x 1000/mm^3)	Baseline	42	0.17 ± 0.15	40	0.20 ± 0.16	0.358
	D28	33	0.21 ± 0.18	31	0.22 ± 0.25	0.802
ALC – Basophils (x 1000/mm^3)	Baseline	42	0.05 ± 0.02	40	0.12 ± 0.47	0.350
	D28	33	0.05 ± 0.03	31	0.05 ± 0.03	0.987
ALC - Platelet count (x 1000/mm^3)	Baseline	44	311.79 ± 81.22	43	297.07 ± 78.22	0.392
	D28	33	392.03 ± 335.55	31	303.81 ± 62.51	0.155
ALC - Mean platelet volume (fL)	Baseline	44	8.99 ± 1.06	43	9.20 ± 0.98	0.327
	D28	33	8.91 ± 0.95	31	11.77 ± 12.89	0.209
Liver Function Test						
AST (U/L)	Baseline	45	25.84 ± 10.38	42	26.07 ± 8.23	0.911
	D28	33	23.97 ± 8.13	32	25.16 ± 7.52	0.542
ALT (U/L)	Baseline	45	23.13 ± 15.97	42	22.55 ± 19.34	0.878
	D28	33	19.88 ± 10.59	32	22.67 ± 13.17	0.350
AST to ALT Ratio	Baseline	45	1.33 ± 0.46	42	1.50 ± 0.55	0.122
	D28	33	3.15 ± 10.39	32	1.33 ± 0.47	0.327
GGTP (U/L)	Baseline	45	21.58 ± 9.61	43	18.65 ± 7.47	0.116
	D28	33	19.86 ± 9.96	32	22.98 ± 14.46	0.313
ALP (U/L)	Baseline	45	78.33 ± 20.89	43	76.72 ± 21.80	0.724
	D28	33	78.73 ± 27.33	32	83.69 ± 27.59	0.469
Bilirubin Total (mg/dL)	Baseline	45	0.58 ± 0.34	43	0.59 ± 0.32	0.902
	D28	33	0.58 ± 0.34	32	0.57 ± 0.30	0.886
Bilirubin Direct (mg/dL)	Baseline	45	0.21 ± 0.12	43	0.22 ± 0.10	0.823
	D28	32	0.21 ± 0.11	32	0.20 ± 0.10	0.830

Parameters	Day	INDCA-NS		PLACEBOCA-NS		P-value*
		N	Mean ± SD	N	Mean ± SD	
Bilirubin Indirect (mg/dL)	Baseline	45	0.37 ± 0.23	43	0.37 ± 0.23	0.949
	D28	32	0.40 ± 0.25	32	0.36 ± 0.21	0.499
Total protein (g/dL)	Baseline	45	7.10 ± 0.47	43	7.19 ± 0.34	0.31
	D28	32	6.88 ± 1.23	32	7.16 ± 0.27	0.214
Albumin (g/dL)	Baseline	45	4.61 ± 0.31	43	4.65 ± 0.25	0.478
	D28	32	4.56 ± 0.26	32	4.58 ± 0.70	0.900
Albumin to globulin ratio	Baseline	45	1.894 ± 0.341	43	1.858 ± 0.275	0.594
	D28	32	1.884 ± 0.309	32	1.845 ± 0.249	0.580
Kidney Function Test						
Urea (mg/dL)	Baseline	45	18.74 ± 4.57	43	21.34 ± 4.60	0.009
	D28	32	18.48 ± 4.69	31	20.90 ± 4.62	0.044
Creatinine (mg/dL)	Baseline	45	0.72 ± 0.18	43	0.73 ± 0.17	0.722
	D28	31	0.73 ± 0.15	31	0.75 ± 0.16	0.664
Uric acid (mg/dL)	Baseline	45	4.73 ± 1.56	43	4.57 ± 1.44	0.631
	D28	32	4.54 ± 1.37	31	4.49 ± 1.29	0.882
Electrolytes						
Calcium (mg/dL)	Baseline	45	9.42 ± 0.46	43	9.40 ± 0.70	0.860
	D28	32	9.41 ± 0.38	31	9.56 ± 0.53	0.210
Phosphorous (mg/dL)	Baseline	45	4.00 ± 1.04	42	4.36 ± 1.31	0.156
	D28	32	4.02 ± 1.01	31	3.93 ± 0.78	0.704
Sodium (mEq/L)	Baseline	45	137.67 ± 2.33	43	137.74 ± 2.19	0.873
	D28	32	138.69 ± 2.68	31	138.23 ± 2.45	0.478
Potassium (mEq/L)	Baseline	45	4.60 ± 0.72	42	4.72 ± 0.73	0.422
	D28	32	4.48 ± 0.54	31	5.48 ± 6.53	0.391
Chloride (mEq/L)	Baseline	45	104.60 ± 2.33	42	103.93 ± 2.07	0.164
	D28	32	104.22 ± 2.45	31	104.10 ± 2.01	0.83

groups). The amount of rescue medication required in the INDCA-NS group (100 mg) was lower than that required in the PLACEBOCA-NS group (700 mg), without difference between the groups. None of patients discontinued the study, and all recovered from the AEs before the completion of their participation in the clinical study.

DISCUSSION

This study evaluated the intranasal INDCA-NS on headache (duration, frequency, and intensity), psychological symptoms (depression, anxiety, and stress), work productivity, ability to perform daily activities, quality of life, stress-related hormones, and safety outcomes in TTH patients. The subacute (28-days) twice daily administration (but not PLACEBOCA-NS) showed a statistically significant reduction within the group in the primary outcome measure (headache duration) and some of the secondary outcome measures (stress score in DASS-21 and loss of work productivity on jobs in working patients). The change in headache duration with and without adjustment for covariates (age and sex) in the INDCA-NS group was significant (vs/ PLACEBOCA-NS). The patients on INDCA-NS treatment showed significant benefits in secondary outcome measures such as improvement of QOL with reduction in the number of headache days, frequency of headache episodes, pain intensity on VAS, and loss of work productivity in regular daily activities (within the group). However, these benefits were also observed in the PLACEBOCA-NS group, with no significant differences between the groups. There was no statistically significant difference between the observation of secondary outcome measures (final total scores of DASS-21 other than stress domain, serum cortisol and DHEA, and scores of Q2 to Q 4 of WPAI: SHP) and safety outcome measures (vital signs, hematology, and biochemistry) between the INDCA-NS and PLACEBOCA-NS groups. The treatments were safe and well tolerated.

The efficacy of the treatments was assessed after 7, 14, and 28 days. A post-treatment efficacy assessment will also be performed on day 42 (excluding serum hormone Cortisol and Serum DHEA). Efficacy assessments were performed as follows:

The duration of headache, number of days with headache, and use of rescue pain medication were recorded in a headache diary. Headache duration was assessed in hours per episode and converted into minutes. A headache day was defined as the beginning of any day when a headache was recorded and ending when a headache-free day occurred [64]. The participant will indicate whether a headache is present each day.

The duration and frequency of headaches are important outcome measures for distinguishing episodic TTH from other types of headaches, such as migraine and cluster headaches [7]. A decrease in the average headache duration per episode and the number of headache days per month indicates the effectiveness of treatment [67-69]. In addition, the frequency of episodic TTH can determine the number of hours lost or reduced productivity in a substantial number of individuals [70, 71].

The patients recorded their headache frequency, duration, and intensity each day in the provided diary, which is reported to be an accurate method for recording headache information [72]. The reduction in pain intensity was recorded in the patient's diary using a 10-point VAS, with scores ranging from 0 (no pain) to 10 (worst pain). The VAS has been validated [65] and is an accurate indicator of the response of patients with pain [73].

Administration of INDCA-NS (but not PLACEBOCA-NS) caused a significant decrease in absolute headache duration as compared with baseline, with significant differences in relative values with or without covariate adjustments (between the group comparisons). These effects were sustained during the follow-up period of 14-days after treatment withdrawal (D42).

However, both INDCA-NS and PLACEBOCA-NS groups showed a reduction of frequency and intensity of headache episodes (within the group). As pain processing involves both neuronal and psychological components in the limbic system [74], a large placebo response is expected during clinical studies involving TTH, where pain is expected. The significant pain relief observed by PLACEBOCA-NS in this study is in agreement with the placebo response reported in earlier reports, especially with the nasal route of administration [75, 76] on stress-related outcomes [77], but not pain-related outcomes [77].

TTH is associated with psychological comorbidities, such depression [78]. Psychological conditions, such as stress are known to contribute to TTH [34]. Poor stress management, chronic stress, negative mood, and pain-related anxiety are specifically linked to TTH [34]. Therefore, the efficacy of the INDCA-NS was evaluated against anxiety, depression and stress in TTH patients using a well-validated questionnaire, the DASS-21 [58].

The DASS-21 is a questionnaire containing three subscales (Depression, Anxiety, and Stress) with seven questions each. DASS-21 collects the responses from the past week to questions in a Likert-scale format from patients (0= "did not apply to me at all" to 3="applied to me very much, or much of the time") [57]. For each subscale, scores were added and then multiplied by two to obtain a total score of 0 to 42. Higher scores directly correlate with symptom severity [57].

Triterpenoids from CA leaves (bioactive marker compounds of INDCA-NS) have been reported to be effective in animal models of various types of stress [41, 79-81], anxiety [79], and depression [82-84]. In the present study, the administration of INDCA-NS (but not PLACEBOCA-NS) to patients with TTH reduced the stress domain scores. However, the INDCA-NS did not have a significant reduction in the scores of the other domains (depression or anxiety) of the DASS-21. These results indicate the involvement of neurological (pain reduction) rather than psychological pathways in the mechanism of INDCA-NS in TTH.

The loss of work productivity is an important consequence of TTH [36, 85-87]. Therefore, the effects on work productivity and daily activity impairment in patients with TTH were assessed using the WPAI-SHP V2.0, a self-reported, validated, and widely used questionnaire with six questions [88]. Responses to Q1 revealed that 13 patients in each of the INDCA-NS and PLACEBOCA-NS groups were working for earnings and answered all questions (effect on work productivity and daily activities), whereas all patients answered Q6 (effect on daily activities). Treatment of TTH patients with INDCA-NS did not affect the number of hours of productivity (Q2-Q4), their estimate of work productivity loss due to TTH (Q5) was significantly lower, whereas PLACEBOCA-NS treatment did not affect the estimate of productivity loss(Q5). However, patients in both groups experienced a significant loss in their ability to perform regular daily activities (Q6). Furthermore, improvements in work productivity have been included in the guidelines of IHS for devices for headache prophylaxis [89], including episodic [90] and chronic [91] migraines. The efficacy of INDCA-NS against loss of productivity and ability of daily activity suggests the prophylactic use of INDCA-NS in nasal spray for the management of headache conditions, such as TTH.

TTH is known to reduce "health-related quality of life"[92]. Coexisting conditions like depression and anxiety intrinsically contribute to decreasing the quality of life for patients with TTH. [93]. So, quality of life using the WHOQOL-BRIEF, a validated questionnaire, containing 26-items in four domains (physical, psychological, social, and environmental) [66]was measured. Subacute administration of both INDCA-NS and PLACEBCA-NS improved scores in all domains of the WHOQOL-BRIEF on D14 or D28, without significant changes except for physical domain scores. This finding is crucial because TTH headaches affect the physical domain more than the mental domains of QoL [92, 94, 95].

Chronic stress, resulting from a prolonged imbalance between situational requirements and individuals' coping resources, is related to TTH [36]. Patients with TTH has neuroendocrine dysregulation, especially alterations in the hypothalamus-pituitary-adrenal (HPA) axis [96]. In patients with TTH, cortisol levels are reported to be maintained compared to normal circadian rhythms [96], which correlates well with adaptation in TTH patients for coping with mental stress [97]. At baseline, serum cortisol levels in patients in both groups were noticeably higher than the reference range of the central pathology laboratory for normal adults (4.30 – 22.40 µg/dL, CLIA kit). Subacute treatment with INDCA-NS reduced serum cortisol levels by 13.58%, whereas patients treated with PLACEBOCA-NS showed a 0.35% increase. The statistically non-significant differences in serum cortisol levels between or within the groups support the absence of a significant drop in headache frequency or intensity.

The role of estrogen in headache pathogenesis has been substantiated, with evidence pointing to increased serotonergic metabolism and activation of endogenous opioid neurotransmission [98]. One such endogenous hormone, DHEA, has been implicated in a variety of pathophysiological processes, including stress- and headache-related disorders, such as migraine [99] and TTH [98, 100]. Chronic stress leads to the overproduction of cortisol, causes desensitization of the HPA axis, and stops the recognition of signals [101]. DHEA has been proposed to cause protective desensitization of the HPA axis, by serving as an opponent to elevated cortisol levels [102-104]. However, under chronic stress-induced conditions, such as TTH, the capacity to produce DHEA is reduced, resulting in lower DHEA levels [105].

The baseline levels of serum DHEA were found 1.96 and 2.02 ng/ml in the INDCA-NS and PLACEBOCA-NS groups, respectively, which were noticeably lower than the normal reference range of the central pathology laboratory for normal adults (0.36-7.82 ng/ml in female and 0.52-5.18 ng/ml in males). The lower serum DHEA in patients are in agreement with earlier reports of reduced DHEA levels under chronic stress conditions [105]. All vital signs and laboratory parameters were within the normal physiological limits in both the INDCA-NS and PLACEBOCA-NS groups. The INDCA-NS was found safe and well tolerated without treatment-related SAE or AEs. None of the patients required higher rescue medication or discontinuation from the study.

A higher occurrence of sleep disturbances (absence, low quality, excessive daytime sleepiness) in patients with TTH has been previously reported [106]. However, no such sleep disturbances were observed in INDCA-NS-treated TTH patient in the present study. Moreover, olfactory dysfunction or anosmia (loss of sense of smell) is associated with repeated use of intranasal medications, such as zinc [107] and macrolide antibiotics [108, 109]. However, none of the patients with INDCA-NS-treated TTH showed olfactory dysfunction in this study, which is corelated with the report of absence of olfactory dysfunction with intranasal INDCA-NS in rats [61].

Intranasal subacute administration of INDCA-NS has been reported to be efficacious against experimental migraine [47]. This study provides first clinical evidence for efficacy of intranasal INDCA-NS in TTH patients. Although many symptoms of TTH resemble those of migraine, INDCA-NS might have potential against migraine as well but need additional clinical evidence.

CONCLUSION

In the present double-blind randomized placebo-controlled study, subacute intranasal treatment with INDCA-NS was found to be efficacious and safe in patients with TTH, without significant AEs.

DATA AVAILABILITY

Data related to this article can be obtained from the corresponding author upon reasonable request.

ABBREVIATIONS

ALC: Absolute Lymphocyte Count; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate aminotransferase; DLC: Differential Leukocyte Count; GGTP: Gamma-glutamyl transpeptidase; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; PCV: Packed cell volume; RBC : Red Blood Cell; RDW: Red cell distribution width; TLC: Total Leucocyte Count; Units: dL: decilitre; fL: Femtoliter; g: grams; L: Litre; mEq: Milliequivalent; mg: Milligram; mm: Millimetre; pg: Picogram; U: Units

SUMMARY

- INDCA-NS is triterpenoid-based standardized Gotu kola extract nasal solution
- INDCA-NS nasal spray was evaluated in patients with tension-type headache (TTH) using a double-blind placebo-controlled design.
- Twenty-eight days of INDCA-NS reduced headache duration and improved work productivity.
- INDCA-NS nasal spray was found safe and well-tolerated without serous AEs.

CONFLICTS OF INTEREST

None.

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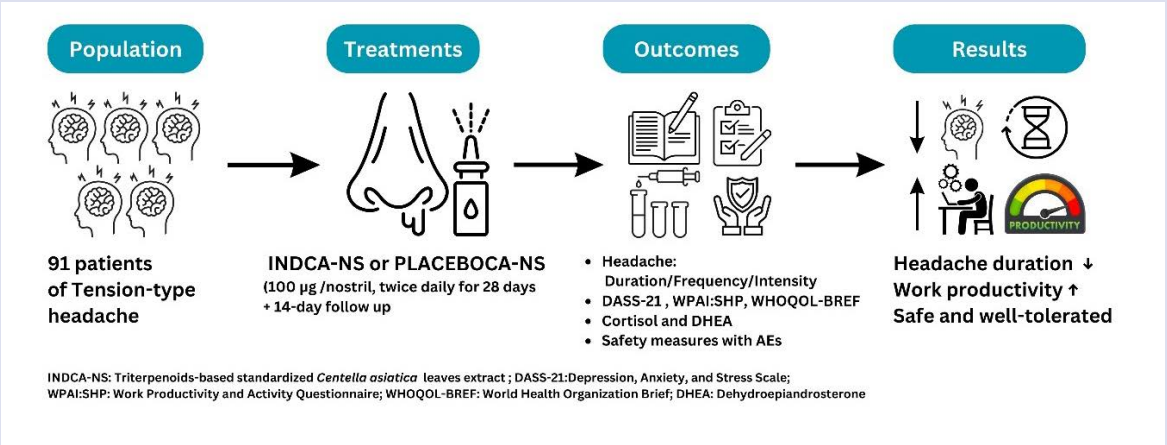
GRAPHICAL ABSTRACT



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