Clinical Case Reports International

Original Research Article
Published: 18 Apr, 2026

Safety and Efficacy of Novel Poly-Herbal Formulations in Alleviating Psychological and Physical Symptoms of Menstrual Disorders: A Randomized Clinical Study

Mohanty B[1], Panda PK[2], Mishra B[3], Satpathy B[2], Reddy YS[3], Mishra S[4], Gupta G[3] and Rajesh AS[3]*

[1]Department of Gynecology and Obstetrics, Shishu Bhawan (Branch of SCB Medical College), Cuttack, India.

[2]Sri Sri College of Ayurvedic Science and Research Hospital, Sri Sri University, Cuttack, India.

[3]Dabur Research and Development Centre, Healthcare Division, Ghaziabad, Uttar Pradesh, India

[4]Independent Consultant, Bengaluru, India

Abstract

Menstruation is a cyclic process right from 1st menstrual cycle of pubertal girl to the age of menopause. Recently, menstrual irregularities and associated psychological, physiological effects are rising among women. Hormonal therapy is a choice but have their own limitations. Natural botanical-based approaches were cited in ancient ayurvedic literature, which needs to prove their scientific efficacy. Current study, aimed to evaluate poly-herbal formulations (DHP Syrup: DRDC/2023/042 and DHP Tablet: 043 and DSS syrup: DRDC/2023/046) efficacy using in vitro system i.e., SKOV3 (human ovarian cancer cells). Antioxidant activity assessed by glutathione-peroxidase (GSH-Px) and catalase levels, whereas anti-inflammatory potential through TNF-α levels. Pilot clinical study was carried out in two groups i.e., Group 1- DHP syrup & tablet and Group 2 - DSS syrup for 3 consecutive menstrual cycles. Menstrual cycle associated signs and symptoms for psychological, physical and menstrual process were assessed. The *in vitro* data suggests antioxidant and anti-inflammatory potential of test compounds (DHP syrup & tablet and DSS syrup). Both groups have shown significant improvement in signs and symptoms associated with menstrual cycle at the end of treatment compared to baseline. Whereas, 'no significant difference' was noted between groups. Neither Adverse Events (AEs) nor Severe Adverse Events (SAEs) were reported by the participants of both groups. Results conclude that the tested herbal formulations have efficacy in countering menstrual associated psychological & physical signs and symptoms. Further, test formulation efficacy can be attributed to quenching of oxidative stress through its antioxidant potential and modulating the TNF-α levels, which are key in pathophysiological process.

Keywords: Menstrual cycle; Psychological signs and symptoms; Antioxidant potential; Anti-inflammatory activity

OPEN ACCESS

Correspondence:
Mishra B, Head, Healthcare R&D, Dabur Research and Development Centre, Dabur India Limited, Site IV, Sahibabad Ind. Area, Ghaziabad, Uttar Pradesh, 201010, India, E-mail: drbnmird13@gmail.com

Received Date: *02 Apr 2026*
Accepted Date: *15 Apr 2026*
Published Date: *18 Apr 2026*

Citation:
Mohanty B, Panda PK, Mishra B, Satpathy B, Reddy YS, Mishra S, et al. Safety and Efficacy of Novel Poly-Herbal Formulations in Alleviating Psychological and Physical Symptoms of Menstrual Disorders: A Randomized Clinical Study. Clin Case Rep Int. 2026; 10: 1767.

Introduction

Menstruation, a periodic process in female life starts with 'menarche', the first menstrual period in adolescent girl, and ends with 'menopause', the natural cessation of menses. The period between menarche and menopause considered as reproductive life span, generally ranges from 30 to 30.13 years. Menstrual cycles normally range from about 24 to 38 days, wherein active bleeding i.e., menses, spans 4 - 8 days. The menses involves prominent changes in uterine tissue architecture and ovary [1]. Cytokines secreted in response of such changes by somatic uterine cells, ovarian cells and migrated leucocytes play crucial role as local regulators of uterine and ovarian function. Studies have shown, interleukin (IL-1) bioactivity in blood plasma showed a menstrual cyclicity with higher activity in the luteal phase than in the follicular phase [2]. In another study it was reported that, Tumor necrosis factor α (TNF-α) levels in peripheral blood varies during the menstrual cycle with lower levels during early luteal phase and higher levels in mid-luteal phase and late follicular phase in comparison to the levels on the day of the luteinizing hormone (LH) surge [1].

In addition to above mentioned, reactive oxygen and nitrogen species levels determines the functioning of the ovaries (ovulation) and the endometrium of the uterus (decidualization, healing after the menstrual phase without scarring), and attributed for inflammatory response regulation

during menstrual cycle [3]. It is very much essential to maintain the balance between oxidants and antioxidants, which in turn lead to proper functioning of uterine physiological processes. Human body has evolved a variety of enzymatic and non-enzymatic antioxidant systems to combat the oxidative stress [4]. Antioxidant enzymes such as Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione Reductase (GSR), and Superoxide Dismutase (SOD) counters the ROS.

On the other hand, menstrual cycle involves periodic change in estrogen, progesterone, luteinizing, follicular stimulating hormones. Such cyclic effects influence women's mental health such as psychological distress and irritability, decreased self-esteem [5]. Studies carried out by Laessle, et al [6] and Owens, et al [7] revealed that women at pre-menstruation and menstruation have shown increased interpersonal conflicts and reduced social engagement, leading to depression and isolation.

Keeping in view of the above Dabur India Ltd., has developed polyherbal / botanical-based syrup / liquid and tablet to counter the menstrual related effects among women. DHP Liquid (DRDC/2023/042) contains actives derived from: Lodhra (*symplocos racemosa*), Manjith (*Rubia cordifolia*), Anantmool (*Hemidesmus indicus*), Bala (*Sida cordifolia*), etc in syrupy, which helps to restore women's health. DHP Tablet (DRDC/2023/043) contain time tested actives like Yashad Bhasma, Abhrak Bhasma, Loh Bhasm, Vach Churna, Tagar Churna (*Valerian Wallichii*) – etc. known to restore women's health. Another test formulation is DSS Syrup (DRDC/2023/046) contains goodness of ingredients like Ashoka (*Saraca ascoca*), Manjishtha (*Rubia cordifolia*), Sariva (*Hemidesmus indicus*), Lajjalu (*Mimosa pudica*), etc that are well documented for beneficial effects on women as well as menstrual health.

In view of the above background, current study aimed to unveil the efficacy using *In Vitro* assays and pilot clinical study, in menstrual health of reproductive age women.

Materials and Methods

Human ovarian cancer (SKOV3) cell line was procured from National Centre for Cell Science (NCCS), Pune, India. Cell culture medium, Mc Coy's, MTT, antibiotic solutions were obtained from HiMedia, India, whereas FBS was procured from Gibco, Brazil.

Test Compounds: Test compounds DHP liquid (DRDC/2023/042) & DHP tablet (DRDC/2023/043) and DSS Syrup (DRDC/2023/046) were obtained from Dabur Research and Development Centre, Ghaziabad, India.

In Vitro Assays

The *in vitro* assays were carried out on human ovarian carcinoma cells i.e., SKOV3 cell lines. The antioxidant and anti-inflammatory potential of test compound was assessed by inducing the oxidative stress through H_2O_2, and inflammation through PGE2 in SKOV3 confluent cells. DHP is prescribed for consumption at of 7 mL Syrup (DRDC/2023/042) along with one tablet (DRDC/2023/043), twice a day. Hence, *in vitro* assays were carried out with syrup, tablet and combination (dissolving 2 tablets in 14 mL of DHP Syrup). Whereas, DSS syrup (DRDC/2023/046) was diluted with medium and used for *in vitro* assays. All the experimental data generated was from three independent experiments for each test category.

Cytotoxic Effect of Test Compound: Cells in the logarithmic growth phase at a concentration 100,000 cells/mL were used for determination of cytotoxicity by the dye exclusion method [8]. Briefly, 0.1 mL of diluted cell suspension was added per well of 96 well plate. Hundred microliters of test compound (1000 - 7.81 µg/mL)/well was added after 24 h incubation at 37°C in humidified 5% CO_2 chamber. The plates were re-incubated in humified chamber for 24 h and then the supernatant was discarded and MTT (100 µL/well) was added followed by gentle shaking at 37°C, 5% CO_2 for 3 h. Then supernatant was removed and formazan was dissolved in 100 µL DMSO and absorbance was recorded at 490 nm. The untreated cells act as control.

Antioxidant Activity: SKOV3 cells grown in 60mm dish were added with test samples (500 and 250 µg/mL) and H_2O_2 and H_2O_2 alone and incubated for 24 h. Cell pellet was obtained by centrifugation at 1000 g / 10 min, sonicated and subjected to freeze thaw cycle. Clear cell lysate supernatant collected by centrifugation (1500g /10 min at 4°C) followed by determination of protein concentration through Bradford Protein Assay.

The cell lysate supernatant was used for Glutathione Peroxidase (GSH-Px) assay (Elabscience, kit), wherein GSH-Px catalyses H_2O_2 to water and oxygen as well as the reduction of peroxide radicals to alcohols and oxygen. As the GSH reacts with dinitrobenzoic acid to form 5-thio-dinitrobenzoic acid anion and measured at 412 nm. Similarly, Catalase activity determined using colorimetric assay kit. Catalase breaks down H_2O_2 but is rapidly halted by ammonium molybdate, producing a yellow-colored complex measured at 405 nm.

TNF-α Inhibitory Activity: SKOV3 cells (1.5 to 2 x 10^5 cells/mL) were seeded into 6 well culture dishes in 10% FBS Mc Coy's medium for 24 h. Then non-toxic concentration of test extracts (500 & 250 µg/mL) along with 1 µg/mL of PGE2 were added and incubated at 37°C with 5% CO_2 for 4 h. Cell supernatant was collected, centrifuged, and TNF-α in the supernatant were determined by ELISA Kit (Elabscience), according to manufacturer's instruction.

Proof of Concept Clinical Study: A randomized, two arm prospective, single centre study was conducted at Sri Sri College of Ayurvedic Science and Research Hospital, Cuttack during July, 2024 and February, 2025. The necessary ethical committee approval was taken (IEC No. IEC/SSU/037/2024) and subsequently trial was registered at Clinical Trial Registry of India vide no. CTRI/2024/06/068685.

A total of 36 women (19- 45 years) were screened by explaining the study procedures and process after obtaining written consent. The study participants were recruited as per the inclusion and exclusion criteria mentioned in Table 1. The dosage regimen, duration and parameters assessed were illustrated in Figure 1.

Investigations: The information such as demographic data, medical history, along with prior and current medications were collected. In addition, physical and systemic examinations was done for all the trial included participants. Blood was collected for haematological analysis includes CBC, Random Blood Sugar (RBS), Liver Function Tests (Serum ALT, AST, Bilirubin), Lipid Profile, Renal Function Tests (Serum Urea & Creatinine) and Thyroid Profile. The Ultrasonography of pelvic region was also assessed. Urine analysis such as UPT, routine and microscopic examinations were carried out.

Psychological and physical signs and symptoms were evaluated followed by validated 'Visual Analogue Score' (VAS). Psychological signs and symptoms include Irritability or anger, Mood swings, Restlessness, Tension, Depression (feeling sad or blue), Anxiety,

Table 1: Inclusion and Exclusion Criteria.

Inclusion Criteria	Exclusion Criteria
1. Healthy females in the age group of 18-45 years with a history of irregular menstrual bleeding.	1. Subjects unwilling to sign ICD
2. Who were not on any hormonal therapy during and one month prior to the trial.	2. Currently pregnant or breastfeeding
3. Willing to practice birth control methods other than hormonal contraception	3. Not willing to practice birth control methods (other than hormonal contraception)
4. Those who signed informed consent.	4. Abnormal laboratory values
	5. Abnormal gynaecological conditions
	6. Presence of chronic systemic diseases
	7. Subjects who in Investigators' opinion will not be able to follow study related procedures.

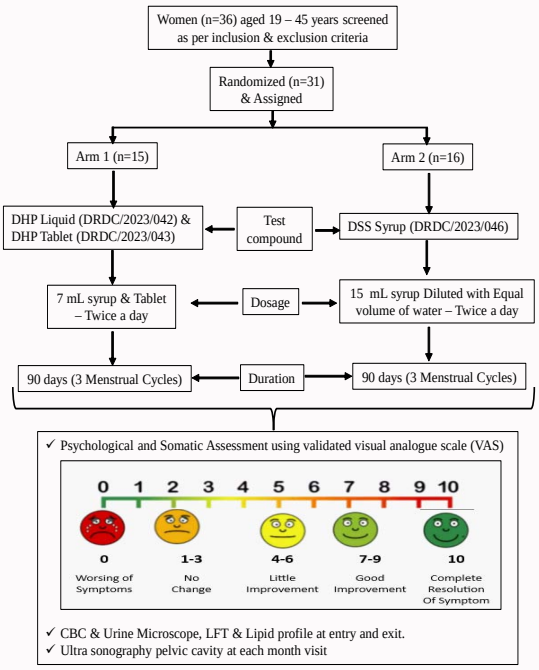

Figure 1: Flow chart of Clinical Proof of Concept study.

Difficulty in concentration, Forgetfulness, crying spell, Fatigue, Nausea/Vomiting etc. Physical signs and symptoms viz. Abdominal cramps or Pelvic Pain, Painful menstruation, Painful breasts, Joint or muscle pain or stiffness, Weight gain (related to water retention), Swelling (e.g., abdomen, breasts, ankles), Hot flashes or Cold Sweat, Bloating of Abdomen etc., were also assessed.

The trial outcome measures were to re-establish the cyclical bleeding, control of excess bleeding occurring in the menstrual cycle, prevention of dysmenorrhoea including backache, pelvic pain, abdominal cramps., leucorrhoea control. The Adverse Effects (AEs) and Sever Adverse Effects (SAEs) if any due to the test compound administration were also recorded during the periodic follow-up visits.

Statistical analysis

The data was compiled and computed to calculate the mean and Standard Deviation (SD) for all the variables. Between two groups, statistical analysis was performed using Wilcoxon Signed Rank Test to assess the differences among groups. Wherever heterogeneity in the data of a particular variable was observed, a non-parametric Mann–Whitney U-test was performed to identify the difference at 95% confidence interval ($p<0.05$).

Results

In Vitro Assays

Cytotoxicity of Test Compound: The cytotoxicity information of test samples i.e., 'DRDC/2023/042; DRDC/2023/043 & their combination (DRDC/2023/042 + 43)', and DSS syrup (DRDC/2023/046) effects on Human Ovarian cancer (SKOV3) cells have shown in Figure 2. Even the highest tested concentration (1000µg/mL) have shown cell viability, 50.20 ± 1.50%; 40.56 ± 1.30%; 51.02 ± 2.81% and 53.92 ± 2.15% for DRDC/2023/042; DRDC/2023/043; combination (Tablet dissolved in syrup), and DRDC/2023/046 respectively. The CTC 50 values of the test sample on the Human Ovary cancer cells was above 500 µg/mL (Figure 2), hence the antioxidant and anti-inflammatory assays were tested with 500 and 250 µg/mL.

Antioxidant Potential of Test Compound: Enzymatic antioxidant potential of test compounds viz. DRDC/2023/042; DRDC/2023/043; their combination (DRDC/2023/042 + 43) and DRDC/2023/046 were depicted in Figure 3. The levels of Glutathione peroxidase (GSH-Px) and Catalase were decreased in SKOV3 cells upon incubation with H_2O_2 compared with cell control. Whereas all the three test compounds (two individual and combination) of DHP and the DSS syrup have shown improved levels of GSH-Px and catalase on par with cell control, suggesting the antioxidant potential of test compounds at tested concentration (Figure 3). Further, a dose dependent effect was also noted for the concentrations of 500µg/mL and 250 µg/mL of DHP and DSS formulations.

Anti-Inflammatory Potential of Test Compound: The SKOV3 cells exposed to PGE2 and subsequently incubated with test

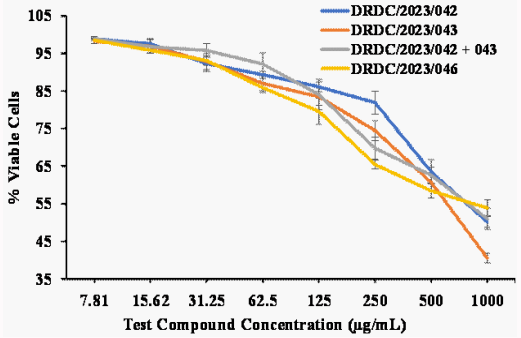

Figure 2: SKOV3 Cell Viability. The cytotoxicity of test compound determined against various test compounds at a concentration range of 7.81 – 1000 µg/mL. Results are expressed as % cell viability, with data sets as mean ± standard deviation of n=3 independent experiments.

Table 2: Signs and Symptoms of Participants.

Sign & Symptom		Group A (n=15)		Group B (n=16)	
		Baseline	End of Treatment	Baseline	End of Treatment
Psychological Signs and Symptoms	Restlessness	3.87 ± 1.51	2.00 ± 1.51*	3.69 ± 1.14	1.56 ± 1.41*
	Tension	3.00 ± 1.60	1.40 ± 1.18*	2.88 ± 1.26	1.31 ± 1.45*
	Depression	3.20 ± 1.70	1.40 ± 1.45*	2.44 ± 1.36	1.44 ± 1.36*
	Anxiety	3.40 ± 1.50	1.80 ± 1.47*	3.44 ± 1.03	1.31 ± 1.35*
	Difficulty in Concentration	3.20 ± 1.08	1.47 ± 1.36*	3.06 ± 1.53	1.56 ± 1.63*
	Forgetfulness	2.80 ± 1.26	1.47 ± 1.19*	2.69 ± 1.45	1.25 ± 1.29*
	Distractable	2.20 ± 1.61	1.40 ± 1.35*	2.44 ± 1.67	1.19 ± 1.11*
	Decreased Efficiency	2.73 ± 1.33	1.53 ± 1.30*	2.88 ± 1.20	1.44 ± 0.96*
	Lowered Judgement	2.47 ± 1.36	1.13 ± 1.25*	2.13 ± 1.31	1.19 ± 1.22*
	Change in Eating Habit	4.07 ± 1.16	1.73 ± 1.62*	3.63 ± 1.67	1.38 ± 1.59*
	Confusion	3.33 ± 1.29	1.33 ± 1.54*	2.88 ± 1.45	1.38 ± 1.36*
	Loneliness	3.33 ± 1.05	1.67 ± 1.29*	3.44 ± 1.26	1.56 ± 1.46*
	Lowered Motor Coordination	2.47 ± 1.13	1.07 ± 1.10*	2.75 ± 1.44	1.19 ± 1.42*
	Lowered Work Performance	2.67 ± 1.18	1.07 ± 1.10*	2.69 ± 1.58	1.25 ± 1.44*
	Change in Libido	0.53 ± 1.41	0.53 ± 1.41	0.38 ± 1.02	0.31 ± 1.01
	Feeling Sleepy	2.73 ± 1.71	1.33 ± 1.45*	2.25 ± 1.69	0.63 ± 1.26*
	Insomnia	2.47 ± 1.81	1.00 ± 1.25*	1.56 ± 1.41	0.75 ± 1.29*
	Affectionate	1.21 ± 1.76	0.80 ± 1.26	1.94 ± 1.91	0.81 ± 0.98*
	Giddiness	3.07 ± 1.53	1.47 ± 1.51*	2.75 ± 1.48	1.13 ± 1.36*
Physical Signs & Symptoms	Joint or Muscle Pian	6.33 ± 1.99	4.07 ± 2.94	5.38 ± 2.28	1.75 ± 2.41*
	Weight Gain	2.87 ± 2.85	2.00 ± 2.27	3.50 ± 2.68	1.69 ± 1.92*
	Swelling	1.60 ± 1.88	1.07 ± 1.83*	2.00 ± 2.53	0.88 ± 1.75*
	Hot Flashes	3.93 ± 2.28	2.33 ± 2.02*	2.81 ± 2.51	1.00 ± 1.59*
	Fatigue	5.40 ± 2.23	2.53 ± 2.42*	5.38 ± 2.03	2.00 ± 2.03*
	Acne-Flares Up	3.47 ± 2.26	2.27 ± 2.19*	4.25 ± 2.41	2.00 ± 1.63*
	Dull Skin	4.13 ± 2.13	2.73 ± 2.12	4.88 ± 2.33	1.75 ± 1.44*
	Headache	3.60 ± 2.47	2.33 ± 1.95	3.38 ± 2.68	1.69 ± 1.70*
	Blind Spots	0.60 ± 1.59	0.47 ± 1.36*	1.44 ± 2.66	0.50 ± 0.89*
	Flatulence	2.87 ± 2.00	1.80 ± 2.04*	2.44 ± 2.03	1.00 ± 1.03*
	Constipation	3.40 ± 2.13	1.93 ± 1.94*	2.25 ± 1.88	0.81 ± 1.22*

The Values represent mean ± SD of percent symptoms at Baseline and End of Treatment. *Indicates Significantly different at p<0.05 between baseline and end of treatment with in Group.

compounds i.e., DRDC/2023/042; DRDC/2023/043; combination (DRDC/2023/042 + 43) and DRD/2023/046 assessed for TNF-α levels were given in Figure 4. Both the test compounds (DRDC/2023/042 and DRDC/2023/043) at tested concentrations of 500 µg/mL inhibited TNF-α production by 17.8 & 23.0 % respectively, in PGE2 treated SKOV3 cells when compared with the cell control. Similarly, SKOV3 cells treated with DRDC/2023/046 have shown inhibition (10.2%) of TNF- α levels compared to controls (Figure 4). Combined test compound (DRDC/2023/042 + DRDC/2023/043), have shown 24.5 %, TNF-α production inhibition at higher concentration (500 µg/mL) in comparison PGE2 treated cells alone (Figure 4).

Proof of Concept Clinical Study: A total of 36 women aged 19 - 45 years were screened as per the inclusion and exclusion criteria mentioned in Table 1. Thirty-one women met the inclusion criteria were randomized and assigned into either of the two groups i.e., Group A (DHP Liquid & Tablet; n=15) and Group B (DSS syrup; n=16). Majority of study participants (42%) were aged 21-25 years, whereas 32% of participants were aged 18-20 years suggesting the study was carried out by recruiting the younger individuals, particularly those in their early twenties.

At screening, Random Blood Sugar (RBS) levels of Group A were 94.9 ± 16.63 mg/dL and Group-B were 89.31 ± 9.99 mg/dL, suggesting the participants were non-diabetic. Similarly, the Thyroid Stimulating Hormone (TSH) levels were assessed among Group A (2.17 ± 1.10 mIU/L) and Group B (2.09 ± 1.05 mIU/L) participants at entry into the study.

The psychological signs and symptoms were given in Table 2 and Figure 5A. Participants administered with DHP liquid and tablet in Group A, as well as DSS syrup used in Group B have shown improvement from irritability & anger, mood swings, crying spell, fatigue and nausea/vomiting signs and symptoms noted during the

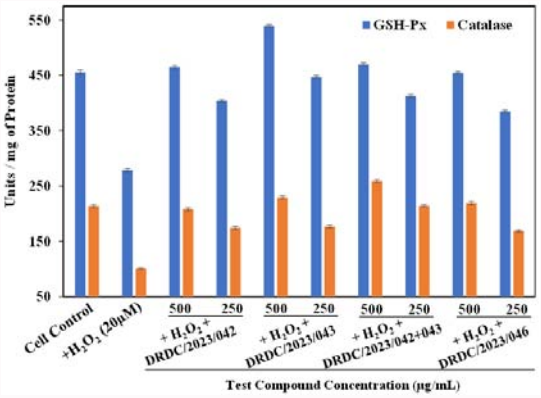

Figure 3: Antioxidant Activity. The bars represent mean±SD of GSH-Px and Catalase levels in SKOV3 cells treated with H_2O_2 to induce oxidative stress, followed by test compound incubation at concentrations of 500 and 250 µg/mL. The higher the levels of antioxidant enzymes indicate ROS quenching potentiality of test compound.

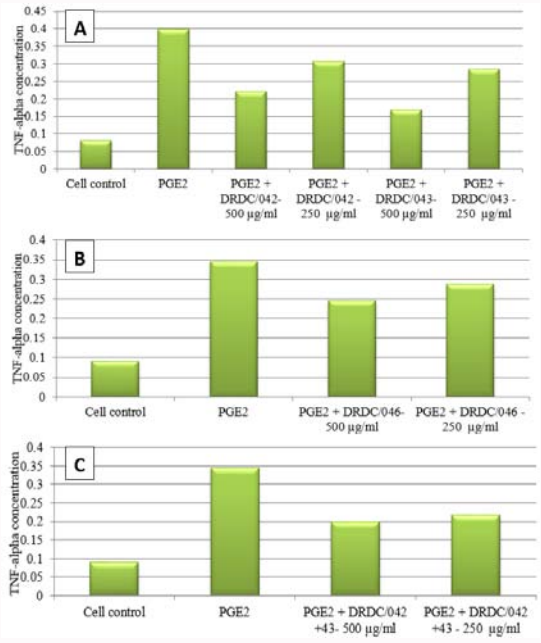

Figure 4: Anti-inflammatory Activity. TNF-α levels in SKOV3 cells treated with PGE2, followed by test compound incubation at concentrations of 500 and 250 µg/mL. A). DHP Liquid; B). DSS Tablet and Syrup and C) Combination of DSS Tablet and Syrup. The bars represent mean of TNF-α levels (in ng/ml).

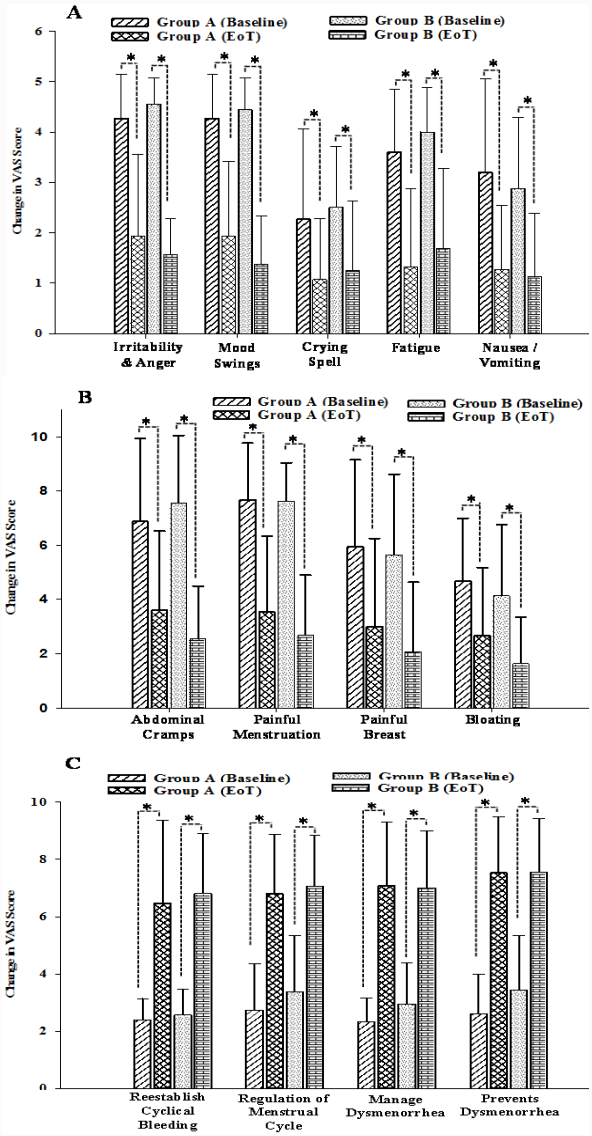

Figure 5: Visual Analogue Scale (VAS) Scores of women with treatment of test compound. **A**. Psychological signs and symptoms of participants. **B**. Physical signs and symptoms of participants; and **C**. Menstrual cycle parameters. Bars represent mean ± SD of VAS Score at baseline, end of treatment for the test compound. *Indicates - Significant different at p<0.05.

menses (Figuere 5A).

Similarly, other psychological signs and symptoms associated with menstrual cycle were improved positively with treatment of DHP liquid & tablet (Group A) and DSS syrup (Group B) (Table 2). The significant difference was noted between the base line and end of treatment with respective test compounds, whereas no significant difference was noted between the groups for the given parameters.

The physical signs and symptoms associated with menstrual cycle viz. abdominal cramps, painful menstruation, painful breast and bloating were also decreased significantly at the end of treatment when compared with baseline (Figure 5B). Both the DHP liquid & tablet group and DSS syrup groups have shown improvement in physical signs and symptoms, but no difference was noted when compared between groups (Table 2 and Figure 5B). Menstrual cycle associated properties such as reestablishment of bleeding, regularization of menstrual cycle, prevention & management of dysmenorrhea, improvement in PCV and control of leukorrhoea was observed among the women of both the Group A and Group B (Figure 5C).

Haemoglobin levels were comparable between baseline and end of treatment among Group A, as well as Group B participants (Table 3). The mean lipid profile levels in Group A at baseline were 148.67 ± 23.72 mg/dL and at end of treatment were 154.20 ± 20.85 mg/dL. Similarly, Group B participant lipid profile levels were comparable at baseline (158.88 ± 26.94 mg/dL) and end of treatment (153.25 ± 18.69 mg/dL) (Table 3). The liver function assessed by ALT and AST at baseline and end of treatment were comparable in both Groups (Table 3). Similarly, renal function parameters viz. serum bilirubin, creatinine and urea levels comparable among the participants of

Table 3: Hematological profile of participants.

S. No.	Parameter	Group – A (n=15)		Group – B (n=16)	
		Baseline	EoT	Baseline	EoT
1	Haemoglobin (g%)	11.87 ± 0.73	12.05 ± 0.67	11.28 ± 1.10	11.73 ± 0.89
2	Alanine Aminotransferase (ALT) (U/L)	19.33 ± 5.04	17.93 ± 5.61	16.81 ± 3.66	16.94 ± 3.15
3	Aspartate Aminotransferase (AST) (U/L)	22.53 ± 5.04	19.33 ± 4.10	20.19 ± 2.86	20.06 ± 3.23
4	Serum Bilirubin (mg/dl)	0.48 ± 0.10	0.46 ± 0.10	0.59 ± 0.16	0.53 ± 0.14
5	Lipid profile	148.67 ± 23.72	154.20 ± 20.85	158.88 ± 26.94	153.25 ± 18.69
6	Serum Urea (mg/dl)	16.33 ± 1.29	14.80 ± 1.52	15.88 ± 2.39	14.56 ± 1.03
7	Serum Creatinine (mg/dl)	0.79 ± 0.06	0.75 ± 0.08	0.76 ± 0.07	0.69 ± 0.08

The values represent mean ± SD. The values were comparable at baseline *vs* EoT (end of treatment) across the Groups.

Group A and Group B at baseline and end of treatment with respective test formulations (Table 3). Ultrasonography (USG) of the pelvis evaluation, suggests Polycystic Ovarian Disease (PCOD) among 5 participants with 4 from Group A, and 1 from Group B. However, no significant changes or variations were noted in the USG findings during the course of the study period, indicating no measurable effect on pelvic ultrasonographic outcomes.

None of the participants have reported the information about the Adverse Events (AEs) and Severe Adverse Events (SAEs) related to use of test compound in any of the three follow up visits. In addition, the serum parameters for liver and rental function were comparable before and end of treatment with respective test formulation suggesting the no-test compound related toxicity on liver and kidneys, respectively (Table 3).

Discussion

Menstrual cycle involves complex and multifactorial functions such as hormonal, physiological, immune-inflammatory activities. In the current study, both test formulations (DHP syrup & tablet and DSS syrup) have shown significant anti-oxidant and anti-inflammatory activity in SKOV3 cell lines [9]. Have reported that the polyherbal formula comprised of *Populus nigra* and *Rosmarinus officinalis* extracts shown antioxidant and anti-inflammatory activity. The results of antioxidant potential of test compound DRDC/2023/042 & 043 (syrup and tablet) and the DSS syrup corroborated the observations of [10]. Similarly, the anti-inflammatory activity in terms of TNF-α levels have decreased in SKOV3 cells treated with DRDC/2023/042; DRDC/2023/043 and DRDC/2023/046 suggesting modulation of inflammation associated with menstrual cycle.

The intended therapeutic use of test compound - DHP syrup and tablet, is twice a day, hence the *in vitro* data of antioxidant and anti-inflammatory potential suggests it's therapeutic efficacy. TNF-α is a pleiotropic and proinflammatory cytokine which is produced by several cell-types and in particularly high amounts by activated macrophages. TNF-α role of in luteal function, is not clear but proposed as it inhibits basal secretion of progesterone and stimulates prostaglandin production [11]. In the current study the SKOV3 cells treated with PGE2 and simultaneously treated with DHP syrup and tablet, suggesting physiological relevance. Similarly, DSS syrup have shown considerable inhibition of TNF-α (10.2%) levels compared to control suggesting it's anti-inflammatory activity (Figure 4).

DHP Liquid formulated with Lodhra (*symplocos racemosa*), Manjith (*Rubia cordifolia*), Anantmool (*Hemidesmus indicus*), Bala (*Sida cordifolia*), etc. with intended use of menstrual cycle regularity, could be due to its antioxidant and anti-inflammatory potential evident through *in vitro* assays. Similar findings were reported by Mishra and Reddy [10] for polyherbal compound Sarvonutra-Diva™ possessing *Asparagus racemosus, Saraka ashoka, Myristica fragrance, Symplocos racemose, Woodfordia fruticosa*, etc. The antioxidant and anti-inflammatory activities were attributed to a range of phytoconstituents present in botanical/poly-herbal extracts. On the other hand, DSS Syrup also comprised of Ashoka (*Saraca ascoca*), Manjishtha (*Rubia cordifolia*), Sariva (*Hemidesmus indicus*), Lajjalu (*Mimosa pudica*), suggesting it's antioxidant and anti-inflammatory potential could be due to the range of phytoconstituents present in these herbs.

Research suggests that menstrual cycle related psychological signs and symptoms vary across individuals as well as with age [12]. Depression symptom worsening during the follicular phase was highest for clinically depressed, moderate for sub clinically depressed, and low for non-depressed women. Current study results are in line with Handy, et al that use of test compound (DHP syrup & tablet and DSS syrup) led to significant improvement for various psychological symptoms associated during the menses among participants (Figure 5A and Table 2).

In addition, current study has revealed that menses associated properties such as bleeding, menstrual cycle, prevention & management of dysmenorrhea, improvement in PCV and control of leukorrhoea has improved. Gama et al (2014) have reported that oil extracted from seeds of the Borago officinalis L. plant is a rich source of ɣ-linoleic acid (GLA), speculated for downregulation of PGE2 production, thereby effective in the treatment of the physical and emotional symptoms of PMS.

The test compounds have shown no toxicity on SKOV3 cells up to 1000µg/ml, wherein the LD_{50} as higher as 500 µg/ml against the individual as well as combination of DHP syrup and tablets. In addition, clinical trial data suggests no AEs and SAEs among any of the participants. Further to this, haematological data specially LFT and RFT are well within the normal range and were comparable at baseline to end of treatment with test formulations. Suggesting neither toxicity nor adverse effects of test compound related among the participants of both the groups.

Conclusion

The test compounds DRDC/2023/042 & 043; DRDC/2023/046 possess antioxidant and anti-inflammatory activity. Both the groups of participants have shown positive response for the signs and symptoms associated with menstrual cycle when compared with

base line and end of treatment. It may be noted that, between two test compounds no significance difference was noted suggesting the efficacy of both the formulations. None of the participants have reported Adverse Events (AEs) and Severe Adverse Events (SAEs) associated with test compound throughout the treatment period. Suggesting the efficacy of botanical based formulations without notable side effects in menstrual cycle.

References

1. Brännström M, Fridén BE, Jasper M, Norman RJ. Variations in peripheral blood levels of immunoreactive tumor necrosis factor α (TNFα) throughout the menstrual cycle and secretion of TNFα from the human corpus luteum. Eur J Obstet Gynecol Reprod Biol. 1999;83(2):213-7.
2. Cannon JG, Dinarello CA. Increased plasma interleukin-1 activity in women after ovulation. Science. 1985;227(4691):1247.
3. Vašková J, Klepcová Z, Špaková I, Urdzík P, Štofilová J, Bertková I, et al. The Importance of Natural Antioxidants in Female Reproduction. Antioxidants. 2023;12(4):907.
4. Agarwal A, Gupta S, Sharma RK. Role of Oxidative Stress in Female Reproduction. Reprod Biol Endocrinol. 2005;3:28.
5. Handy A, Greenfield SF, Yonkers KA, Payne LA. Psychiatric Symptoms Across the Menstrual Cycle in Adult Women: A Comprehensive Review. Harward review of psychiatry. 2022;30(2):100-17.
6. Laessle RG, Tuschl RJ, Schweiger U, Pirke KM. Mood changes and physical complaints during the normal menstrual cycle in healthy young women. Psychoneuroendocrinology 1990;15(2):131-8.
7. Owens SA, Eisenlohr-Moul TA, Prinstein MJ. Understanding when and why some adolescent girls attempt suicide: an emerging framework integrating menstrual cycle fluctuations in risk. Child Dev Perspect. 2020;14(2):116-23.
8. Srivalliputturu SB, Yathapu SR. Effects of zinc status on cell cycle and viability: An in vitro study in monocytes (THP-1) and muscle (Rhabdomyosarcoma) cells. Indian J Pharmacol. 2025;57(3):187-192.
9. Grigore A, Vulturescu V, Neagu G, Ungureanu P, Panteli M, Rasit I. Antioxidant–Anti-Inflammatory Evaluation of a Polyherbal Formula. Pharmaceuticals. 2022;15(2):114.
10. Mishra B, Reddy YS. *In Vitro* Efficacy Evaluation of Sarvonutra-DivaTM: The Herbal Pre-Mix for Women Wellbeing. Int J Res Eng Sci. 2022;10(1):15-21.
11. Wuttke W, Jarry H, Pitzel L, Knoke I, Spiess S. Luteotrophioc and luteolytic actions of ovarian peptides. Hum Reprod. 1993;8(2):141-6.
12. Kiesner J. One woman's low is another woman's high: paradoxical effects of the menstrual cycle. Psychoneuroendocrinology. 2011;36(1):68-76.
13. Gama C.R.B, Lasmar R, Gama G.F, et al. Premenstrual Syndrome: Clinical Assessment of Treatment Outcomes Following Borago officinalis Extract Therapy. RBM, 2014; 71, 211-217.