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Original Research Article (Experimental)

Development and evaluation of aphrodisiac potential of a classical ayurvedic formulation, '*Kaamdev ghrita*' in rat model

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ABSTRACT

Background: *Ghee* is widely considered as the Indian name for clarified butterfat and processing of *ghee* with therapeutic herbs i.e. *ghrita* is renowned for augmenting their medicinal properties. *Kaamdev ghrita* (also known as '*Vajikarana Rasayana*') is cow *ghee* based classical Ayurvedic formulation from the aphrodisiac category, which is used to ameliorate and potentiate sexual performance and also in the treatment of sexual dysfunctions, infertility, and premature ejaculation.
Objective: Present research work deals with the organoleptic, physicochemical, and biological assessment of *Kaamdev ghrita* for its aphrodisiac activity using *in-vivo* animal models.
Material and methods: *Kaamdev ghrita* was prepared using Indian cow's *ghee* as per standard Ayurvedic classical texts and subjected to organoleptic (color, odor, taste, texture, touch), physicochemical (acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, extractive values, refractive index, and specific gravity) analyses as per the standard pharmacopeial procedures. The aphrodisiac potential of *ghrita* in rat model was evaluated by monitoring sexual behavioral performance using different parameters (mount frequency and latency, intromission frequency and latency, anogenital grooming and sniffing) at the dose of 150 and 300 mg/kg body weight.
Results: The physicochemical evaluation of *Kaamdev ghrita* showed higher acid value, iodine value, refractive index, and specific gravity whereas the lower saponification and peroxide value than the plain *ghee*. *Kaamdev ghrita* revealed the presence of flavonoids, alkaloids, saponins, sterols, terpenoids, coumarins, tannins, and showed remarkable antioxidant activity by *in-vitro* assays. It augmented the sexual performance in a dose-dependent manner as indicated by significant improvement ($P < 0.05$) in mount frequency and latency, intromission frequency and latency, anogenital grooming, and sniffing as compared to plain *ghee* treated control group. The present investigation has corroborated the ethnopharmacological claim of *Kaamdev ghrita* for its aphrodisiac potential.
Conclusion: *Kaamdev ghrita* exhibited aphrodisiac activity which may be attributed to the presence of antioxidant herbs present in it. It is the first scientific report on validation of the traditional claim of *Kaamdev ghrita* for its aphrodisiac potential.

1. Introduction

Ayurveda describes a good number of formulations containing '*panchgavya*' (cow *ghee*, milk, urine, curd, and dung) components either individually as well as conjointly with other substances of herbal, mineral or animal origin. Several formulations based on each one of these five components with medicinal claims are reported in the Indian traditional system of medicines [1]. *Ghee* (clarified butter) is usually prepared from cow milk and described as a traditional adjuvant/vehicle in *Ayurveda*. Processing of '*ghee*' with plant material/materials i.e. *Ghrita* is well acclaimed for

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enhancing the therapeutic efficacy of plant ingredients [2,3]. *Ghrita* is one of the popular Ayurvedic formulations prepared by boiling cow *ghee* (*Sneha dravya*), a paste of crude plant powder in water (*Kalka*), fresh juice of plant (*Svarasa*) and water (*Drava dravya*) in specific proportions as per the formula and procedures are given in Ayurvedic texts [1,4].

In India, the practitioners of *Ayurveda* recognized the vital importance of virility and designed '*Vajikarana*' therapy [1,4]. *Vajikarana* is well defined in the *Rigveda* and the *Yajurveda* (the first written texts of medicine) and close to 'aphrodisiac' in modern context [5]. *Kaamdev ghrita* (KG) also known as a '*Vajikarana ghrita*', important *ghee* based Ayurvedic formulation is mentioned under the aphrodisiac category of drugs i.e. nourishment to '*shukradhatu*' [4,6]. KG comprises various '*Vajikarana Rasayana*' (aphrodisiac herbs which ameliorate and potentiate sexual performance and are useful in treating sexual complaints) having ethnopharmacological relevance viz. *Withania somnifera* (L.) Dunal, *Tribulus terrestris* L., *Asparagus racemosus* (Willd), *Pueraria tuberosa* (Willd.) DC., *Desmodium gangeticum* (L.) DC., *Zingiber officinale* (Roscoe), *Tinospora cordifolia* (Willd) Miers, *Sida cordifolia* (L.), *Boerhaavia diffusa* (L.), *Nelumbo nucifera* Gaertn [5,7–9]. The name *'Kaamdev ghrita'* is assigned from the name of '*Kaamdev*', a divine personality mentioned in ancient texts who symbolizes strength, vigor, and excellent health. As per the '*Charak Samhita*' and other Ayurvedic references, the use of KG endows an individual with good physique, potency, strength, and complexion and sexual exhilaration and potency which is helpful in many common sexual dysfunctions, including infertility, premature ejaculation and erectile dysfunction [10]. The regular and long-term use of KG not only provides nourishment to the body to ensure a healthy life but also improves fertility in both males and females [4].

Despite the traditional use of KG for aphrodisiac purposes, there are no systematic and scientific studies to delineate its aphrodisiac potential. Hence, present research work was aimed to develop KG as per Ayurvedic reference texts, evaluate KG on organoleptic and physicochemical grounds and validate the ethnopharmacological claims for its aphrodisiac activity. Oxidative stress is considered as one of the leading causes of infertility, therefore antioxidant studies of KG were also evaluated by *in-vitro* methods.

2. Material and methods

2.1. Procurement of cow ghee and herbal ingredients

An authentic and fresh sample of cow *ghee* (from Indigenous cow breed i.e. *Bos indicus*) was procured from *Go-Vigyan Anusandhan Kendra*, Deodapar, Nagpur, Maharashtra, India. The herbal raw material was procured from experts at Shri-Shail Medifarms, Nagpur, Maharashtra, India, identified and authenticated [11–13] at the Department of Botany, Rashtrasant Tukdoji Maharaj University, Nagpur, Maharashtra, India (Voucher Specimen No. 8961/21 to 8961/40).

2.2. Preparation of Kaamdev ghrita (KG)

The KG was prepared by using previously authenticated and stated herbs in the prescribed manner (Table 1) as per the pronounced procedure described in the reference text (Fig. 1) [1,4]. Briefly, a specified quantity of *W. somnifera* roots, *T. terrestris* fruits, *P. tuberosa* roots, *A. racemosus* bulbs, *T. cordifolia* stems, *Z. officinale* rhizomes, *D. gangeticum* leaves, *S. cordifolia* roots, *B. diffusa* whole plant and *N. nucifera* seeds were collected, dried, powdered and passed through sieve number 44 to get a coarse powder. This coarse powder mixture was boiled with water (25 lit) and concentrated to 6 lit followed by filtration *(Kwath)*. The remaining herbs (50 g each) i.e. *Piper longum* fruits, *Hemidesmus indicus* roots, *Lilium polyphyllum* bulbs, *Abutilon indicum* whole plant, *Roscoea purpurea* tubers, *Cinnamomum tamala* leaves, *Pterocarpus santalinus* heartwood, *Vigna mungo* cereal, *Vitis vinifera* fruits were dried, cleaned, powdered separately and passed through sieve number 85 to get a fine powder. The powdered ingredients were transferred to wet grinder and grounded with sufficient quantity of water to get homogenous mass/paste i.e. *Kalka* (fine paste) which was added to the previous filtrate (*Kwath*) along with cow *ghee* (1.5 kg), *Saccharum officinarum* juice (1.5 lit) and sugar cubes (100 g). This whole mixture was heated in a thick bottomed big vessel with continuous stirring on low to moderate fire till complete evaporation of water and appearance of specific characters of *ghee*. Further, the contents, before cooling, were filtrated through a two-fold muslin cloth and the filtrate i.e. KG was collected in a clean autoclaved glass bottle and stored for further studies.

2.3. Organoleptic and physicochemical evaluation

The plain *ghee* and KG samples were analyzed for various organoleptic (color, odor, taste, texture, touch) and physicochemical parameters (acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, extractive values, refractive index, and specific gravity) as per standard procedures described in reference books and published reports [2,3,14–16].

2.4. Biological activity evaluation

2.4.1. In-vitro anti-oxidant evaluation

Antioxidant activity of freshly prepared test samples i.e. plain *ghee* and KG on the same day (by preparing the test solution in DMSO) was evaluated using various *in-vitro* methods [3,17–19]. The antioxidant potential was expressed as IC_{50} i.e. the concentration of test samples that inhibited the formation of free radicals by 50%. Ascorbic acid was used as a reference standard in all methods.

2.4.1.1. DPPH radical scavenging assay. The hydrogen-donating ability of plain *ghee* and KG was measured using the established 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method [3,17–19]. Briefly, 3.0 mL of 10–100 μg mL^{-1} concentration of test solutions and 1.0 mL of 0.1 mM solution of DPPH in ethanol were mixed, the reaction mixture was kept in the dark for 30 min and then absorbance was measured at 517 nm using UV spectrophotometer (UV-1700, Shimadzu Corporation, Japan). Lower absorbance indicates a higher free radical-scavenging activity.

2.4.1.2. Nitric oxide (NO) radical scavenging assay. NO generated in Griess reaction was used to evaluate free radical scavenging activity of test drugs i.e. plain *ghee* and KG. Briefly, 3.0 mL of test samples at the concentration of 10–100 μg mL^{-1} and sodium nitroprusside (5 mM) in phosphate-buffered saline were mixed, allowed to incubate at 25 °C for 150 min and further reacted with Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride). The chromophore formed during the diazo-coupling of nitrite with sulphanilamide and naphthyl ethylenediamine was subjected for absorbance measurement at 546 nm. The reaction mixture without the test sample but, with the same quantity of distilled water was served as control [3,17–19].

2.4.1.3. Hydrogen peroxide scavenging assay. The test samples were subjected to Hydrogen peroxide (H_2O_2) scavenging assay based on replacement titration [3,17–19]. Briefly, 1.0 mL of 10–100 μg mL^{-1} concentration of test samples, 2 drops of 3% ammonium molybdate, 10 mL of 2 M H_2SO_4, 1.0 mL of 0.1 mM H_2O_2, and 7.0 mL of 1.8 M KI

Table 1
Herbal composition of *Kaamdev Ghrita*.

Name	Family	Common name	Voucher Specimen No.	Part of plant	Quantity taken (kg)
Withania somnifera (L.) Dunal	Solanaceae	*Ashwagandha*	8961/21	Roots	2.5
Tribulus terrestris L.	Zygophyllaceae	*Gokhru*	8961/22	Spiky fruits	1.25
Pueraria tuberosa (Willd.) DC.	Fabaceae	*Vidarikand*	8961/23	Roots	0.5
Asparagus recemosus (Willd)	Liliaceae	*Shatavari*	8961/24	Bulbs	0.5
Tinospora cordifolia (Willd) Miers	Menispermaceae	*Guduchi*	8961/25	Stems	0.5
Zingiber officinale (Roscoe)	Zingiberaceae	*Ginger*	8961/26	Rhizomes	0.5
Desmodium gangeticum (L.) DC.	Papilionaceae	*Shalparni*	8961/27	Leaves	0.5
Sida cordifolia (L.)	Malvaceae	*Bala*	8961/28	Roots	0.5
Boerhaavia diffusa (L.)	Nyctaginaceae	*Punarnava*	8961/29	Whole plant	0.5
Nelumbo nucifera Gaertn.	Nymphaeaceae	Lotus	8961/30	Seeds	0.5
Piper longum (L.)	Piperaceae	*Pippali*	8961/31	Fruits	0.05
Hemidesmus indicus (L.) R. Br. ex Schult.	Asclepiadaceae	*Sariva*	8961/32	Roots	0.05
Lilium polyphyllum D. Don	Liliaceae	*Kshirkakoli*	8961/33	Bulbs	0.05
Abutilon indicum (L.) Sweet	Malvaceae	*Atibala*	8961/34	Whole plant	0.05
Roscoea purpurea Sm.	Zingiberaceae	*Kakoli*	8961/35	Tubers	0.05
Cinnamomum tamala (Buch.-Ham.) T.Nees and Eberm.	Lauraceae	*Tejpan*	8961/36	Leaves	0.05
Pterocarpus santalinus L.f.	Fabaceae	*Lalchandan*	8961/37	Heartwood	0.05
Vigna mungo (L.) Hepper	Black-gram	*Urad*	8961/38	Cereal	0.05
Vitis vinifera L.	Vitaceae	Raisins	8961/39	Fruits	0.05
Saccharum officinarum L.	Poaceae	Sugarcane (ikshu rasa)	8961/40	Stem juice	0.05

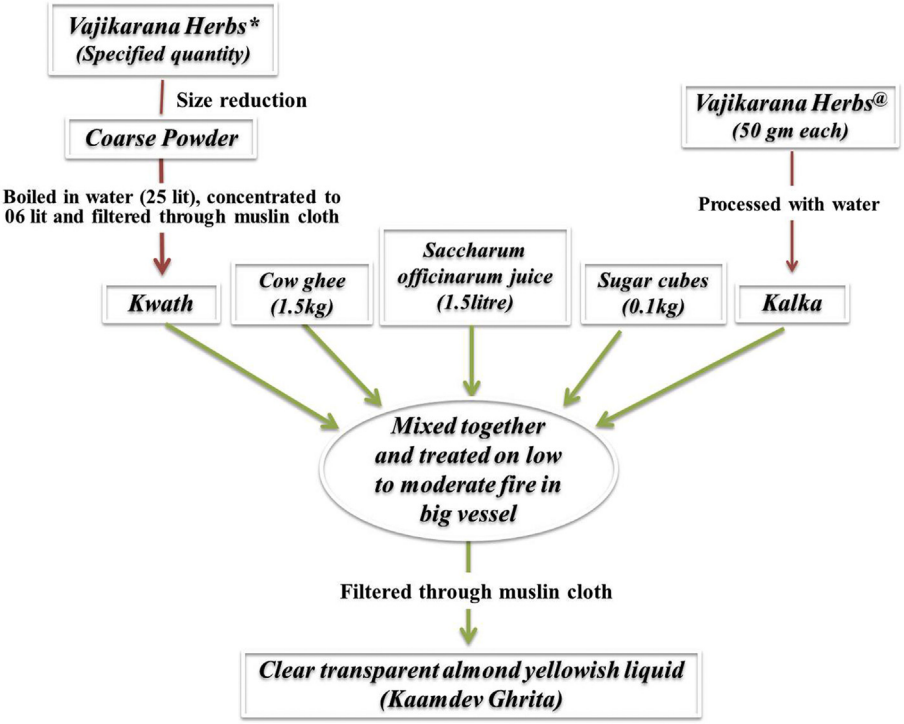

Fig. 1. Flowchart for preparation of *Kaamdev ghrita* (KG). In Figure, ***Vajikarana* Herbs***- *Withania somnifera* roots, *Tribulus terrestris* fruits, *Pueraria tuberosa* roots, *Asparagus recemosus* bulbs, *Tinospora cordifolia* stems, *Zingiber officinale* rhizomes, *Desmodium gangeticum* leaves, *Sida cordifolia* roots, *Boerhaavia diffusa* whole plant and *Nelumbo nucifera* seeds. ***Vajikarana* herbs@**- *Piper longum* fruits, *Hemidesmus indicus* roots, *Lilium polyphyllum* bulbs, *Abutilon indicum* whole plant, *Roscoea purpurea* tubers, *Cinnamomum tamala* leaves, *Pterocarpus santalinus* heartwood, *Vigna mungo* cereal, *Vitis vinifera* fruits.

were mixed and the resultant solution was titrated with 5.09 mM sodium thiosulphate ($Na_2S_2O_3$) till complete disappearance of yellow color. Hydrogen peroxide scavenging potential was calculated as

$$\% \text{ Inhibition} = (V_0 - V_1)/V_0 \times 100$$

Where, V_0 was the volume of $Na_2S_2O_3$ solution used to titrate the control sample in the presence of H_2O_2 (without *ghee*/*ghrita*) and V_1 was the volume of $Na_2S_2O_3$ solution used in the presence of the *ghee*/*ghrita*.

2.4.2. Invivo animal studies

2.4.2.1. Experimental animals. Albino rats of Sprague–Dawley strain weighing 150–200 g of either sex were housed in polypropylene cages in an air-conditioned area at 25 ± 2 °C with 12/12 h light/dark cycle. All animals had free access to a standard pellet diet and clean water *ad libitum*. All the procedures in the present investigation were carried out under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines for the care and use of laboratory animals. A preceding approval was obtained from the Institutional Animal Ethics Committee (IAEC) of Government College of Pharmacy,

Karad, Maharashtra, India which is registered under CPCSEA, Government of India with Registration No. 209/GO/a/2000/CPCSEA.

2.4.2.2. Acute toxicity study. A single-dose acute toxicity study was carried out as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines [18,20,26]. Male Sprague Dawley rats (n = 6 rats, 3 at preliminary and 3 at the confirmatory stage) were selected, kept fasting for overnight providing only water followed by oral administration of KG at the dose of 10 mg/kg body weight and monitored for 7 days. If mortality observed in 2 out of 3 animals, then the administered dose was assigned as a toxic dose whereas if mortality observed in 1 animal, then the same dose was repeated to confirm the toxic dose. In absence of mortality, the procedure was repeated for further higher doses such as 100, 500, and 2000 mg/kg body weight, and animals were observed daily for a week to distinguish any physical alterations.

2.4.2.3. Aphrodisiac potential evaluation

2.4.2.3.1. Preparation of animals for study. Male rats were given the test drugs (plain cow *ghee* and KG) by suspending in 0.1% sodium carboxymethyl cellulose for oral administration and trained for sexual experience (copulatory behavior), three times a day at an interval of every 3 h over 10 days. Before testing for copulatory performance, each male rat was exposed for 30 min to a female rat (used as mating stimulus) in behavioral estrous for several days to provide sexual experience. The male rat, which failed to show any sexual interest during the test period was noted as inactive and discarded from the study. The female rats were treated with a single subcutaneous dose of estrogen benzoate (2 μg/kg body weight) and progesterone 500 μg/kg body weight at 48 h and 6 h before experimentation, respectively to bring them in estrous phase [21,22].

The experimental animals were categorized into four groups consisting of six animals each; Group I received vehicle (plain cow *ghee*) and served as negative control; Group II received 150 mg/kg body weight of KG and group III was administered with 300 mg/kg body weight of KG orally, while Group IV served as positive control i.e. sildenafil, 4.5 mg/kg body weight [21]. Doses of KG were selected based on the acute toxicity study performed.

2.4.2.3.2. Sexual behavior analysis. The aphrodisiac activity was performed in a silent room under dim red light in specially designed transparent cages measuring 50 × 30 × 30 cm. Any jerking movement of the mating area was avoided to enable the rats to chase each other. After each trial, the mating area was cleaned to remove urine trails left by the earlier rat to avoid possible alteration in sexual behavior [21–24]. Briefly, each male rat was trained for 15 min at a time till elicitation of sexual behavior followed by administration of test formulations/drugs and exposure to receptive females kept in groups (1 male with 5 females). After 30 min of administration of test samples, sexual behavior of animals was monitored individually for 60 min by noting different parameters like mount latency (time duration for the first mount following the introduction of a female into the cage), genital grooming and anogenital sniffing, intromission frequency (total number of intromission preceding ejaculation), mount frequency (total number of mounts preceding ejaculation), intromission latency (time duration for first intro-mission following the introduction of the female into the cage).

2.5. Phytochemical screening

The KG was subjected to phytochemical screening by various phytochemical tests for the presence of various primary and secondary metabolites using standard protocols [25–28].

2.6. Statistical analysis

The results are expressed as mean ± SEM and data from all the groups were evaluated using a One-way analysis of variance (ANOVA) followed by Newman–Keuls test using GraphPad PRISM (Version 5).

3. Results

3.1. Organoleptic evaluation of plain ghee and KG

Specific sensory peculiarities of plain *ghee* and KG are presented in Table 2. Various organoleptic characters (color, odor, and taste) of plain *ghee* found to be different than KG. The taste and odor (aroma) were observed as best for freshly prepared and warm *ghee/ghrita* samples. Plain *ghee* and KG exhibited almost identical touch and texture i.e. smooth and soft touch and greasy texture.

3.2. Physicochemical evaluation of plain ghee and KG

Physicochemical analysis comprising acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, extractive values, refractive index, and the specific gravity of plain *ghee* and KG are shown in Table 3.

KG showed the higher acid value and iodine value than plain *ghee* whereas the saponification value and peroxide value of KG was found to be lower than plain *ghee*. Changes in the unsaponifiable matter of plain *ghee* and *ghrita* samples were found insignificant. Plain *ghee* and KG exhibited higher hexane soluble extractive values than ethanol soluble and ether soluble extractive values. The present study revealed a lower refractive index (i.e. 1.4448) and specific gravity (i.e. 0.9124) of plain *ghee* than that of KG whereas very minor changes were recorded in ash values (total ash, acid insoluble ash, water-soluble ash) of plain *ghee* and KG.

3.3. Biological activity evaluation

3.3.1. Antioxidant evaluation

Plain *ghee*, KG, and ascorbic acid exhibited concentration-dependent (10–100 μg mL^{-1}) free radical scavenging activity. KG revealed the IC_{50} value of 17.23 ± 7.42 μg mL^{-1} by DPPH method whereas plain *ghee* showed IC_{50} value as 40.76 ± 5.02 μg mL^{-1} (Table 4). In NO method, IC_{50} for KG was found to be 20.32 ± 6.52 μg mL^{-1}, whereas plain *ghee* demonstrated an IC_{50} value of 41.43 ± 6.22 μg mL^{-1}. Plain *ghee* and KG exhibited dose-dependent H_2O_2 scavenging activity with the IC_{50} of 43.13 ± 4.43 μg mL^{-1} and 21.78 ± 5.76 μg mL^{-1} respectively. Ascorbic acid proved to have excellent antioxidant potential by all *in-vitro* methods.

3.3.2. Acute toxicity study

Neither any toxicity nor mortality was shown by KG up to a maximum dose of 2000 mg/kg of body weight. There was no change in cage side clinical and neurological observations and weekly body weight for 14 days.

3.3.3. Aphrodisiac activity of KG

Pronounced enhancement in overall sexual behavior performance as evidenced by various parameters was recorded for the KG at the selected doses of 150 mg/kg and 300 mg/kg body weight (Fig. 2). One-way ANOVA showed a significant increase ($P < 0.05$) in mount frequency and latency, intromission frequency and latency, anogenital grooming, and sniffing in KG treated group as compared to the untreated control group. Mount latency time, the sign of physical exhaustion during a sexual act was reduced by 46% in KG

Table 2
Organoleptic (Sensory) evaluation of plain *ghee* and *Kaamdev Ghrita*.

Characters	Plain *ghee*	KG
Color	Golden yellow	Almond yellowish
Odor	Aromatic, pleasant, characteristic	Aromatic, pleasant, characteristic
Taste	Characteristic	Slightly bitter, astringent, slightly pungent
Touch, Texture	Smooth, soft, greasy	Smooth, soft, greasy

Table 3
Physicochemical evaluation of plain *ghee* and *Kaamdev Ghrita*.

Physicochemical parameter	Plain *ghee*	KG
Acid value	0.334 ± 0.05	1.3202 ± 0.05
Peroxide value	1.581 ± 0.2	1.450 ± 0.4
Iodine value	38.98 ± 4.1	48.56 ± 3.2
Saponification value	227.1 ± 21	202.78 ± 18
Unsaponifiable matter	0.201 ± 0.02	0.108 ± 0.02
Ethanol-soluble extractive (%)	15.9 ± 2.12	12.25 ± 2.65
Hexane-soluble extractive (%)	93.5 ± 3.02	75.88 ± 2.12
Ether-soluble extractive (%)	6.85 ± 0.82	10.28 ± 0.34
Refractive Index	1.4448 ± 0.01	1.4539 ± 0.02
Specific gravity	0.9124 ± 0.03	0.9582 ± 0.02
Total ash (%)	0.34 ± 0.03	0.52 ± 0.03
Acid insoluble ash (%)	0.12 ± 0.01	0.11 ± 0.01
Water soluble ash (%)	0.02 ± 0.01	0.02 ± 0.02

All values are mean of three independent repeated experiments and expressed as mean ± SEM, KG- *Kaamdev Ghrita*.

treated group at the dose of 150 mg/kg body weight whereas 59% reduction was observed at 300 mg/kg body weight dose as compared to control group. Intromission frequency was significantly increased ($P < 0.05$) at both doses of KG in comparison to the untreated group. KG exhibited 56% ($P < 0.05$) and 68% ($P < 0.05$) reduction in intromission time at the dose of 150 mg/kg and 300 mg/kg body weight, respectively whereas standard sildenafil showed 72% reduction when compared to control. A significant ascendant shift ($P < 0.05$) in anogenital sniffing and genital grooming was recorded with KG at the dose of 300 mg/kg body weight. The effect of KG at both doses was found to be as potent as sildenafil ($P < 0.005$). Promising improvement in the sexual behavior of rats treated with KG at both doses is the positive sign of effective aphrodisiac action.

3.4. Phytochemical screening

The results from the various phytochemical analysis of KG revealed the presence of mainly; flavonoids (Shinoda test), alkaloids (Mayer, Hager, Dragendorff, and Wagner test), saponins (foam test), sterols and terpenoids (Salkowski and Liebermann–Burchard test), tannins (Ferric chloride test), amino acids (Ninhydrin test), proteins (Biuret test), and sugars (Molisch test, Fehling test, Benedict test).

4. Discussion

4.1. Organoleptic evaluation

The carotenoids from cow *ghee* are responsible for its distinctive golden yellow color. The more residual moisture content during low heating temperature decreases the keeping quality of *ghee* whereas high heating temperature darkens the color with the increase in the keeping quality of resultant *ghee* and reduction in the vitamin A [2,3]. The color of a *ghrita* is also governed by the nature of herbal ingredients, processing, clarification temperature, and heating duration, etc. [2]. *Ghee* possesses the characteristic aroma which is contributed by more than one hundred flavour compounds present in it and free fatty acids, carbonyls, and lactones are the major groups of compounds contributing to *ghee* flavour. However, the complex mixture of compounds produced during the various stages of processing contributed to the characteristic aroma and taste of the *ghrita* formulations [2,3].

4.2. Physicochemical evaluation

Quantity of potassium hydroxide required to neutralize free acids expresses the acid value of a fat or oil. In the present investigation, the acid value represented the amount of free fatty acids formed in *ghee* during processing. Over some time, with the rancidification of oil/fats, triglycerides get converted into fatty acids and glycerol and thus an increase in the amount of acids is observed. Freshly prepared plain *ghee* had not undergone much hydrolysis and therefore showed lower acid value whereas several herbs processed together in KG, dissolving different phytoconstituents as revealed by higher acid values [2,3].

Fatty acid composition and storage conditions determine the extent of lipid peroxidation, which is assumed as a major deteriorative change commonly observed in fat [2,3]. Peroxide value is an index of the degree of auto-oxidation, giving initial evidence of rancidity in unsaturated fats and oils. High acidity in butterfat signifies high peroxide value. Ganguly and Jain reported easier and quicker oxidation of the free unsaturated acids than the same acids in intact glycerides [29]. KG showed lower peroxide value than plain *ghee,* which might be contributed by antioxidant components of herbs used in the preparation of KG.

The quantity of iodine absorbed at unsaturation defines the iodine value which signifies the degree of unsaturation of the *ghrita*

Table 4
In-vitro antioxidant evaluation of plain *ghee* and *Kaamdev Ghrita*.

IC_{50} Values ($\mu g.mL^{-1}$)			
Method	Plain *ghee*	KG	Ascorbic Acid
DPPH Method	40.76 ± 5.02 (0.9877)	17.23 ± 7.42 (0.9763)	14.38 ± 4.33 (0.9762)
NO Method	41.43 ± 6.22 (0.9732)	20.32 ± 6.52 (0.9712)	17.82 ± 5.66 (0.9826)
H_2O_2 Method	43.13 ± 4.43 (0.9839)	21.78 ± 5.76 (0.9829)	20.21 ± 5.11 (0.9458)

All values are mean of three independent repeated experiments and expressed as mean ± SEM.
Values in bracket indicate r* i.e. regression coefficient, KG- *Kaamdev Ghrita*.

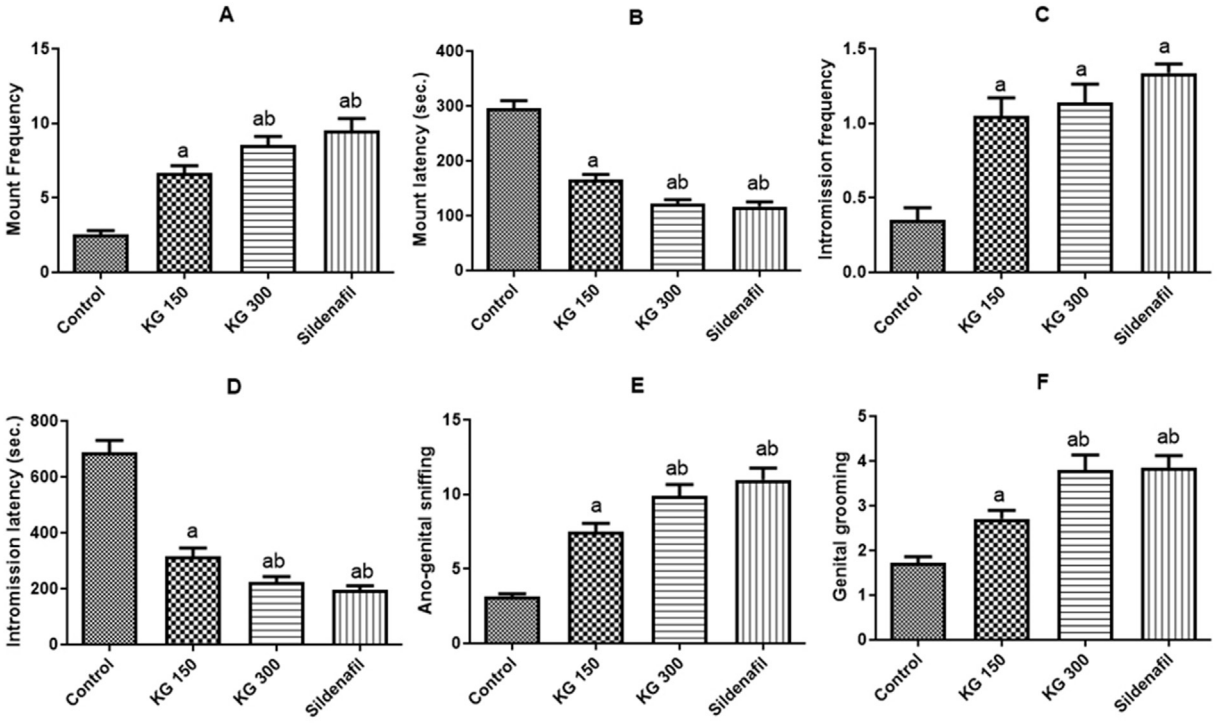

Fig. 2. Aphrodisiac activity of *Kaamdev Ghrita*. In figure, **A: Mount Frequency, B: Mount latency (sec.), C: Intromission frequency, D: Intromission latency (sec.), E: Ano-genital sniffing and F: Genital grooming**; All values are expressed as mean ± SEM, n = 6, where a = $P < 0.05$ vs. Control and b = $P < 0.05$ vs.KG150 (One-way ANOVA followed by Newman–Keuls *post hoc* test).

formulations [2,3,30]. KG, due to the presence of different herbs revealed more iodine value than plain *ghee* suggesting the beneficial role of numerous phytoconstituents in lowering the degree of saturation [2,3].

The saponification value is directly proportional to the fatty matter content and assumed as an index of mean molecular weight (or chain length) of all the fatty acids of glycerides present in fat. It specifies the number of reactive terminal acid groups in the fat. Rancidity in *ghrita* corresponds to the fatty matter content or the carboxylic functional groups per unit mass, which affects the shelf life and therapeutic efficacy of a *ghrita* [2,3]. Possibilities of some interactions between phytoconstituents and *ghee* components might have contributed to the lower saponification value of KG compared to plain *ghee*.

Unsaponifiable matter can be estimated by the extraction of the saponified substance under examination with an organic solvent. The unsaponifiable matter is the portion of *ghee* or *ghrita* formulation consisting of substances (lipids of natural origin such as sterols, pigments, vitamins, higher aliphatic alcohols, and hydrocarbons as well as any non-volatile foreign organic matter) which are not saponifiable by alkali hydroxides [2,3].

Extractive values of a crude drug represent the nature of chemical class and solubility of phytoconstituents extracted in respective solvents.

The refractive index is a characteristic feature of the fatty materials as well as the formulations containing them and often used to determine its purity or measure the concentration. The higher refractive index shows more concentration of light facilitating the rancidity of *ghrita* i.e. decomposition of *ghrita*. The refractive index escalates with the decrease in the chain length whereas a double bond upraises the refractive index [2,3] which may be due to interactions between *ghee*-components and phytoconstituents.

The specific gravity symbolizes solid to liquid ratio in the formulations and eventually density to water which can be ominously used to enhance the consistency of formulations. The less liquid content in formulation increases the life span and thus its therapeutic value [2,3]. The solid extractives originated from the added herbs during the formulation process of KG might be responsible for the ascendant shift in specific gravity.

From the physicochemical analysis of plain *ghee* and KG, it can be inferred that plain *ghee* had undergone certain major physicochemical changes as depicted in Table 3. It can be anticipated that oxidation is the main reason for alterations in physicochemical properties and ultimately rancidity of *ghee*-based formulations. KG herbs i.e. *W. somnifera, P. tuberosa, A. recemosus, Z. officinale, D. gangeticum, B. diffusa, N. nucifera, T. cordifolia,* are the source of polyphenolic compounds comprising phenolic acids, flavonoids, coumarins, tannins with proven antioxidant potential [1,31] which might have exhibited protection against oxidative damage in KG.

4.3. Biological activity evaluation

Oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from the deficient natural antioxidant defense. Antioxidants on interaction with DPPH (a stable free radical) either transfer an electron or hydrogen atom to DPPH or thus neutralize its free radical character resulting in decolorization. Such stoichiometric decolorization with respect to the number of electrons taken up and the substance capable of reducing DPPH could be considered as an antioxidant and radical scavenger. NO is a diffusible free radical that plays a role as an effector molecule in biological systems including neuronal messenger and vasodilation. Sodium nitroprusside spontaneously generates NO (at physiological pH and in aqueous solution) which interacts with oxygen to produce nitrite ions that can be estimated

by the Griess reagent. Scavengers of NO compete with oxygen, leading to reduced production of nitrites. H_2O_2, a weak oxidizing agent, crosses cell membranes and can react with Fe^{2+} and Cu^{2+} ions to form a hydroxyl radical, which may be the origin of many of its toxic effects. Hence, the drug having the potential of controlling DPPH, NO, and H_2O_2 concentration could serve as an antioxidant [17–19]. In the present study, the antioxidant potential of test samples by all *in-vitro* methods was found in increasing order i.e. Plain *ghee* < KG < Ascorbic acid. Various antioxidant principles reported in herbs present in KG might be responsible for synergistic effect and potent antioxidant activity.

As compared to the hormonal induced estrous phase in ovariectomized rats, aphrodisiac evaluation using male and female rats in the natural proestrus phase of the estrous cycle is more advantageous, hence the aphrodisiac effect of drugs like '*Safed Musli*' (*Chlorophytum borivilianum*) could be due to the stimulation of dopaminergic neurons comprising the probable role of neurotransmitters in controlling sexual behavior [32]. It is reported that enhancement in serotonergic level has an inhibitory role on sexual function, whereas dopaminergic level facilitates copulatory and extra copulatory genital responses, noradrenergic responses control sexual arousal and cholinergic activity facilitates ejaculation [33].

The aphrodisiac potential of KG was evaluated in terms of sexual performance/behavior in rats. The results clearly revealed that sexually active KG fed animals showed enhanced sexual performance at a dose of 300 mg/kg body weight as compared to untreated control. The observed effects might be attributed to the synergistic effects of plants from KG with proven aphrodisiac potential. The literature documents numerous scientific reports of the aphrodisiac potential of *W. somnifera, T. terrestris, A. racemosus, P. tuberosa, D. gangeticum, Z. officinale, T. cordifolia, S. cordifolia, B. diffusa N. nucifera* and *Passiflora incarnata* [7–9,34–38]. *Ashwagandha,* one of the major components of KG reported for its capability of combating stress-induced infertility and its protective effect on some reproductive endocrine dysfunctions in male rats [39,40]. It improves sexual function and behavior in male rats via increasing the testosterone levels, activation of Nrf2/HO-1 and inhibition of NF-κB levels [41].

Oxidative stress is considered as one of the mechanisms which lead to infertility [42]. The plants with antioxidant activity preserve the nitric oxide needed to dilate the blood vessels in genital tissues, which in turn causes sexual arousal. These are also delivered in the form of lubricant to sensitive genital skin, energize nerve endings, and improve sexual pleasure and intimacy [43]. The present study has revealed the antioxidant potential of KG by *in-vitro* methods. Hence, there is a possibility that potent free radical scavenging ability of different KG components might have played role in the aphrodisiac activity of KG. However, the exact mechanism of action of KG for its aphrodisiac potential needs to be explored further.

5. Conclusion

In conclusion, the present study substantiates the traditional/ethnopharmacological attribute of *Kaamdev ghrita* as a '*Vajikarana Rasayana*'. It is the first scientific report on the aphrodisiac potential of the '*Kaamdev ghrita*' formulation established by *in-vivo* studies.

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Conflict of interest

None.

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