Research Article

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EXPERIMENTAL EVALUATION OF SOURCE PLANTS OF DUGDHKA (*EUPHORBIA HIRTA* LINN. AND *EUPHORBIA THYMIFOLIA* LINN.) FOR ANABOLIC ACTIVITY: AN *IN VIVO* STUDY

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ABSTRACT

Rajakshavaka a drug, mentioned in Brimhaniya Maha Kashaya Gana of Charaka is mentioned as Dugdhika by Chakrapani in his commentary and in Bhava Prakasha KC Chunekar commentary, 4 sources are mentioned for the same. Among 4 sources only 2 sources are locally available i.e., *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn. These 2 sources were selected to evaluate Brimhana (Anabolic) action experimentally to understand the better source plant for Dugdhika among locally available sources. The objective of the Study is to experimentally evaluate the drug Dugdhika (*Euphorbia hirta* Linn. *Euphorbia thymifolia* Linn) with respect to its anabolic activity. Anabolic activity was estimated in Wistar rats by administering Hydro-alcoholic extract of *Euphorbia hirta* Linn (HAEH) and *Euphorbia thymifolia* Linn (HAEET) with Normal and High Protein Diet (HPD: Protein – 39.4%, Fat – 10.0%, Fibre – 4.3%, Carbohydrates - 7.0 %). Anabolism was evidenced by physical parameters (Weight, Nose to Anus length, Chest Circumference, Abdominal circumference, BMI), Lean body mass (LBM), Fat body mass (FBM) and Biochemical and Haematological parameters. Both the sources of Dugdhika (*Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn) showed significant anabolic action. But, among the two sources *Euphorbia thymifolia* Linn was more significant compared to *Euphorbia hirta* Linn with HPD. The obtained results revealed that, among the two locally available sources of Dugdhika (*Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn) *Euphorbia thymifolia* Linn is a better source plant for Brimhana action.

Keywords: Rajakshavaka, Dugdhika, *Euphorbia hirta* Linn, *Euphorbia thymifolia* Linn, Brimhana, Anabolic activity

INTRODUCTION

India has a very rich plant biodiversity. Plants are one of the main sources of medicine since time immemorial. Ayurveda, the science of life emphasizes on use of plants as medicine and it is mentioned that each and every Dravya in this universe possesses medicinal property.

Dravyas (naturally occurring drugs) has been attributed to many pharmacological activities, Brimhana is one such activity which leads to sthoolata/bruhataakaara of Shareera. Charaka categorized ten dravyas (drugs) which are having Brimhana actions and mentioned it as Brimhaniya Maha Kashaya Gana in Shadvirechana Shatasritiya adhyaya.

Rajakshavaka a drug, mentioned in Brimhaniya Maha Kashaya Gana of Charaka which is said as Dugdhika by Chakrapani¹.

Source plants considered for Dugdhika are ^{2,3}

Dugdhika - *Euphorbia hirta* Linn

Choti Dudhi - *Euphorbia thymifolia* Linn

Choti Dudhi - *Euphorbia microphylla* Linn

Choti Dudhi - *Euphorbia hypericifolia* Linn

Among above, *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn are the locally available sources.

That which provides nourishment to the body and helps to increase the muscle bulk is considered as Brimhana.⁴ Anabolic activity is one of the activities attributed to the action of Brimhana drugs in previous research works.⁵ Anabolic activity/process of Anabolism is the set of metabolic pathways that construct molecules from smaller units and these complex molecules will be utilized to form cellular structure which in term responsible for the growth, nourishment and repair of the bodily tissues.⁶ Brimhana being an important action attributed to the plant Dugdhika, locally available source plants (*Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn) need evaluation to understand the right source plant for Dugdhika. There are very less research works conducted on the plant Dugdhika but, the references regarding it in Ayurvedic system of medicine and the usage of drug as a green leafy vegetable by rural people highlights its importance as a medicine and as well as a food. Hence the study is intended to experimentally evaluate *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn for Anabolic action to understand the locally available source plant for Dugdhika.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Whole plant of *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn were collected during Sharad Ritu (October-November) from in and around the campus of SSCASR, Bangalore and

authenticated by Dr. Shivamanjunath M.P, Senior scientist, Dept. of Dravya guna at SSCASR, Bangalore.

Preparation of Hydro-Alcoholic Extract *Euphorbia hirta* Linn (HAEEH) and *Euphorbia thymifolia* Linn (HAEET)

The whole plant of *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn were cleaned and removed adhered mud and dust particles. It was dried under shade and made into coarse powder with the help of pulveriser. Soxhlet extraction was done at Dravya guna Laboratory of SSCASR, Bangalore by using 50.0 percent of alcohol (ethanol) as a solvent. Solvent was added 3 times the quantity of raw material and heated under reflux at a temperature between 80-85°C for 3-4 hours. After 3-4 hours the extract was filtered through Whatmann filter paper No. 42. Further, concentrated filtrate was obtained by filtering the extract each time into the same vessel. Filtrate was evaporation using water bath at the temperature between 80-85°C and dried. Obtained dry powder of extract is collected and stored in air tight container for further use. Stock solution was prepared by triturating test drug with 0.1 g of 2% gum acacia and purified water (i.e., 1 ml/100 g of body weight) for oral administration to animals.⁷

Experimental Animals

Healthy Wistar rats required for the study were procured from Biogen Laboratory Animal Facility, Bangalore. Animals were housed at Department of Pharmacology Acharya and BM Reddy College of Pharmacy, Bangalore. The animals were kept in polypropylene cages (6 in each cage) (Figure 1) and followed the standard guidelines for the housing and feeding of the animals mentioned in the OECD (Organization for economic co-operation and Development) 425 Guidelines (i.e., at an ambient temperature 24°C and humidity with 12 hours light and 12 hours dark cycle) and had free access to food and water. The study was approved by the Institutional Animal Ethics Committee of Department of Pharmacology, Acharya & B.M Reddy College of Pharmacy and Bangalore (IAEC/ABMRCP/2019-2020/12). Ethical norms were strictly followed during the experiment.

Acute Toxicity Study

Acute oral toxicity – Limit test of hydro-alcoholic extract of whole plant of Dugdhika (*Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn) was carried out according to OECD guidelines 425.

Nulliparous, non-pregnant, healthy Wistar rats of 10-12-week-old, weighing about 180-200 g were selected.

A single dose of HAEEH and HAEET (2000 mg/kg body weight – p. o.) was administered to the animals respectively and observed for 14 days.

A single dose of HAEEH and HAEET (5000 mg/kg body weight – p. o.) was administered to the animals respectively and observed for 14 days.

After dosing animals were observed once in 30 minutes during first 4 hours, periodically during first 24 hours and thereafter, daily for total 14 days.

Observed for

Behavioural profile: Spontaneous, restlessness, irritability and fearfulness.

Neurological profile: Spontaneous activity, reactivity, touch-response, pain response and gait.

Autonomic profile: Defecation and urination.

Other changes: Change in skin or fur, eyes, mucosa and also respiratory, Somatomotor and tremors, convulsion, diarrhoea, lethargy, sleep, coma, changes in the weight and any mortality.

Anabolic Activity

The female Wistar rats were selected based on the inclusion and exclusion criteria as follow

Inclusion criteria

Healthy Wistar rats of either sex.

Wistar rats weighing 140- 180 g

Exclusion criteria

Wistar rats which are pregnant

Wistar rats showing the signs of infection during the course of study

Wistar rats those who are under other experiments.

Wistar rats younger than 45 days.

The selected animals were divided into 6 groups: Group I served as control group, Group II and III were test group treated with HAEEH and HAEET at 500 mg/kg p. o. respectively with normal diet, for 28 days. Group IV administered with HPD, Group V and VI again test group treated with HAEEH and HAEET at 500 mg/kg p. o. respectively with HPD, until 28 days of the study. During and after the study various physical, biochemical and haematological parameters were accessed in all 6 groups.

Group	Drug used	No. of rats
G1	Normal diet control group	06
G2	Hydro-alcoholic extract of <i>Euphorbia hirta</i> Linn	06
G3	Hydro-alcoholic extract of <i>Euphorbia thymifolia</i> Linn	06
G4	High-protein diet control group	06
G5	High-protein diet + Hydro-alcoholic extract of <i>Euphorbia hirta</i> Linn	06
G6	High-protein diet + Hydro-alcoholic extract of <i>Euphorbia thymifolia</i> Linn	06

Physical Parameters

Before the Drug administration (1st day), once in 7 days during the study (i.e., 7th, 14th, 21st day) and at the end of the study (28th day) weight and anthropometric measurements i.e., Chest circumference (immediately behind the foreleg) (Figure 2), Abdomen circumference (anterior to the hind leg) (Figure 3) and length (measured from tip of the nose to anus) (Figure 4) were noted.⁸

By measuring body fat percentage (Skin fold thickness at 5 sites Right Triceps, Right Scapular region, Right Abdomen, Right Iliac crest and Right Leg, 3 readings at each site and means was calculated) and weight - fat mass and lean mass of each animal were calculated at the end of the study (28th day) using formulas.

Fat Body Mass (FBM) = Body fat % × Weight

Lean Body Mass (LBM) = Body weight (BW) – Body fat (BF)

Haematological Study

After 28 days of drug administration, on 29th day animals were anaesthetised using short acting ether and few drops of blood samples were collected in EDTA tubes from retro orbital region

of each animal for the Haematological analysis (complete blood count). Soon after the collection, blood was subjected to Haematological analysis using GB-325 Automated Haematology Analyzer and readings were recorded.

Biochemical Parameters

On 29th day of the study 2 ml of blood was collected from retro orbital region of each animal using EDTA tubes and clear serum was separated by centrifuge at 4000 rpm for 10 min and the following biochemical investigation was carried out in Bio system 350 semi-automatic analyser using commercially available text kits (Bio system) and readings were recorded. Biochemical parameters are i.e.

- Glucose level
- Lipid profile – Cholesterol, triglycerides, HDL
- Liver function test – SGOT, SGPT, total bilirubin, albumin and protein.

Statistical Analysis

Data expressed as Mean $\pm$ SEM. The level of statistical significance was taken at P value – $p < 0.001$, $p < 0.01$ and $p < 0.05$, using one-way analysis of variance (ANOVA) test with Post HOC Dunnett test to detect the significance of differences between each group and control. IBM SPSS software version 20 was used for the analysis.

RESULT

Acute Toxicity Study (Table 1 to 6)

Acute toxicity study of *Euphorbia hirta* Linn revealed that: At 2000 mg/kg – rats were drowsy, from 1st hour of dosing till 24 hours and at 5000 mg/kg – rats were drowsy for 2 days after the administration of drug. Other than that, no changes were found in behavioural, neurological, autonomic profile and also no mortality till 14 days of toxicity study.

Acute toxicity study of *Euphorbia hirta* Linn revealed that: No changes in behavioural, neurological, autonomic profile and also no mortality.

Table 1: Observation of Behavioural changes: *Euphorbia hirta* Linn. - Vehicle control

Signs and Symptoms	Basal	30 min	1 hour	2 hours	3 hours	4 hours	24 hours	48h till 14 th day
General impression	N	N	N	N	N	N	N	N
Increased motor activity	-	-	-	-	-	-	-	-
Convulsion: Tonic	-	-	-	-	-	-	-	-
Clonic	-	-	-	-	-	-	-	-
Straubs reaction	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-
Catatonias	-	-	-	-	-	-	-	-
Opisthotonos	-	-	-	-	-	-	-	-
Hyperaesthesia	-	-	-	-	-	-	-	-
Decreased motor activity	-	-	-	-	-	-	-	-
Muscle relaxation	-	-	-	-	-	-	-	-
Anaesthesia	-	-	-	-	-	-	-	-
Arching and rolling	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-
Writhing	-	-	-	-	-	-	-	-
Salivation								
Viscid	-	-	-	-	-	-	-	-
Watery	-	-	-	-	-	-	-	-
Respiration								
Stimulation	-	-	-	-	-	-	-	-
Depression	-	-	-	-	-	-	-	-
Failure	-	-	-	-	-	-	-	-
Skin colour								
Blanching	-	-	-	-	-	-	-	-
Cyanosis	-	-	-	-	-	-	-	-
Vasodilatation	-	-	-	-	-	-	-	-
Grip strength	N	N	N	N	N	N	N	N
Visual placing response	N	N	N	N	N	N	N	N
Tail pinch response	N	N	N	N	N	N	N	N
Auditory response	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N
Pilo erection	N	N	N	N	N	N	N	N

Table 2: Observation of Behavioural changes: *Euphorbia hirta* Linn at dose -2000 mg/kg

Time interval	Rat No. 1	Time after drug administration	Exit us	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h	1	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	N
2h	1	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	N
3h	1	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	N
4h	1	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	N
24h	1	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	N
48h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3 rd d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
5 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
6 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
8 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
9 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
10 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
11 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
12 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
13 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
14 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 3: Observation of Behavioural changes: *Euphorbia hirta* Linn at dose -5000 mg/kg

Time interval	Rat No. 1	Time after drug administration	Exit us	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h	1	-	-	++	-	-	-	-	+	-	-	-	-	-	-	-	-	N
2h	1	-	-	++	-	-	-	-	+	-	-	-	-	-	-	-	-	N
3h	1	-	-	++	-	-	-	-	+	-	-	-	-	-	-	-	-	N
4h	1	-	-	++	-	-	-	-	+	-	-	-	-	-	-	-	-	N
24h	1	-	-	++	-	-	-	-	+	-	-	-	-	-	-	-	-	N
48h	1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3 rd d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
5 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
6 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
8 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
9 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
10 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
11 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
12 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
13 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
14 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 4: Observation of Behavioural changes: *Euphorbia thymifolia* Linn – Vehicle control

Signs and Symptoms	Basal	30min	1h	2h	3h	4h	24h	48h till 14 th day
General impression	N	N	N	N	N	N	N	N
Increased motor activity	-	-	-	-	-	-	-	-
Convulsion: Tonic	-	-	-	-	-	-	-	-
Clonic	-	-	-	-	-	-	-	-
Straubs reaction	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-
Catatonia	-	-	-	-	-	-	-	-
Opisthotonos	-	-	-	-	-	-	-	-
Hyperaesthesia	-	-	-	-	-	-	-	-
Decreased motor activity	-	-	-	-	-	-	-	-
Muscle relaxation	-	-	-	-	-	-	-	-
Anaesthesia	-	-	-	-	-	-	-	-
Arching and rolling	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-
Writhing	-	-	-	-	-	-	-	-
Salivation								
Viscid	-	-	-	-	-	-	-	-
Watery	-	-	-	-	-	-	-	-
Respiration								
Stimulation	-	-	-	-	-	-	-	-
Depression	-	-	-	-	-	-	-	-
Failure	-	-	-	-	-	-	-	-
Skin colour								
Blanching	-	-	-	-	-	-	-	-
Cyanosis	-	-	-	-	-	-	-	-
Vasodilatation	-	-	-	-	-	-	-	-
Grip strength	N	N	N	N	N	N	N	N
Visual placing response	N	N	N	N	N	N	N	N
Tail pinch response	N	N	N	N	N	N	N	N
Auditory response	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N
Pilo erection	N	N	N	N	N	N	N	N

Table 5: Observation of Behavioural changes: *Euphorbia thymifolia* Linn at dose -2000 mg/kg

Time interval	Rat No. 1	Time after drug administration	Exit us	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Prosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3 rd d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
5 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
6 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
8 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
9 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
10 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
11 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
12 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
13 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
14 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 6: Observation of Behavioural changes: *Euphorbia thymifolia* Linn at dose -5000 mg/kg

Time interval	Rat No. 1	Time after drug administration	Exit us	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3 rd d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
5 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
6 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
8 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
9 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
10 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
11 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
12 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
13 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
14 th d	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 7: Effect of HEEH and HEET on Body weight

S. No	Group	Body Weight in g
1	Control	13.42 ± 3.04
2	HAEH + ND (500 mg/kg, p. o.)	17.40 ± 3.03*
3	HAEET + ND (500 mg/kg, p. o.)	24.92 ± 1.94***
4	HPD	17.04 ± 4.84*
5	HAEH + HPD (500 mg/kg, p. o.)	17.76 ± 2.14
6	HAEET + HPD (500 mg/kg, p. o.)	24.09 ± 1.87***

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where * = p < 0.05, ** = p < 0.01 and *** = p < 0.001 compared to control group (Group

1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet.

Table 8: Effect of HEEH and HEET on Anthropometric measurements

S. No.	Group	Chest Circumference	Abdominal circumference	Nose to anus length
1	Control	7.53 ± 0.71	5.64 ± 1.27	2.40 ± 0.90
2	HEEH + ND (500 mg/kg, p. o.)	11.67 ± 1.92	9.64 ± 2.16	6.27 ± 1.45*
3	HEET + ND (500 mg/kg, p. o.)	15.80 ± 0.71**	14.27 ± 2.06**	12.56 ± 0.83***
4	HPD	7.10 ± 2.52	6.13 ± 1.81	8.85 ± 0.82***
5	HEEH + HPD (500 mg/kg, p. o.)	11.85 ± 1.32	12.68 ± 1.15*	12.44 ± 0.86***
6	HEET + HPD (500 mg/kg, p. o.)	13.22 ± 2.04	14.63 ± 1.57**	14.42 ± 1.01***

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where * = p < 0.05, ** = p < 0.01 and *** = p < 0.001 compared to control group (Group

1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet.

Table 9: Effect of HEEH and HEET on FBM and LBM

S. No.	Group	FBM	LBM
1	Control	1.55 ± 0.09	171.78 ± 10.14
2	HEEH + ND (500 mg/kg, p. o.)	2.18 ± 0.10	187.81 ± 10.77
3	HEET + ND (500 mg/kg, p. o.)	2.42 ± 0.13*	197.07 ± 12.82**
4	HPD	3.45 ± 0.25***	186.54 ± 11.01
5	HEEH + HPD (500 mg/kg, p. o.)	2.69 ± 0.23***	183.97 ± 9.92
6	HEET + HPD (500 mg/kg, p. o.)	4.67 ± 0.25***	201.99 ± 10.57***

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where * = p < 0.05, ** = p < 0.01 and *** = p < 0.001 compared to control group (Group

1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet.

Table 10: Effect of HAEH and HAEET on Haematological studies

S. No.	Group	RBC	Hb	WBC
1	Control	6.45 ± 0.4	11.77 ± 0.1	9.77 ± .56
2	HEEH + ND (500 mg/kg, p. o.)	8.26 ± 0.1**	12.73 ± 0.2*	8.72 ± .73
3	HEET + ND (500 mg/kg, p. o.)	7.22 ± 0.5*	13.12 ± 0.1***	8.22 ± .82
4	HPD	9.7 ± 0.5***	12.63 ± 0.2*	8.00 ± .42
5	HEEH + HPD (500 mg/kg, p. o.)	8.24 ± 0.1**	11.88 ± 0.3*	8.45 ± .79
6	HEET + HPD (500 mg/kg, p. o.)	8.65 ± 0.4***	13.90 ± 0.3***	10.48 ± .27

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where * = p < 0.05, ** = p < 0.01 and *** = p < 0.001 compared to control group (Group 1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet

Table 11: Effect of HAEH and HAEET on Blood Glucose level

S. No.	Group	Glucose
1	Control	88.67 ± 4.33
2	HEEH + ND (500 mg/kg, p. o.)	80.50 ± 2.96
3	HEET + ND (500 mg/kg, p. o.)	101.50 ± 10.86
4	HPD	95.67 ± 6.89
5	HEEH + HPD (500 mg/kg, p. o.)	93.00 ± 1.37
6	HEET + HPD (500 mg/kg, p. o.)	87.83 ± 3.86

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where ns = No significant when compared to control group (Group 1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet

Table 12: Effect of HAEH and HAEET on Lipid Profile

S. No.	Group	Cholesterol	Triglycerides	HDL
1	Control	164.83 ± 1.4	73.33 ± 6.7	54.33 ± 1.2
2	HEEH + ND (500 mg/kg, p. o.)	161.67 ± 1.4	87.17 ± 9.1	59.83 ± 0.8**
3	HEET + ND (500 mg/kg, p. o.)	158.17 ± 1.1 *	135.00 ± 19.7	69.67 ± 1.5***
4	HPD	163.50 ± 1.6	62.83 ± 4.7	59.83 ± 1.2**
5	HEEH + HPD (500 mg/kg, p. o.)	163.83 ± 1.6	88.83 ± 8.5	56.50 ± 0.67*
6	HEET + HPD (500 mg/kg, p. o.)	155.33 ± 2.0 **	77.17 ± 7.3	76.00 ± 0.8***

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where * = p < 0.05, ** = p < 0.01 and *** = p < 0.001 compared to control group (Group 1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet

Table 13: Effect of HAEH and HAEET on Liver function test (LFT)

S. No.	Group	SGOT	SGPT
1	Control	32.00 ± 4.1	33.67 ± 11.9
2	HEEH + ND (500 mg/kg, p. o.)	17.57 ± 14.1	33.33 ± 10.2
3	HEET + ND (500 mg/kg, p. o.)	19.63 ± 16.9	31.67 ± 11.2
4	HPD	43.38 ± 9.9	37.17 ± 2.5
5	HEEH + HPD (500 mg/kg, p. o.)	38.65 ± 11.8	29.83 ± 9.2
6	HEET + HPD (500 mg/kg, p. o.)	21.31 ± 4.9	23.67 ± 8.6

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where ns = No significant when compared to control group (Group 1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet.

Table 14: Effect of HAEH and HAEET on LFT

S. No.	Group	Total bilirubin	Total protein	Albumin
1	Control	0.4 ± 0.4	6.57 ± 0.4	3.19 ± 0.2
2	HEEH + ND (500 mg/kg, p. o.)	0.67 ± 0.2	7.33 ± 1.2	3.70 ± 0.4
3	HEET + ND (500 mg/kg, p. o.)	0.57 ± 0.4	6.5 ± 0.9	3.71 ± 0.3
4	HPD	0.65 ± 0.1	5.67 ± 0.1	3.44 ± 0.2
5	HEEH + HPD (500 mg/kg, p. o.)	0.6 ± 0.2	6.28 ± 0.7	4.33 ± 0.1
6	HEET + HPD (500 mg/kg, p. o.)	0.58 ± 0.2	5.9 ± 0.3	4.00 ± 0.2

Values are expressed in Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Post HOC Dunnett test. Comparisons were done between Group I versus Group II, III, IV, V and VI. Where ns = No significant compared to control group (Group 1). SEM: Standard Error of Mean, HAEH: Hydro-alcoholic extract of *Euphorbia hirta* Linn, HAEET: Hydro-alcoholic extract of *Euphorbia thymifolia* Linn, ND: Normal diet, HPD: High Protein Diet.

Figure 1

Figure 2

Figure 3

Figure 4

Effect of HAEH and HAEET on Physical Parameter

Effect of HAEH and HAEET on Body Weight and Anthropometric Measurements

Table 7 and 8 shows the effect of HAEH and HAEET on the body weight and Anthropometric measurements in various groups after 28 days of intervention. Results were clearly showed that there was a significant increase in weight of animals in Group II & IV. Whereas, highly significant in Group III & VI when compared it with the control and HPD group (Group IV). Among anthropometric measurements there is a significant increase in Chest circumference in Group III, significant increase in Abdominal circumference in Group III, V, VI and highly significant increase in length of the animals in Group III, IV, V, VI and significant increase in Chest circumference in Group II were seen in comparison with the control group.

Effect of HEEH and HEET on FBM and LBM

Table 9 shows the effect of HAEH and HAEET on FBM and LBM in various groups after the 28 days of intervention. Results were clearly showed that there is a significant increase in FBM in Group III, IV, V & VI and significant increase of LBM in Group III & VI in comparison with the Control group.

Effect of HAEH and HAEET on Haematological Studies

Table 10 shows the effect of HAEH and HAEET on the Haematological studies in various groups after the 28 days of intervention. Results were clearly showed that there is a significant increase in RBC and Hb in all test groups. There is no significant increase or decrease in WBC count.

Effect of HAEH and HAEET on Biochemical Studies

Table 11 show the effect of HAEH and HAEET on the Blood Glucose level in various groups. Normal Blood Glucose level

with no significant increase or decrease were seen in all 6 groups' animals.

Table 12 shows the effect of HAEH and HAEET on the Lipid profile. Significant decreases in cholesterol level in Group III and VI when it compared with the control group. In all 6 groups Total serum Cholesterol level was found to be in normal limits (< 200 mg/dl). Significant increases in Triglycerides level were seen in Group III but it was maintained in its normal limit (< 160 mg/dl). Increase in HDL- Cholesterol level was seen in all test Groups in comparison with control group. Among them group III and VI was considered to be highly significant.

Table 13 and 14 shows the effects of HAEH and HAEET on the LFT. There is no significant increase or decrease in LFT.

DISCUSSION

Acute toxicity study revealed that, both the drugs *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn were found to be non-toxic up to 5000 mg/kg dose.

During acute toxicity study mild sedative and hypnotic activity along with hypoactivity of the rats was observed in the group administered with *Euphorbia hirta* Linn, it is due to the sedative pharmacological action of the drug.

In this study animals of first 3 groups were provided with normal diet and further 3 groups of animals were provided with the high protein diet, in order to rule out the importance of Protein diet in the anabolic activity; as protein, component of diet responsible for the Anabolism.

RBC and Hb

Haemoglobin is a special protein present in Red blood cells. High protein diet decreases the osmotic fragility of Red blood cells and helps to increase RBC and Hb which in turn helps in anabolism.

In this study it was noted that, the groups administered with trial drug (Group II, III, IV, V & VI) showed increase in the RBC and Hb percentage suggestive of anabolic activity of both the drugs.

Biochemical Parameters

Normal values of WBC, Platelet, LFT and Glucose level in the present study revealed that the animals included in this experiment were healthy and devoid of any pathological conditions, which in turn helped proper physiological growth and development of animals suggesting anabolic activity.

Lipid Profile

In the present study, highly significant increase of HDL was found in all the groups with drug intervention (Group II, III, IV, V & VI) and also decreased level of Serum Cholesterol in the groups administered with *Euphorbia thymifolia* Linn with normal and High Protein Diet respectively (Group III and Group VI). This is suggestive of presence of FFA in the blood, as increase of FFA in the blood is because of the lipolysis (which occurs due to the anabolic process). Hence, Lipid profile value found also is suggestive of anabolic activity of the drugs.

Physical parameter

Anabolic activity can be considered as an overall growth of an individual i.e., increase in weight, length and also in size (Anthropometric measurements). Hence, weight and Anthropometric measurement of each rats were noted before, after and once in a week during the study. In the current study it was observed that the linear mass of animals was increased.

Anabolic activity results in increase of LBM and decrease of FBM. LBM has been described as an index superior to total body weight and it also helps in the assessment of metabolic disorder. The percentage of LBM and FBM should be 60-90% and 10-40% respectively.

Both the Biochemical and Physical parameters revealed that, both the sources of Dugdika (*Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn) with normal and High Protein Diet produced significant improvement in growth which suggestive of Anabolic activity. Alkaloid, Steroid and Proteins present in the drugs may be responsible for the above result.

Among *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn, *Euphorbia thymifolia* Linn was found to be more significant with normal and as well as High Protein Diet. The quantitative analysis of both the drug revealed that, the percentage of Alkaloid, Protein and Steroids is comparatively higher (i.e., 65.4%, 7.70% and 21.8%) in *Euphorbia thymifolia* Linn which is supportive for the experimental result found. However, the Hydro-alcoholic extract of *Euphorbia thymifolia* Linn with High Protein diet has given the

most significant anabolic activity, giving an impetus that protein diet also plays a significant role in Anabolic activity along with responsible phytoconstituents of the drug.

CONCLUSION

In the current experimental study *Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn both exhibited anabolic activity. *Euphorbia hirta* Linn was more significant with normal diet ($P < 0.05$) whereas, *Euphorbia thymifolia* Linn was significant with both Normal and High Protein Diet with the P value < 0.01 and < 0.001 respectively. Among the two sources *Euphorbia thymifolia* Linn showed better anabolic activity than *Euphorbia hirta* Linn. Thus, the obtained results revealed that, among the two locally available sources of Dugdika (*Euphorbia hirta* Linn and *Euphorbia thymifolia* Linn) *Euphorbia thymifolia* Linn is a better source plant for Brimhana action.

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