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Comparative antipyretic activity of ethanolic extracts of some species of *Cynodon* in rabbits

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Abstract
To investigate and compare the antipyretic activity of the Ethanolic extracts of *Cynodon dactylon* and *Cynodon barberi* in rabbits. The antipyretic activity of Ethanolic extract at a dose of 400 mg/kg and 600 mg/kg was tested be milk induced pyrexia in rabbits. Paracetamol (100 mg/kg) was used as standard drug and control group received distilled water. Rectal temperature of the rabbits was recorded till 4 hrs at an interval of 1 hr using digital thermometer. The investigation showed that the 600 mg/kg dose of both the drug showed a reasonable decrease in temperature ($p<0.05$) when compared to the standard drug. The result showed that the Ethanolic extract of two species of *Cynodon* possess significant antipyretic effect in maintaining reducing the milk induced elevated body temperature in rabbits and their effect were comparable to that of the standard antipyretic drug paracetamol.

Keywords: *Cynodon dactylon, Cynodon barberi*, antipyretic activity, ethanolic extract, paracetamol

1. Introduction
The plants have been used as the natural source of medicine from ancient times. The various plant extract have been used during the ayurvedic times. The medicinal property of various plants can be studied in various books like rig-vedas, charaka samhita, sushrusha samhita etc [1]. Both *Cynodon dactylon* and *Cynodon barberi* belong to the family poaceae. They belong to the same genus of Cynodon. It is commonly known as the Durva Grass, Bermuda Grass, Dog's Tooth Grass, Bahama Grass Devil's Grass etc. In sansktrit it is known as the Durva, which means it is cut or eaten by the animals. It is a scared plant in India [2]. This plant is a perennial grass and is rapidly growing in nature. They grow in almost a type of soil and mainly grow in area having high nitrogen eve. They grow in moist area along the river side [3]. Antipyretics are the drug that reduces the increased body temperature by acting centrally on the temperature regulation center in the brain and act peripherally through the vasodilatation or by heat dissipation which occurs by prostaglandin synthesis inhibition [4]. When the body temperature rises above the "average" body temperature, such condition is known as fever or pyrexia. It occurs when bacteria or virus triggers the body defense mechanisms [5]. Pain is an unpleasant sensation in the body which occurs during fever but is beneficial to the human being. This pain sensation occurs only when the body is undergoing any type of protective mechanism. This occurs only when there is any type of tissue damaging occurs in the body [6]. Pyrexia is linked with symptoms of sickness behavior which consist of lethargy, depression anorexia, and sleepiness and is unable to concentrate. This leads to shivering and muscle tone. Now a day's most of the herbal medicines are used to treat the diseases as it has very less side effect as compared to the synthetic drugs [7].
According to ayurveda, fever is mainly originated from the combination of factors like indigestion, seasonal variation and any type of significant alteration in daily routine. In developing countries, factors like poor hygiene practice and malnutrition etc. children suffer from infection which finally has a symptom of fever [8].

2. Materials and methods
2.1 Method of collection
Fresh leaves, roots and stems of both *Cynodon dactylon* and *Cynodon barberi* were collected in the month of January from the locality of the Azara, Guwahati, Assam and were washed under tap water. It was shade dried and was sieved through sieve having mesh size 40 to get fine powders. It was authenticated by the head of botany dept. Guwahati University Dr P.P. Baruah (Acc.No.1812).

2.2 Extraction of the plant drug
The dried power of the plants was extracted by cold maceration process. The drug was dissolved in alcohol and was kept for three days. The whole mixture was shaken occasionally. The aqueous extract was prepared by maceration in alcohol (72hrs).The macerate was filtered through Whatman No.1 filter paper and concentrated in a rotary flash evaporator at a temperature not exceeding 50 ºC [9].

2.3 Drug
Paracetamol and ethanol was collected from Krishna Chemicals Pvt. ltd, Panbazar and was used as known antipyretic agent. The dose of paracetamol was maintained 100 mg/kg body weight [10].

2.4 Animals
The experiment was carried out on albino rabbits. They were 13-15 months old of both sexes weighing between 1.5-1.6 kg. They were collected from the Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS) animal house. The rabbits were kept in iron cages (considering group), were fed with cauliflower, cabbage, banana and tap water for 40 days before experiment to adjust with environment. Food and water were withdrawn 6 hours prior to the experiment. Treatment of different groups (each group contains four rabbits) of animals were as follows (A Khan *et al*; 2008) -

Group 1: Treated with Ethanolic fraction of *Cynodon dactylon* (400 mg/kg).
Group 2: Treated with Ethanolic fraction of *Cynodon dactylon* (600 mg/kg).
Group 3: Treated with Ethanolic fraction of *Cynodon barberi* (400 mg/kg).
Group 4: Treated with Ethanolic fraction of *Cynodon barberi* (600 mg/kg).
Group 5: Treated with standard antipyretic agent Paracetamol (+ control)
Group 6: Treated with solvent (- control).

2.5 Study of physicochemical parameters of plants
The physiochemical parameters such as moisture content, extractive value, acid insoluble ash, water soluble ash and total ash content of the test drug powder was analyzed according to the standard procedures [11, 12].

2.6 Moisture content
The powdered material (10gm) was placed in a moisture disc and dried to a constant weight in a oven in 100-105^{0}C.The loss of weight (in mg/g) of air dried was calculated as follows.

$$\% \text{ Moisture content} = \frac{\text{Weight loss}}{\text{Weight of the sample}} \times 100$$

2.7 Total Ash
3 gm of drug was weighed and incinerated in a China dish at a temperature not exceeding 450ºC until free from carbon, cooled and weighed, until a constant weight was obtained for three successive readings. Percentage of ash was calculated with reference to air dried drug.

$$\text{Total Ash} = \frac{\text{Weight of ash}}{\text{Weight of the drug}} \times 100$$

2.8 Acid-Insoluble Ash
The total ash was obtained by boiling for 5 min with 25 ml of dilute hydrochloric acid; the insoluble matter was collected in a Gooch crucible, the insoluble matter was washed with hot water and ignited to constant weight. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

2.9 Extractive values of the drug powder
2.9.1 Alcohol-soluble extractive: 5 gm of accurately weighed powdered drug was taken in a stoppard conical flask and add 100 ml of 90% alcohol, and shake constantly for 6hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:

$$\text{Alcohol-Soluble Extractive} = \frac{\text{Weight of extractive}}{\text{Weight of the drug}} \times 100$$

2.9.2 Water Soluble extractive: 5 gm of accurately weighed powdered drug was taken in a stoppard conical flask and add 100 ml of chloroform water, and shake constantly for 6hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:

$$\text{Water -Soluble Extractive} = \frac{\text{Weight of extractive}}{\text{Weight of the drug}} \times 100$$

2.10 Phytochemical screening
Preliminary phytochemical screening of the different constituents like alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, fats and oils, proteins and amino acids was performed following standard procedures [13, 14].

2.11 Antipyretic test procedure
The study protocol was approved by the institute's ethical committee (GIPS/IACE/B.Ph/2016/02). Before experimentation rectal temperature of rabbits were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Care was taken to insert it to the same depth each time (about 6 cm). Milk was collected from local cow and had been boiled and when temperature of the boiled milk equilibrates to room temperature then milk was injected through intraperitoneal route at the dose of 0.5 ml/kg body weight, to induce pyrexia. Induction of fever was taken about one to two hour. Test 1 group of animals received 300 mg/kg of Ethanolic extract, test 2 group received 600 mg/kg of Ethanolic extract, standard group received 100mg/kg paracetamol and control group was treated with solvent. Finally, rectal temperatures were recorded at 1 hour intervals up to 4 hour [15, 16].

3. Results and discussion
3.1 Physicochemical analysis: Different physicochemical parameters of both the plants were evaluated and results were shown in Table 1.

Table 1: Values of different physicochemical parameters of plants *Cynodon dactylon* and *Cynodon beriberi*

Sl. No	Parameters	Value (*Cynodon dactylon*)	Value (*Cynodon berberi*)
1	Loss on drying	53.5%	50.5%
2	Total ash value	40.66%	43.66%
3	Acid insoluble ash	71.64%	65.64%
4	Water soluble ash	48.55%	51.55%
5	P^H (1%)	6.32	6.22
6	P^H (10%)	6.08	6.19
7	Alcohol soluble extractive value	14.4%	13.8%
8	Water soluble extractive value	12.6%	14.9%

Ash values are used to determine the quality and purity of crude drug indicating presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicates contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent [17].

3.2 Phytochemical analysis: Results of Phytochemical test were shown in Table 2.

Table 2: Results of Phytochemical test

Sl. No.	Chemical tests	Result (*Cynodon dactyon*)	Result (*Cynodon barberi*)
1	**Test for carbohydrate:**		
	Molish test	Present	Present
	Iodine test	Present	Present
	Benedict test	Present	Present
	Fehling test	Present	Present
2	**Test of proteins and free amino acid:-**		
	Million's test	Present	Present
	Biuret's test	Present	Present
	Ninhydrine test	Present	Present
3	**Test for saponin:-**		
	Forth formation test	Present	Present
4	**Test for flavanoids:-**		
	NaOH test	Present	Present
	$FeCl_3$ test	Present	Present
5	**Test for alkaloids:-**		
	Mayer's test	Present	Absent
	Wragner's test	Present	Absent
	Dragondroff test	Present	Absent
	Hager's test	Present	Absent
6	**Test for glycosides:-**		
	Killer killani test	Absent	Absent
	Borntrager's test	Absent	Absent
7	**Test for sterols and terpenoids:-**		
	Liberman buchard test	Present	Present
	Salkowaski test	Present	Present
8	**Test for tannin and phenolic compound:**		
	$FeCl_3$ test	Present	Present
	Gelatin test	Present	Present
	Iodine test	Present	Present

The secondary metabolites are mainly of medicinal use, and the examination of the plant constituent can only show those compounds that have accumulated at a specific organ of a given plant. The presence or absence compounds depend largely on the extent of deposition, the amount of plant material used and the analytical method employed [18]. Phytochemical screening is based on colour formation, precipitate formation, interfacial film formation, and frothing or emulsion production [19].

3.3 Antipyretic activity: The results of antipyretic activity of treated different groups are given in Table 3 and Fig 1.

Table 3: Results of antipyretic activity of treated different groups

Drug	Initial (before injection of milk)	2 hrs after milk injection	Rectal temperature at particular time interval				
			1 hrs after drug dosing	2 hrs after drug dosing	3 hrs after drug dosing	4 hrs after drug dosing	% reduction after 4hrs
Group 6 (-Control)	36.12±0.063	38.95±0.082	38.75±0.087	38.53±0.047	38.60±0.065	38.23±0.065	1.84%
Group 5 (+Control)	36.10±0.038	38.97±0.068	38.75±0.087	37.72±0.080	37.05±0.106	36.25±0.045*	6.97%
Group 1	36.09±0.059	38.98±0.099	38.72±0.046	38.48±0.048	38.19±0.067	37.99±0.053	2.53%
Group 2	36.11±0.078	38.95±0.056	38.47±0.065	37.92±0.069	37.01±0.011	36.33±0.095*	6.27%
Group 3	36.29±0.048	38.76±0.078	38.64±0.057	38.42±0.028	38.29±0.063	37.92±0.057	2.16%
Group 4	36.19±0.083	38.86±0.045	38.43±0.056	37.89±0.048	37.12±0.016	36.48±0.086*	6.12%

All values are expressed as mean ± SEM (n = 4), percentage reduction in rectal temperature is given within parentheses. * P<0.05 significant compared to control.

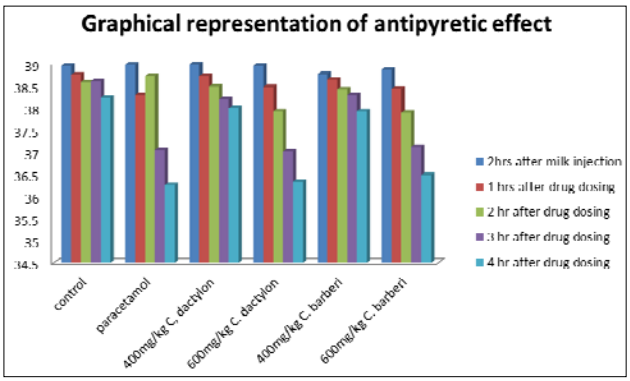

Fig 1: Graphical representation of antipyretic activity

There are some endogenous substances like prostaglandins which lead to the rising in body temperature asp known as pyrexia. All type of antipyretic rugs has the ability to inhibit the formation of prostaglandins [20]. Infection, tissue damage, inflammation, malignancy etc are some of the secondary factors that lead to rise in body temperature or pyrexia. These factors leads to formation of pro-inflammatory mediators for example cytokinines like interleukin 1β, α, β and TNF-α. These mediators lead to increase in synthesis of prostaglandins [21]. Most of the antipyretic drug reduces the temperature by inhibiting the COX-2 expression which finally inhibits the PGE2 synthesis. The synthetic antipyretic drugs are toxic to the hepatic cells but are highly selective in nature, whereas the natural compounds are less toxic and are less selective in nature [22]. The phytochemicals investigation shows the presence of flavanoids and sterols and the ethanol extract of two plants of *Cynodon* possesses a significant antipyretic effect in maintaining reducing milk-induced elevated body temperature in rabbits and their effects were comparable to that of the standard antipyretic drug paracetamol.

4. Conclusion

The phytochemical investigation of the plant shows the presence of flavanoids and sterols which could contribute to the antipyretic effect of the plants. The *Cynodon dactylon* showed the significant antipyretic effect in comparison to *Cynodon barberi* at the same concentration of 600 mg/kg. Further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

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