



ISSN 2320-3862
JMPS 2016; 4(5): 104-107
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Received: 16-07-2016
Accepted: 17-08-2016

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Journal of Medicinal Plants Studies

www.PlantsJournal.com

Evaluation of central activity of ethanolic flower extract of *Sorghum halpense* on Albino Rats

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Abstract

Evaluation of Central activity of *Sorghum halpense* on albino rats. The *Sorghum halpense* has anti-inflammatory, catarrh, febrifuge, relieving headache, properties. Recent study identified that flowers have Central activity. The ethanolic flower extract of *Sorghum halpense* prepared by soxhlet extraction. The crude extract dissolved in dimethyl sulfoxide (DMSO) and subjected to screening. For study young swiss albino rats about weighing 220-250g were taken from the animal house of Geethanjali college of Pharmacy maintained under described environmental conditions. The experiment met the national guidelines on the proper care and use of animals and the CPCSEA approved the experimental protocol. Evaluation studies done by Digital analgesimeter, Writhing test and Carragenan induced paw oedema models.

Keywords: *Sorghum halpense*, central activity, dimethyl sulfoxide, Actophotometer Rota-rod, inclined plane

1. Introduction

1.1 Plant material

Sorghum is a genus of grasses with about 30 species, one of which is raised for grain and many of which are used as fodder plants, either cultivated or as part of pasture. The plants are cultivated in warm climates worldwide. They are native to the tropics and subtropics of the Old World and one species is endemic to Mexico; a number have been introduced into other parts of the world.

Sorghum halepense is adapted to a wide variety of habitats including open forests, old fields, ditches and wetlands. It spreads aggressively and can form dense colonies which displace native vegetation and restrict tree seedling establishment. *Sorghum halepense* has naturalized throughout the world, but it is thought to be native to the Mediterranean region. It was first introduced into the United States in the early 1800s as a forage crop.

Sorghum plant varieties are countless, with over 200 natural and cultivated species, one would definitely be spoiled for choice. A genus of shrubs and vines, *Sorghum* belongs to the *Oleaceae* (olive) family. *Sorghum* is native to the old world.

2. Material and Method

The evaluation of Central activity of *Sorghum halepense* was done on albino rats. This research would need different material and equipment's.

2.1 Plant material

Sorghum halepense plant flowers are collected and flowers were shade dried for several days to remove moisture. After drying, the dried flowers were ground into coarse powder by 'mixer grinder'.

Table 2.1: Other Materials

Ingredients	Quantity
Dried powder	200g
Ethanol	500ml
Methyl paraben	1.0g
Di methyl sulfoxide	50ml

2.2 Equipment

Equipment
Soxhlet apparatus
Heating mantel
Mixer grinder
Rotary evaporator

2.3 Evaluation equipments

Actophotometer
Rota-rod
Inclined Plane Test

2.4 Soxhlet apparatus

A soxhlet apparatus is a piece of laboratory apparatus invented in 1879 by Franz von soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The solid material comes in this flask it take hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated. The chamber containing the solid material slowly fills warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded.

2.5 Extraction procedure

About 200 g of powdered flowers were taken in a clean muslin cloth and pack it and put into a Soxhlet apparatus. In the container pour into a 500 ml of 80% Ethanol and with its contents was seal and kept for a Period of 6 days. Extraction was comes down with solvent, and the solvent is evaporated and recycled in the process of condensation. After 6 days the extraction was taken out for further procedure such as phytochemical and pharmacological screening and drying process.

The drying process is done by using a Rotary evaporator to get the crude extract. The crude extracts were dissolved in Di methyl sulfoxide (DMSO) and subjected to screening.

2.6 Phytochemical Screening

The Ethanol extract of *Sorghum halepense* was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% w/v solution of the extract in ethanol was used unless otherwise specified.

2.6.1 Tests for Reducing Sugar

Benedict's test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Result: Green to Red colour precipitate was obtained.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

Result: Yellow to Brick red precipitate was obtained. Reducing sugars are present.

2.6.2 Tests for Tannins

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Result: Dark green color precipitate was obtained. It indicates presence of Tannins.

2.6.3 Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to 5 ml of the extract.

Result: Solution turns to Pink color. It indicates the extract was present Flavonoids.

2.6.4 Test for Saponins

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

Result: Foam was not obtained so that Saponins was not present.

2.6.5 Test for Gums

5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

Result: Red color precipitate is not obtained so that extract not resent any Gums.

2.6.6 Tests for Steroids

Liebermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Liebermann- Burchard reagent was added to it.

Result: Dark greeny blue color was obtained it indicates presence of Steroids.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

Result: Green color precipitate was obtained. It indicates presence of Steroids.

2.6.7 Tests for Alkaloids

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

Result: Creamy color precipitate was obtained.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Result: Reddish brown color precipitate was obtained.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Result: Yellow precipitate was obtained. It indicates presence of Alkaloids.

2.6.8 Tests for Glycosides

i. A small amount of extract was taken in 1 ml water. Then few drops of aqueous sodium hydroxide were added.

Result: Yellow precipitate is considered as an indication for the presence of glycosides.

ii. A small amount of extract was taken in 1 ml water and boiled with 5 ml Fehling's solution in a boiling water bath.

Result: Brick-red precipitate is considered as an indication for the presence of glycosides.

iii. A small amount of extract was boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with 5 ml Fehling's solution in a boiling water bath.

Result: Brick red precipitate is considered as an indication for the presence of glycosides.

2.7 Chemical Group Test

Results of different chemical group tests on the ethanol extract of roots of *Sorghum halepense* showed the presence of Reducing Sugar, Alkaloids, Tannins, Flavonoids and Glycosides (Table.2.4).

Table 2.4: Different components of ethanolic extract of roots of *Sorghum halepense*

Component	Indication
Alkaloids	+
Glycosides	+
Saponins	-
Flavonoids	+
Gums	-
Reducing sugars	+
Steroids	+
Tannins	+
+ : Presence, - : Absence	

2.8 Animals

For Central activity study, young Swiss-albino rats of either sex, weighing 220 to 250 g, taken from the Animal house of Geethanjali College of Pharmacy.

The animals were kept at separate shelves in the same animal house of pharmacy discipline, Geethanjali college of pharmacy, for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature $25.0 \pm 2.00^\circ\text{C}$ and with light-dark cycle). All rats were fed with standard diets and had free access to tap water.

2.9 Drugs

Tramadol

2.10 Methods

2.10.1 Actophotometer

The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Tramadol (2mg/kg) intraperitoneally. Group III

treated orally with aqueous extract of 400 mg/kg body weight. The animals were individually placed on the actophotometer maintained at 55°C , one hour after their respective treatments. The locomotor activity scores in 5 minutes time was noted.

2.10.2 Forced Swim test

When the animal is forced to swim, it tries to escape by making rigorous movements. When it cannot escape from the enclosure or jar the animal surrenders to the situation and floats making very little or no movements in jar. Thus forced swimming –induced immobility is considered as helplessness or a state of depression animals. Anti-depressant drugs reverse or reduce this immobility period.

2.10.3 Rotarod test

The test is used to evaluate the activity of drugs interfering with motor coordination, Skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. This forced motor activity has subsequently been used by many investigators.

2.10.4 Inclined Plane test

Plain glass was used to assess this test. Groups of mice (n=6) were left on a plain glass, inclined at 30° . The rats, which tried to move out of the plane glass without sliding off, were used for the test. The test was performed at 15 and 30min after administration of extract and diclofenac and normal saline as control drugs. (Rudzik et al 1973)

3. Results and Discussion

3.1 Actophotometer

Animals: Albino rats

Table 3.1: Weight of rats

Animals	Weight (g)
Rat. I	250
Rat. II	220
Rat. III	200
Rat. IV	200

Extract: *Sorghum halepense* flower extract.

Drugs: Tramadol (2mg/kg).

Equipment: Actophotometer.

Animal group /Treatment	Locomotor Activity Scores in 5 min
Group-1 (Control)	397
Group-2 (Tramadol 2mg/kg)	210
Group-3 (EESH150mg/kg)	173
Group-4 (EESH300mg/kg)	166

All Values are expressed as mean \pm S.E.M (Number of trails, n=6).

A cut off time of 20 sec. is taken as maximum Central response to avoid damage due to heat.

3.2. Forced Swim Test

Animal group /Treatment	Immobility time in Sec
Group-1 (Control)	150
Group-2 (Tramadol 2mg/kg)	120
Group-3 (EESH150mg/kg)	110
Group-4 (EESH300mg/kg)	108

All Values are expressed as mean \pm S.E.M (Number of trails, n=4).

All values obtained by i.p administration

3.4 Rota-rod Test

Drugs: Tramadol

Equipment: Rota-rod apparatus

Animal group/treatment	Basal reaction time (sec)
Group-1(control)	6.5±1.01
Group-2 (Tramadol 2mg/kg)	11±0.05
Group-3 (EESH 150mg/kg)	8.04±1.24
Group-4 (EESH 300mg/kg)	10.07±0.09

All Values are expressed as mean ± S.E.M (Number of trails, n=6).

All values obtained by i.p administration

3.5 Inclined Plane Test

Drugs: Tramadol

Equipment: Inclined Plane

Animal group/treatment	Falloff time (sec)
Group-1 (control)	12.55±1.01
Group-2 (Tramadol 2mg/kg)	16±0.05
Group-3 (EESH 150mg/kg)	10±1.24
Group-4 (EESH 300mg/kg)	12±0.09

All Values are expressed as mean ± S.E.M (Number of trails, n=6).

All values obtained by i.p administration

4. Conclusion

From the preliminary Phytochemical & Pharmacological investigations carried out on the flowers of *Sorghum halpense* following conclusions can be made:

The phytoconstituents present in the extracts of flowers are carbohydrates, phenolic compounds, flavonoids, tannins, alkaloids & glycosides.

The central activity is being proven by actophotometer, rotarod apparatus, forced swim test,

Inclined plane test, the extract has also shown to have central analgesic activity which is shown by Rota rod test and Inclined plane test,

The activity of the extract may be due to the presence of chemical constituents like flavonoids the presence of flavonoids was witnessed by the phytochemical screening. Thus the extract is found to have both peripheral and central analgesic activity.

Thus further research can be carried out to establish the central activity of the drug.

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