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Pharmacological Evaluation of Analgesic Activity of Ethanolic Extract of *Pennisetum Glauccum* seeds in Swiss Albino Mice.

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ABSTRACT:

In recent years, the use of herbal medicines has significantly increased due to their safety, efficacy, cost-effectiveness, and favorable therapeutic outcomes when compared to synthetic drugs. *Pennisetum Glaucum*, traditionally valued in folk medicine, is believed to possess analgesic properties. The present study aimed to scientifically evaluate the analgesic potential of *Pennisetum glaucum* seeds based on traditional claims.

Ethanolic extraction of the dried seeds was carried out using a Soxhlet apparatus, yielding 28.27% w/w of extract. Initial phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, carbohydrates, and proteins.The analgesic activity of the extract was assessed in Swiss albino mice using Eddy's Hot Plate and Tail Flick methods, with diclofenac sodium (9 mg/kg) and aspirin (100 mg/kg) serving as standard drugs.

The extract, administered orally at doses of 400 mg/kg and 800 mg/kg, demonstrated significant, dose-dependent analgesic activity. The maximum effect was observed at the 800 mg/kg dose, evidenced by increased reaction time and tail flick latency. These findings validate the traditional use of *Pennisetum glaucum* seeds for pain relief and suggest its potential as a natural analgesic agent.

Keywords: *Pennisetum Glauccum*, Analgesic activity, Hot plate method, Tail flick method.

Introduction

Natural drugs are defined as a branch of science that involves the use of herbs, plants, or their parts to alleviate various diseases and health conditions . It may also be called as herbal drugs or phyto-medicines. In the mid- twentieth century, the natural drug was the structure of the main medical services, since no analgesics or uninfected agents were found. With the allopathic disposition of the pharmacological approach, the natural prescription has constantly lost its reputation among individuals, which depends on the rapid aid activities of synthetic drugs.[1-4]

Pain is defined as a distressing sensory and emotional experience associated with real or perceived tissue injury, or described in terms of such harm. It typically begins in response to harmful stimuli and is conveyed through specific neural pathways to the central nervous system, where it is perceived as pain. This reaction functions as a protective mechanism to safeguard the body against injury.Like wise, inflammation is a protective reaction triggered by both the innate and adaptive immune systems in response to harmful stimuli like infections or tissue injuries.

Nevertheless, these drugs are linked to numerous side effects and toxicities, including gastric irritation, ulcers, impaired kidney function, changes in blood pressure, liver damage, and inhibition of platelet function, which can increase the risk of bleeding. The use of NSAIDs, particularly COX-2 inhibitors, is also associated with a higher risk of cardiovascular complications. Similarly, opioid analgesics can lead to adverse effects such as drowsiness, nausea, vomiting, itching, constipation, hormonal imbalance, hearing loss, tolerance, physical dependence, addiction, and respiratory issues. Therefore, there is a growing need to enhance research on medicinal plants that are traditionally believed to be effective in managing pain.[4-5]

Material and Methods

Plant Selection: – The plant was chosen based on its traditional use in medicine. Pharmacognostical studies serve as a key criterion for identifying herbal drugs derived from plants. Medicinal plants form a large group of economically important plants that gives the basic raw materials for indigenous pharmaceuticals approach to the discovery of new drugs.

Collection, identification and Authentication of plant material

Seeds from were gathered from an Indore local market, and were authenticate by a botanist at APS University, Rewa (M.P.), India.

Preparation of extracts

The extraction was carried out utilizing Soxhlet extractor. The air- dried fine powder of the *Pennisetum Glauccum* seeds were exposed to soxhlation for 24 hour by hot extraction at around 60°C at room temperature using ethanol as a solvent. The extract was distilled in the porcelain evaporating dish and was evaporated to dryness on boiling water bath to achieve dark semi solid brown mass. [7]

Phytochemical Property: -

Qualitative assessment of ethanolic extract of *Pennisetum Glauccum* seeds was performed for major classes of phytochemical namely alkaloids, saponins, tannins, flavonoids, glycosides, phenolic content, terpenoids and volatile oils.

Experimental Animals: – Swiss albino mice weighing between 20–25 grams were procured from the animal facility of Swami Vivekanand College of Pharmacy, Indore, India. The animal were kept individually in the large spacious hygienic cages at 22c +-3 c following 12 hour light and 12 hour dark cycle, allowed them, free access to water ad libitum and food. The animal were allowed to acclidimize for seven days before being used for the study. The experimental procedure received approval from the Institutional Animal Ethics Committee of our institute. (Approval No: IAEC/SVCP/2024/JULY/04) and were strictly in accordance with the norms of CCSEA.

Acute oral Toxicity

According to literature ethanolic extract of *Pennisetum Glauccum* seeds was found safe at 300 mg/kg and 2g/kg body weight, no mortality was observed at both doses and therefore, LD50 of ethanolic extract of plant was reported 4000 mg/kg body weight.[7]

Grouping of experimental animals

Healthy Swiss albino mice of both sexes were utilized for the study. After one week of acclimatization, the mice were divided into four group comprising total 24 mice in different group of animals.

Group A- Normal control
Dose: Normal saline (0.9%Nacl) 1ml/100ml kg
Group B- Low dose of ethanolic extract
Dose: 400 mg/kg
Group C: High dose of ethanolic extract
Dose: 800 mg/kg
Group D: Standard group
Dose: Diclofenac Na (9mg/kg) and Aspirin (100mg/kg)

Evaluation Analgesic Activity

Eddy's hot plate method

Procedure:

The Mice were weighed and marked individually. All the Mice except normal control was pre-treated with low dose and high dose of ethanolic extract of *Pennisetum Glauccum* (400mg/kg; 800mg/kg) and standard preparation of diclofenac sodium (9mg/kg). The test solution and the reference drug were constituted in normal saline as vehicle and was administered orally through oral gavage. The volume of the drug and the test solution to be administered was calculated based on the body weight of animals following OECD guidelines. Group1 received normal saline and were placed on hot plate maintained at 55°C±1°C within the restrainer. The reaction time (in seconds) or latency period was determined as licking their paw or jumping. The treatment group 2 and 3 was pretreated with low dose (400mg/kg) and high dose (800mg/kg) of ethanolic extract of *Pennisetum Glauccum* and the reaction time was recorded at initial (0 min) and at 15, 30, 45 and 60min. interval. The cycle was repeated with all the animals of the particular group and the reaction time was recorded. The 4thtreatment group was administered the reference standard drug Diclofenac sodium (9mg/kg) orally through the oral gavage and the reaction time was recorded. The cycle was repeated with all the animals of the treatment group. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. If the reading exceeds 45 sec, it would be considered as maximum analgesia. The results of the test extract of low dose, high dose and negative control were compared with the normal control group and standard reference drug Diclofenac sodium. [8-9]

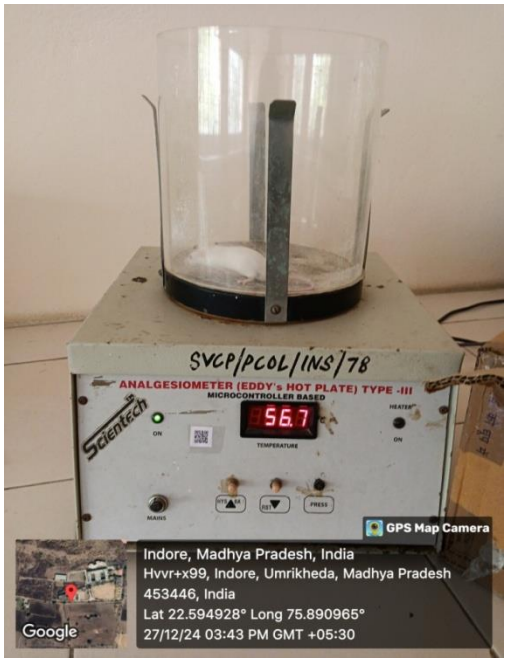

Figure: 1 Determination Analgesic activity in Swiss Albino mice.

The maximum possible analgesia (MPA) will be calculated as follows

$$MPA = \frac{Reaction\ time\ for\ treatment - reaction\ time\ for\ saline}{45\ \text{sec} -\ \text{reaction time for saline}} \times 100$$

Tail Flick Method

Procedure

The Mice were weighed and marked individually. All the Mice except normal control was pretreated with low dose and high dose of ethanolic extract of *Pennisetum Glauccum* (400mg/kg; 800mg/kg) and standard preparation of Aspirin (100mg/kg). The test solution and the reference drug were constituted in normal saline as vehicle and was administered orally through oral gavage. The volume of the drug and the test solution to be administered was calculated based on the body weight of animals following OECD guidelines. The albino mice of either sex divided into 4 groups of 6 animals each. Group 1 received 0.1 ml saline orally and served as control group. Group II received 400mg/kg ethanolic extract orally and served as low dose of test group. Group III received 800mg/kg ethanolic extract orally and served as high dose of test group. Group IV received 100mg/kg of Aspirin orally as a standard drug treated group.[10-11]

The analgesic potential was evaluated by tail flick method (Radiant heat). The mice were placed in a restrainer with an opening for their tails. The proximal third of the tail was exposed to radiant heat. The response of mice either as withdrawal of tail (tail flick) or turning of head to one side was taken as reaction time. The reaction time (in second) was recorded before treatment &0, 15, 30, 45 &60 min. after the administration of the treatment. The maximum time was fixed at 15 sec. to prevent any tail tissue injury.

Statistical analysis

The results are reported as mean ± SEM The statistical analysis were performed using one way analysis of variance (ANOVA) followed by turkey HSD test. The outcomes were compared to these of the normal saline treated and standard group. For all tests, difference with values of $P<0.05$ were considered significant.

Results

Extraction

The % yield of ethanolic extract of ***Pennisetum Glaucum*** seeds extracted compound was 28.27% w/w.

Qualitative phytochemical analysis of ethanolic extract of *Pennisetum Glaucum* seeds:

The qualitative phytochemical extract indicated that the ethanolic extract of seeds of ***Pennisetum Glaucum*** contain alkaloids, flavonoids, glycosides, volatile oils, saponins ,tannins and phenolic compounds.

Hot plate reaction time in mice:

While evaluating the analgesics activity of the ethanolic extract using hot plate, it was observed that normal 0.9% Nacl solution (group-1) did not have

any significant changes in basal reaction time, the low dose and high dose of ethanolic extract of *Pennisetum Glauccum* seeds showed highly significant effect at 15, 30, 45 and 60 min as compared with standard group. The ethanolic extract at a dose of 400mg/kg was found to have significant difference in basal reaction time at different time period. The ethanolic extract at a dose of 400mg/kg showed peak analgesic effect 39.8±0.18 at 60 min when compared to diclofenac sodium at 60 min.

Table 1: Maximum Possible Analgesia in *Pennisetum Glaucum* and Diclofenac sodium treated mice.

Maximum Possible Analgesia (%)							
Group name	**Treatment**	**Dose**	**0min.**	**15min.**	**30min.**	**45min.**	**60min.**
Test 1	EPG	400mg/kg	2.29	2.76	2.07	4.37	5.72
Test 2	EPG	800mg/kg	9.84	10.14	10.07	10.83	13.73*
Standard	Diclofenac sodium	9mg/kg	22.88	22.35	23.89	23.50	24.71

All values are mean ± SEM, Statistical analysis by one-way ANOVA followed by Turkey's post-hoc test, *P<0.05, EPG (Ethanolic *Pennisetum Glaucum)* seeds, SEM: Standard Error Mean.

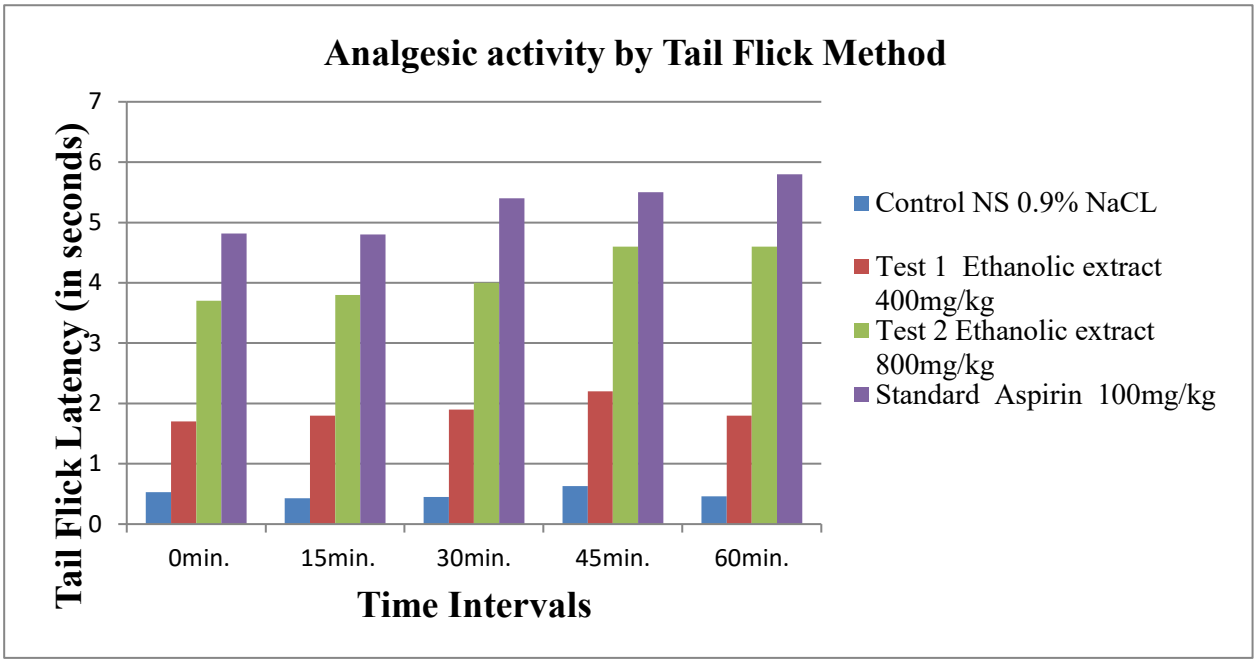

Figure 2: Increase in reaction time of mice at different time intervals of EPG relative to control and standard group.

Tail Flick method reaction time in mice:

While evaluating the analgesics activity of the ethanolic extract using Tail flick method, it was observed that normal 0.9% Nacl solution (group-1) did not have any significant changes in basal reaction time, the low dose and high dose of ethanolic extract of *Pennisetum Glaucum* seeds showed highly significant effect at 0, 15, 30, 45 and 60 min as compared with standard group. The ethanolic extract at a dose of 400mg/kg was found to have significant difference in basal reaction time at different time period. The ethanolic extract at a dose of 400mg/kg showed peak analgesic effect 39.8±0.18 at 60 min when compared to Aspirin at 60 min.

Table 2: Maximum Possible Analgesia in *Pennisetum Glaucum* and Aspirin treated mice.

Tail Flick Latency in seconds (mean ± SD)					
Drugs and doses (mg/kg)	**0 minutes**	**15 minutes**	**30 minutes**	**45 minutes**	**60 minutes**
Control (Normal saline 0.9% Nacl)	1.1±0.5	1.2±0.5	1.3±0.4	1.4±0.5	1.5±0.6
Low dose of T.E. (400 mg/kg)	1.7±0.65	1.8±0.75	1.9±0.45	2.2±0.8	1.8±0.65
High dose OF T.E. (800 mg/kg)	3.7±0.95	3.8±0.46	4±1.3	4.6±0.95	4.6±1.0
Standard Aspirin(100 mg/kg)	4.81±1.25	4.8±1.05	5.4±1.35	5.5±0.75	5.8±O.

All values are mean ± SEM, Statistical analysis by one-way ANOVA followed by Turkey's post-hoc test, *P<0.05, EPG(Ethanolic *Pennisetum Glaucum*) SEM: Standard Error Mean.

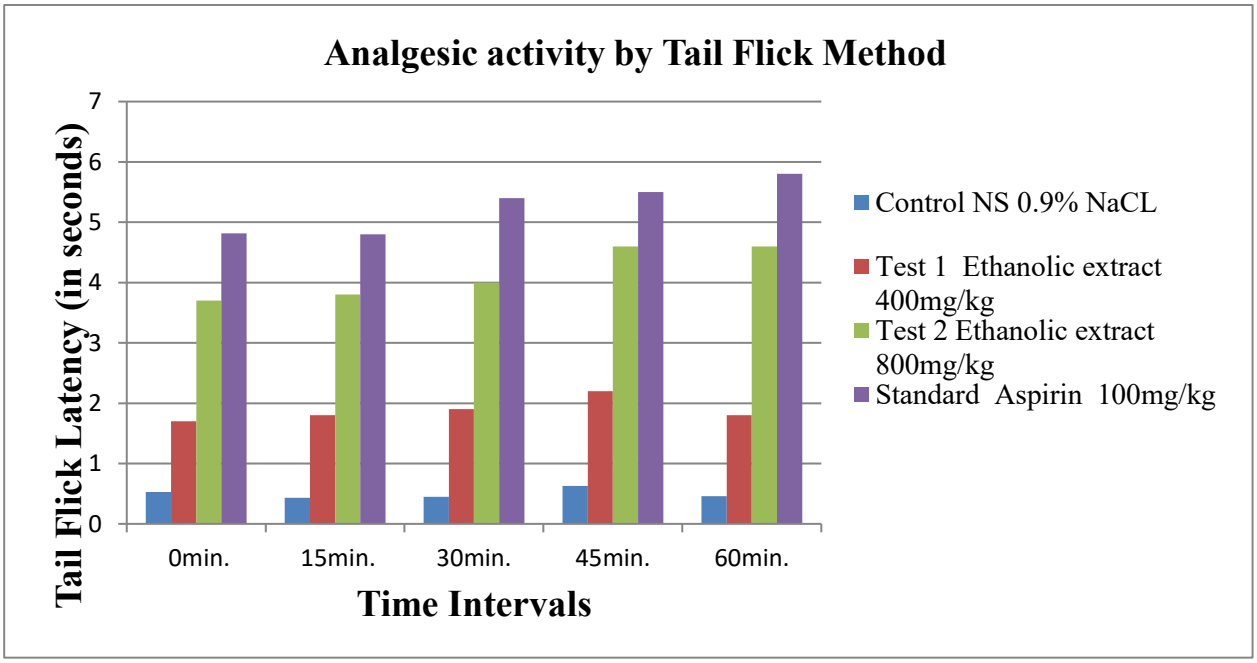

Figure3: Increase in tail flick latency of mice at different time intervals of ethanolic extract of EPG relative to control group.

Discussion

The study evaluated the *analgesic effects of ethanolic seed extract of Pennisetum glaucum* (EPG) using *Eddy's Hot Plate* and *Tail Flick* methods in *Swiss albino mice*, with *aspirin and diclofenac sodium* as reference drugs. The ethanol extract yielded *28.27%* and tested positive for *alkaloids, flavonoids, carbohydrates, saponins, and proteins*.

Both models confirmed that EPG exhibited *significant central analgesic activity*, with *peak effect at 60 minutes* post-administration, especially at *800 mg/kg*. The effect pattern mirrored that of aspirin, suggesting that *EPG may act via central mechanisms*, potentially involving *prostaglandin synthesis inhibition* and *arachidonic acid pathway blockade*.

Conclusion

The study demonstrated that *ethanolic seed extract of Pennisetum glaucum* exhibits *significant, dose-dependent central analgesic activity* in Swiss albino mice, as shown through *Eddy's Hot Plate* and *Tail Flick* methods. At *400 mg/kg and 800 mg/kg*, the extract notably increased *basal reaction time* and *tail withdrawal latency*, showing efficacy *comparable to standard drugs* like diclofenac sodium and aspirin.

This is the *first report* of such analgesic potential from *Pennisetum glaucum* seeds. *Phytochemical screening* revealed the presence of *alkaloids, flavonoids, saponins, cardiac glycosides, phenolics, and tannins*, which may contribute to the observed effects. Further studies isolating active constituents are recommended to confirm the *mechanism of action and therapeutic potential*.

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