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Evaluation of Analgesic Activity of Isolated Flavonoids from *Mucuna pruriens* Seeds

Naima Javed, Saadia Shahzad Alam, Habib Subhani, M. Shoaib Akhtar and Abdul Hameed Khan
Department of Pharmacology, Sargodha Medical College, Sargodha

ABSTRACT

Medicinal plants have been used as analgesics since millenia. **Purpose of the study**: Seeds of *Mucuna pruriens* have shown to possess analgesic activity and we planned to test flavonoids and alkaloids to find the active principles because both of them are found in relatively higher concentration in seeds of *Mucuna pruriens* plant. **Materials & Methods:** This study has been carried out in four groups of eight albino mice each, group I (control), group II (standard), group III (flavonoids) and group IV (alkaloids). Formalin paw licking test has been used to evaluate the analgesic activity by recording the number of lickings for 30 minutes after formalin injection in dorsal surface of left hind paw. **Results:** Flavonoids have shown significant analgesic activity (mean no. of lickings 24.25 ± 8.97) $p < 0.05$. While alkaloids have not shown any significant analgesic activity (mean number of lickings 39 ± 6.78). **Conclusion:** Isolated flavonoids from Mucuna pruriens seeds have showed significant analgesic activity whereas alkaloids have not shown any significant analgesic activity. So, flavonoids are responsible for the analgesic effect of Mucuna pruriens.

Key words: Mucuna pruriens, flavonoids, alkaloids, analgesics, formalin paw licking test.

INTRODUCTION

The plants have been widely used in the folk medicine of many countries to treat number of painful conditions like fever, dysmenorrhea and muscular pains[1]. *Mucuna pruriens* is a plant of family Leguminoseae and has been used in a number of diseases. It has been shown to reduce pain, fever and inflammation as well[2]. Its seed powder has been evaluated previously for its analgesic activity and has shown good results[3]. Keeping this in mind, we planned to carry out this work forward and find the active principal responsible for this analgesic activity. To begin with, we planned to isolate flavonoids and alkaloids and then test them. We selected them as both are well known to have many pharmacological actions and are found in relatively higher concentration in *Mucuna pruriens* seeds.

Purpose of the study

To evaluate the analgesic activity of isolated flavonoids from *Mucuna pruriens* seeds.

MATERIALS AND METHODS

Material required

a. Seeds of *Mucuna pruriens*: Seeds of the *Mucuna pruriens* Linn were purchased, identified and authenticated and kept at normal room temperature.
b. Aspirin (Manufactured By RECKITT BENCKISER, Pakistan Ltd.) purchased from local pharmacy.
c. Formalin (Formaldehyde solution 37% manufactured by Merck, Germany)was purchased from local pharmacy.

Animals required

A total of 32 Wister albino mice of either sex weighing 20-25 gm were used. They were housed in standard polypropylene cages and kept under controlled room temperature (25±10°C; relative humidity 60-70%) and fed with standard laboratory diet with water *ad libitum*.

Grouping of the animals

Four groups of animals were used for the

experiment.

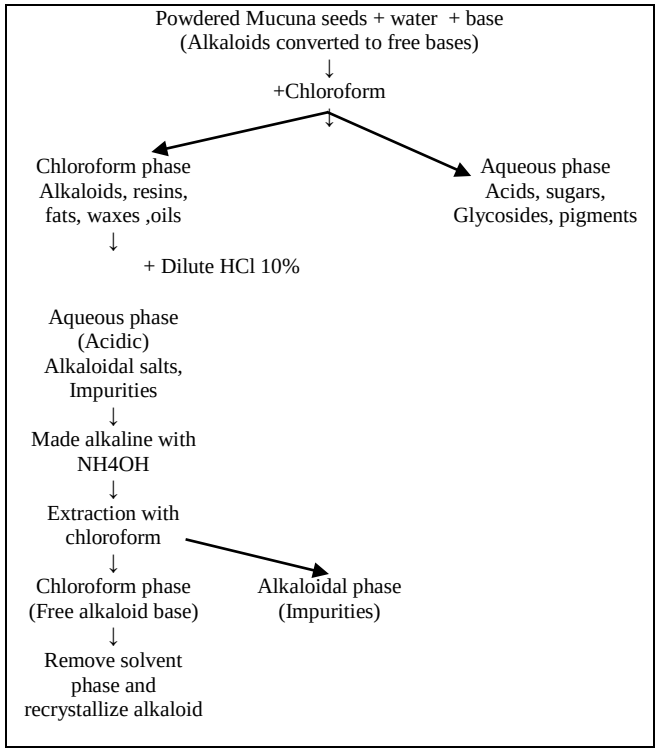
Method

The seeds were powdered with an electric grinder and the flavonoids and alkaloids isolated by the method described below[4]. They were stored in air-tight glass containers at room temperature.

Extraction of flavonoids

Powdered *Mucuna* seeds
↓
20g of powdered seeds + 100ml petroleum ether
↓
Refluxed for 1 hour & Filtered while hot
↓
Dried the residue & added 100ml Methanol
↓
Refluxed for 2 hours & Filtered while hot
↓
Washed the residue with 20ml methanol
↓
Concentrated under reduced pressure

Extraction of Alkaloids:



Analgesic activity

Formalin induced paw-licking test:

Animals of group I (control), group II (standard), group III and group IV were administered orally gum tragacanth solution (1ml/kg/p.o), aspirin (100mg/kg/p.o), flavonoids and alkaloids(equivalent to 3 grams seed powder) respectively[5]. One hour later, the analgesic activity was determined by using formalin test[6]. In this the mice were observed for 30 minutes after injection of fifty microlitres of 2.5% formalin into the dorsal surface of the left hand paw and the number of lickings during the 30 minutes observation period were recorded.A change in the number of lickings was taken as a measure of the analgesic activity of the administered compound.

Statistical analysis

The data was analysed as mean±s.d and Duncan's multiple range test (ANOVA) was applied (Table 1) p-value<0.05 was taken as statistically significant between the groups.

Table 1: Analgesic activity of Flavonoids evaluated by ANOVA.

Group	Mean no. of lickings ± S.D
I (control)	44.125 ± 5.88
IV (alkaloids)	39 ± 6.79
III (flavonoids)	24.25 ± 8.97*
II (standard)	17.75 ± 4.39*

Standard error (SE) = 2.82
*The mean values were statistically significant ($p < 0.05$)

RESULTS

To determine the analgesic activity, number of lickings were recorded in all the four groups (Table 2, Fig. 1). and p values < 0.05 were used to make comparison between the groups.

Table 2: Number of lickings in four groups of mice

No. of animal	Group I Control	Group II Standard	Group III Flavonoids	Group IV Alkaloids
1	40	12	14	49
2	53	22	30	45
3	50	24	16	31
4	36	16	31	37
5	44	15	40	40
6	47	19	25	29
7	45	21	17	43
8	38	13	21	38

Figure 1 shows mean number of lickings in all groups of mice.

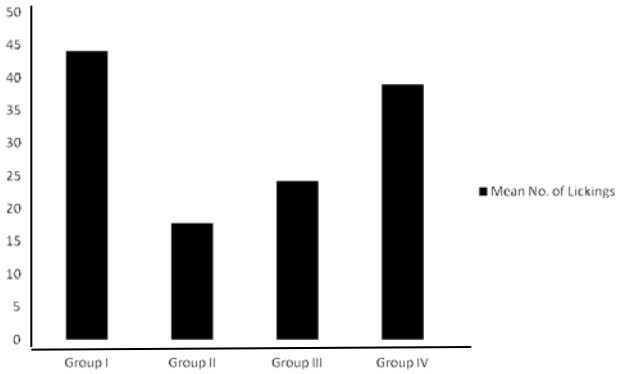


Fig. 1. Mean number of lickings in all groups.

DISCUSSION

Pain and inflammation is associated with pathophysiology of various clinical conditions like arthritis, gout and muscular pains. *Mucuna pruriens* is known to contain a number of different constituents like alkaloids, flavonoids, saponins in addition to its major constituent L-dopa. The results obtained indicated that isolated flavonoids of *Mucuna pruriens* possessed significant analgesic activity as shown in statistical analysis. p values for the group III (flavonoids) being < 0.05 indicated significant analgesic activity whereas alkaloids failed to show any significant analgesic activity $p < 0.05$.

Flavonoids are the class of phenolic compounds widely distributed in plants and have medical functions as well. They are known to target prostaglandins which are involved in the late phase of pain perception[7,8]. So, in this study we were able to move a step forward *i.e.,* to isolate and evaluate the active principal the flavonoids. This work can be extended further by chromatography and finding the active fraction. Pure drug can be isolated from that active fraction which will possess the analgesic action.

As we know that although number of analgesics are available like NSAIDS (non-steroidal anti-inflammatory drugs) or opioids they possess partial activity and large number of unwanted side effects like gastric ulcer, nephropathy, sedation, respiratory depression, constipation and addiction[9]. A huge number of patients awaits an efficacious and safe analgesic which the flavonoids of Mucuna pruriens seeds appear to be in this preclinical research .

CONCLUSION

On the basis of our study and statistical analysis, it could be concluded that isolated flavonoids from Mucuna pruriens seeds showed significant analgesic activity whereas alkaloids failed to show such effect. We did not observe any adverse effects in groups receiving either flavonoids and alkaloids. In the light of our study, there is scope of further work on plant and flavonoids with the possibility to isolate the pure drug which may be more potent and free of adverse effects as well.

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The Authors:

Naima Javed
Assistant Professor
Department of Pharmacology
Sargodha Medical College,
Sargodha.

Saadia Shahzad Alam
Associate Professor
Department of Pharmacology
Sheikh Zayed Post-graduate Medical Institute,
Lahore.

Habib Subhani
Associate Professor
Department of Medicine
Sargodha Medical College,
Sargodha.

M. Shoaib Akhtar
Professor
Department of Pharmacology
University of Sargodha,
Sargodha.

Abdul Hameed Khan
Professor
Department of Pharmacology,
Sheikh Zayed Postgraduate Medical Institute,
Lahore.

Corresponding Author:

Naima Javed
Assistant Professor
Department of Pharmacology
Sargodha Medical College,
Sargodha.