Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **04 Year 2023** Page **224:229***

An *In Vitro* Pharmacognostical Study on Gluconeogenesis and Glucose

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Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 11 Nov 2023

Abstract

Traditional medicine over 60% of the world's population used for health care name as Mukia maderaspatana (L.) M. Roem. (Cucurbitaceae) (Mukia) is extensively important medicine as an anti-inflammatory plant. It is rich in content of phenolics that exerts various medicinal properties. Mukia extract and its derivatives phenolics such as quercetin and phloroglucinol are investigated for their in vitro anti-inflammatory activity. Materials used was Quercetin, phloroglucinol, and methanol which extract of the dried whole plant (0.55 and 0.6 mg/ml) were studied for the inhibition of gluconeogenesis and glucose Phenolics of Mukia were analyzed by HPLC and UV-spectroscopy. Results obtained were Glucose (1.5 mg/g/h) was synthesized from pyruvate, the synthesis was whole inhibited by insulin (1 U/ml). Quercetin at 0.35 and 0.6 mg/ml caused 60% and 90% inhibition (0.43 mg/g/h and 0.12 mg/g/h glucose). Addition of insulin did not increase inhibition. Phloroglucinol inhibited 100% glucose production with or without insulin. Mukia (0.20 mg/ml) inhibited gluconeogenesis (0.65 mg/g/h) by 40%, and with insulin, inhibition increased to 50% (0.59 mg/g/h). At 0.6 mg/ml, glucose synthesis was stimulated by1.3-fold, but with insulin gets inhibited by 90% (0.12 mg/g/h glucose). Mukia possessed no effect on glucose uptake, in case it potentiated the activity of insulin mediated glucose uptake (153.82 ± 12.30 mg/dl/g/30 min) compared with insulin control (122.41 ± 9.14 mg/dl/g/30 min) ($p < 0.02$). HPLC analysis revealed the presence of phenolics. Results were concluded scientific rationale for the use of Mukia in medicine as an anti-inflammatory nutraceutical.

Keywords: *Anti-inflammatory, nutraceutical, Glucose synthesis, Phloroglucinol, medicinal property.*

1. Introduction

The most important and has a major role in all traditional medical system especially in Ayurveda, Siddha, Unani, Homeopathy, Naturopathy and Chinese Medicine. The plants which is enough to possessing health promoting capacities and their bio active compounds or active-ingredients cure the diseases. It is important to study the pharmacology activity of individual plants with their bio active compounds deed in treating diseases. Mukiamaderaspatana (L.) M. Roem.is a species of plant many of year it possessing for cooking and medicinal remedies (Cleide de souse, 2004).

Plant profile

Kingdom: Plantae
Division: Sermatophyta
Sub-division: Angiospermae
Class: Dioctyledonae
Sub-Class: Polypetalae
Series: Calyiflorae
Order: Passiflorales
Family: Cucurbitaceae
Genus: Mukia
Species: maderaspatana

Synonyms: Cucumismaderaspatanus (L.), Melothriamaderaspatana (L.) Cogn.,Melothriaaltaeoides (Ser.), Mukiaalthaeoides (Ser.) (M.Roem.), Mukiarottleri (M.Roem.), Mukiascabrella (L.) (Arn.), Trichosanthesdioica (Wall.), Bryoniaalthaeoides (Ser.), Bryoniacallosa (Rottler.), Bryoniagracilis (Wall.), BryoniahispidaSalisb.), Bryoniamaderaspatana (L.), (Lam.), BryoniamicranthaHochst (Cogn.), Bryoniamicropoda (E.mey.), Bryoniarottleri (Spreng.), Bryoniascabra (Rottler.), Bryoniascabrella (L.)

Mukia maderaspatana is highly valued herbs in ayurvedic system of medicine for cure of various ailments. The herbs reported to have activities such as hepatoprotective, anti-flatulent, anti-inflammatory, antidiabetic, expectorant, diuretic, stomachic. This research study was taken to investigate anti-inflammatory activity of methanolic extract of roots of *Mukia maderaspatana* in alloxan induced diabetic rats. Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent cells and tissues. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only the sign of inflammation (Debra Manzella, 2009).

2. Materials And Methods

Mukia maderaspatana (Linn.) Roem, Swiss albino mice female weighing 20-25gm were used for the study. Animals were fed a standard pellet (Pranav Agro Industries ltd; sangli) and water ad libitum and maintain at 24-28°C temperature, 60-70% relative humidity and 12 hours, day and night cycle. Animals described as fasted were deprived of food for 16 hrs., but had free access to water.

Plant Treatment

Petroleum ether extraction

About 75g of dried plant material was extracted with 375 ml of petroleum ether by using a separating funnel with occasional shaking for 16 hours. The extract was concentrated to 1/4th of its original volume by evaporation at room temperature. Each time before extracting with the next solvent, the residue was air dried thoroughly to remove the solvent used (Gerhard Vogel, 2002).

Chloroform extraction

The dried residue was extracted with chloroform by occasional shaking for 16 hours.

Water extraction

Finally, the above dried residue was extracted with water by occasional shaking for 16 hours.

Animals Treatment

Group-I: The rats received only the 10 ml/kg vehicle; these animals serve as a healthy control.

Group-II: The methanolic extract of *Mukia maderaspatana* at a dose of 200 mg/kg body Weight to the normal served as experimental control first.

Group-III: The methanolic extract of *Mukia maderaspatana* at a dose of 400 mg/kg body Weight to the normal served as experimental control second.

Group IV: The rats were treated with 10 mg/kg of indomethacin as a standard drug.

Evaluation of extracts "*Mukia maderaspatan"*

Phytochemical Screening The various extracts of roots of *Mukia maderaspatana* subjected to the following chemical tests for the identification of its various active constituents.

Liebermann – Burchard Reagent

5ml of acetic anhydride and 5ml concentrated sulphuric acid were added carefully to 50 ml absolute ethanol, while cooling in ice.

Dragendroff's reagent

Solution (A): Mix 0.85g basic bismuth nitrate in 10 ml glacial acid and 40 ml of distilled water under heating. If necessary, filter.

Solution (B): Mix 8gm of potassium iodide in 30 ml of distilled water. Stock solution: Solution (A) + (B) are mixed 1:1.

Wagner's Reagent

Mixed 1.27g Iodine and 2g of potassium iodide in 5ml of water and make up to volume 100ml with distilled water.

Animal Treatment

Animals were feed standard pellets. Swiss albino mice female weighing 20-25gm were used for the study. Animals were fed a standard pellet (Pranav Agro Industries ltd; sangli) and water ad libitum and maintain at 24-28˚C temperature, 60-70% relative humidity and 12 hours, day and night cycle. Animals described as fasted were deprived of food for 16 hrs., but had free access to water.

Methods: Acute oral toxicity studies were performed according to OECD-423 GUIDELINES (acute toxic class method). Swiss female mice (n=3/each dose) by random sampling technique were employed in this study. The animals were fasted for 4hr with free access to water only. The extracts (suspended with 0.5%w/v, CMC) were administered orally at a dose of 5mg/kg, to separate group of mice and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of three animals, then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 50, 300 and 2000mg/kg. The following general behaviors were observed for first 2 hour and after 24 of test drug administration (Ecobichon *et al*., 1997)**.**

Acute toxicity studies revealed the nontoxic nature of methanolic extract of *Mukia maderaspatana.* There were no lethality or toxic reactions found at any of the doses selected until the end of the study period. All the animals were alive, healthy and active during the observation period.

Table No. 2: General action was testing after the test drug administration

S. No.	Actions potentials	Drug		
		Animal G- 1	Animal G- 2	Animal G- 3
1.	Weight & Marking	25	25	20
2.	Tremor	-	-	-
3.	Convulsion	-	+	-
4.	Straub reaction	-	-	-
5.	Pilo erection	-	-	-
6.	Catatonia	-	-	-
7.	Loss of Righting Reflex	+	-	-
8.	Decreased Motor Activity	-	-	-
9.	Increased Motor Activity	-	-	-
10.	Sedation	-	+	-
11.	Muscle Reaction	-	-	-
12.	Analgesia	-	-	-
13.	Ptosis	-	-	-
14.	Lacrimation	-	+	-
15.	Salivation	-	-	-
16.	Diarrhea	+	-	-
17.	Change in skin color	-	-	-

Meaning: **"-" Absent and "+" Present**

Anti-Inflammatory Study

Principle: The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. (Gerhard vogel, 2002).

Reagent composition

A. Sterilized cotton pellet (Each weighing 20 mg implanted s. c.).

B. Indomethacin 10 mg/kg, p.o. prepared as a stock solution.

Method: For every treatment, six animals were used. After oral treatment of extract 50 and 100 mg/kg and standard indomethacin 10 mg/kg for 7 days, the rats were sacrificed. The pellets were dissected and dried at 60˚C overnight to determine the dry weight. The increase in weight of cotton pellet was determined and used for further calculation. Four sterilized cotton pellets, each weighing 20 mg were

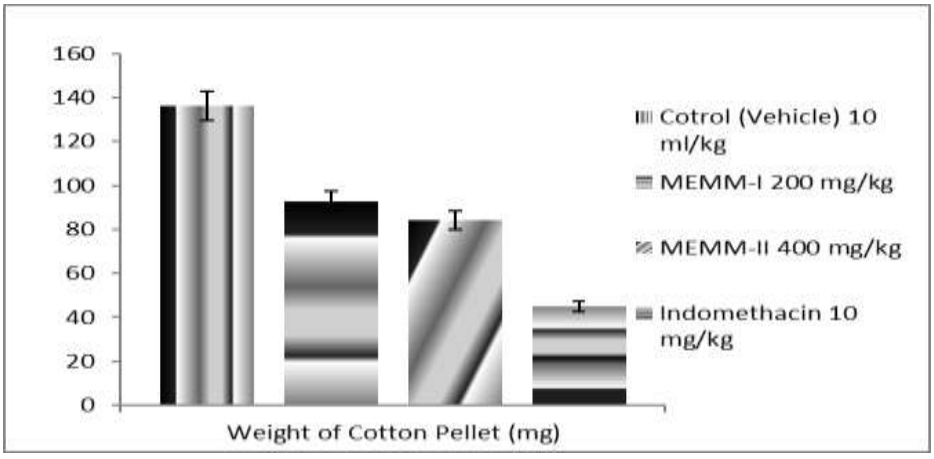
implanted subcutaneously one in each axilla and groin of anesthetized rats weighing between 150 and 200 g using the method of (D'Arcy *et al*., 1960).

Table No. 8: *Mukia maderaspatana* (MEMM) on cotton pellet implantation in rats.

Groups	Concentrations	Wt. cotton pellet (mg)	(%) of inhibiting
Control(vehicle)	10 ml/kg	136.17 ± 7.75	-
MEMM	200 mg/kg	92.85 ± 1.32**	31.9 %
MEMM	400 mg/kg	84.33 ± 1.43**	38.1 %
Indomethacin	10 mg/kg	44.86 ± 2.36**	67.1 %

The data represents the mean ± S.D. (n=6), **p< 0.01 values were significant as compared to Control.

Fig. 14: Effect of methanolic roots extract of *Mukia maderaspatana* (MEMM) on cotton pellet implantation in rats.



3. Results and Discussion

There was dose-dependent significant reduction in carrageenan-induced rat paw edema at 200 and 400 mg/kg of extract and at 10 mg/kg indomethacin over a period of 4 hrs.

In these models, root methanol extract showed significant anti-inflammatory activity against carragen an induced phlogistic response. Moreover, Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin and slow reacting substances. The results of carrageenan experiment showed maximum activity at first and fourth hour after the injection. This explains the inhibition of first phase of inflammation which is mediated by histamine and serotonins.

The values of inhibitory action of the methanol extract against granuloma formation induced by pellet implantation are shown in table no. 7. The extract was found to significantly reduce the weight of cotton pellet induced granuloma formation in rats. Indomethacin (10 mg/kg) and the methanol extract of *M. maderaspatana* (200 mg/kg and 400 mg/kg) showed significant ($p<0.01$) anti-granuloma activity, but less active than indomethacin. The inhibition % of extract was 31.9 % and 38.1 % respectively 200 mg/kg and 400 mg/kg but for indomethacin it was 67.1 %.

The results showed that the methanol extract was inhibitory in its action and is proportion to the doses employed, thus proving its activity in the proliferative phase of inflammation (Seyle, 1949).

Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts during glandular tissue formation (Gupta *et al*., 2003).

4. Conclusion

The present study was focused on the antihyperglycemic and anti-inflammatory activities of methanolic root extract of *Mukia maderaspatana* in alloxan induced diabetic rats. Different extract of the *Mukia maderaspatana* roots were subjected to phytochemical screening out of which methanolic extract was found to contain a greater number of phytochemicals such as carbohydrates, proteins, alkaloids, tannins, saponins, flavonoids and coumarins. Hence, further study was done with methanolic root extract of *Mukia maderaspatana* in animal's models to investigate the antidiabetic and anti-inflammatory activity.

The rats were categorized into five groups. Rats in the group first treated normally and served as control. In group second, third and fifth rats' diabetes was induced by giving alloxan. Among this, rats of group third treated with methanolic extract of the roots of *Mukia maderaspatana.* The fourth group without inducing diabetes treated with methanolic extract of roots of *Mukia maderaspatana*. The fifth group rats were treated with standard drug (glibenclamide). Alloxan, which is a chemical used for the induction of diabetes in animals, has been shown to damage pancreatic ß-cells by the liberation of oxygen radical.

The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Winter et al., 1962). The first phase occurs within an hour of injection and is partly due to the trauma of injection and also to the serotonin component (Crunkhorn and Meacock, 1971). Prostaglandins (PGs) play a major role in the development of the second phase of reaction which is measured around 3 hours times (Di Rosa, 1972). The presence of PGE2 in the inflammatory exudates from the injected foot can be demonstrated at 3 hours and period thereafter (Vinegar *et al.,* 1969). The carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis (Phadke, 1988). Based on these reports it can be inferred that the inhibitory effect of extract on carrageenan-induced inflammation in rats could be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

The values of inhibitory action of the methanol extract against granuloma formation induced by pellet implantation. The extract was found to significantly reduce the weight of cotton pellet induced granuloma formation in rats. Indomethacin (10 mg/kg) and the methanol extract of *M. maderaspatana* (200 mg/kg and 400 mg/kg) showed significant anti-granuloma activity, but less active than indomethacin. Based on the results of this study we came to the conclusion that the methanol extract of the root of the *Mukia maderaspatana* may have potential anti-inflammatory activity, against both exudative and proliferative phases of inflammation. However further study needed for the exploration of phytoconstituents present in *Mukia maderaspatana* the Anti-diabetic and Anti-inflammatory activities which was responsible for this above said reference screening.

Acknowledgements

All the authors are great full for the research center and management of Bhagwant University, India to provide all the necessary equipment and facilities to perform the research.

Conflict of Interest

All the authors declare that he has no conflict of interest. This article does not contain any studies with human subjects performed by the any of the authors.

Funding Source

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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