Short Comunications

Inflammation Modulation with Methanol *Zea Mays* Cob Husk on an Experimental Model in Albino Rats

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ABSTRACT

Introduction: Several parts of *Zea mays* (maize) have been used for the treatment of several ailments including inflammatory conditions.

Objective: to evaluate the modulatory effect on carrageenan-induced inflammation of the methanol extract of *Zea mays* cob husk in Albino rats.

Methods: 9.45% maize cob husks methanol extract was obtained by soxhlet extraction and the phytochemical screening was done. The carrageenan-induced paw edema model of acute inflammation was carried out to 30 Albino rats which were divided into five groups, two controls (negative and positive) and three experimental. A single oral methanol extract (100, 200 or 400mg/Kg) were administered to the experimental groups. Indomethacin 10mg/Kg was used as positive control. The paw volume up to the tribiotural articulation was measured at 0, 1, 3 and 6 th hours. Inflammation was expressed as an increase in paw volume due to carrageenan injection and the percentage inhibition produced by the extracts was calculated to assess the anti-inflammatory

activity. Acute toxicity study was conducted. ANOVA test was applied and *P* value less than 0.05 were considered significant.

Results: saponins, tannins and polyphenols were present in the plant extract. Doses below 5000mg/Kg were considered safe. Significant anti-inflammatory activity was shown at 400mg/Kg after sixth hours.

Conclusions: The anti-inflammatory effect of *Zea mays* cob husk extract may be attributed to the presence of saponins, tannins and polyphenols. Percentage inhibition of inflammation by the extracts showed dose and time dependence. The anti-inflammatory effect was found significant for safe concentrations of the plant extract.

Keywords: *Zea mays*, anti-inflammatory activity, carrageenan-induced inflammation.

METHODS

The anti-inflammatory effect of *Zea mays* cob husk methanol extract was assessed by determining the percentage inhibition of inflammation induced by a carrageenan injection.

Plant material

A sample of fresh maize cob husks was collected from Mayuge, Uganda, taken to Mbarara University of Science and Technology, Faculty of Science, for identification by the botanist Dr. Eunice Olet and it was given a voucher number: THADEUS 001.

Preparation of Z. maiz methanol cob husk extract

One kilogram of fresh cob husks were washed with distilled water and shade dried. Then were powdered by a blender and weighed; 129g of the powdered obtained was soxhlet extracted[1] in 80 % methanol for 24 h and then filtered; 12.2g of crude methanol extract was obtained at a percentage yield of 9.45% w/w.

Phytochemical screening

Phytochemical analysis was performed on 9.45% methanol extract using some chemical reactions to identify the presence of secondary metabolites: saponins (foam), alkaloids

(Dragendorff), phenols and tannins (ferric chloride: $FeCl_3$), glycosides (keller-Killiani), terpenoids (Libermann-Buchard), flavonoids (Shinoda), reducing sugars (Fehling). [2]

Animals

Forty-two Albino rats from 170 to 350g of weight were used. The rats were obtained from the Animal house of MUST and were kept for two months under controlled conditions, 23 ± 0.5 ^{o}C, relative humidity around 50 %, in a 12h:12h alternate light-dark cycle, food and water add libitum. The Guide for the care and use of laboratory animals was strictly followed.[3]

Pain, suffering or distress was minimized both in duration and magnitude to the greatest possible extent without jeopardizing the aim of the experiment. The method used for animal´s euthanasia was overdose of anesthesia (three times the anesthetic dose of sodium pentobarbital) as described by the American Veterinary Medical Association Guidelines for the Euthanasia of Animals.[4]

Anti-inflammatory effect

The experiment was carried out following the carrageenan-induced rat hind paw edema animal model of acute inflammation.[5] Thirty rats were used and were divided into five groups of six rats each.

Acute inflammation was provided by injection of 0.1mL of 1% carrageenan into the sub-plantar surface of rat hind paw. Different doses of the plant extract, the standard and the negative control where administered 30 minutes before injection of the carrageenan as a single oral dose:

Group I: indomethacin (10mg/Kg) (standard or positive control)

Group II: *Zea mays* methanol extract (100mg/Kg)

Group III: *Zea mays* methanol extract (200mg/Kg)

Group IV: *Zea mays* methanol extract (400mg/Kg)

Group V: NaCl 0.9% (10mL/Kg) (negative control)

The paw volume up to the tribiotural articulation was measured at 0, 1, 3 and 6th hours. Inflammation was expressed as an increase in paw volume due to carrageenan injection and the percentage inhibition produced was calculated.

Acute toxicity screening

The acute toxicity of the plant was determined by the Lorke´s method [6]:

Phase 1: Nine rats were divided into three groups of three rats each. Each group were administered different doses (10, 100 and 1000 mg/Kg) of test substance.

Phase 2: Three rats were divided into three groups of one animal each. The rats were administered higher doses (1600, 2900 and 5000 mg/Kg) of test substance.

In both phases the animals were placed under observation for 24 hours to monitor their behavior as well as mortality. LD_{50} was calculated.

Statistical analysis

Statistical analysis was carried out using SPSS (version 16.0) software. The mean differences were calculated by two-way ANOVA test and *P* value less than 0.05 was considered significant.

RESULTS

Phytochemistry

The phytochemical screening of the plant extract found a positive reaction to polyphenols, tannins and saponins. Reducing sugars were present in trace quantities.

Acute toxicity

No mortality was observed within 24 hours. The animals did not show any physiological signs of toxicity. Therefore the LD_{50} of the plant extract was considered to be higher than 5000mg/Kg.

Anti-inflammatory study

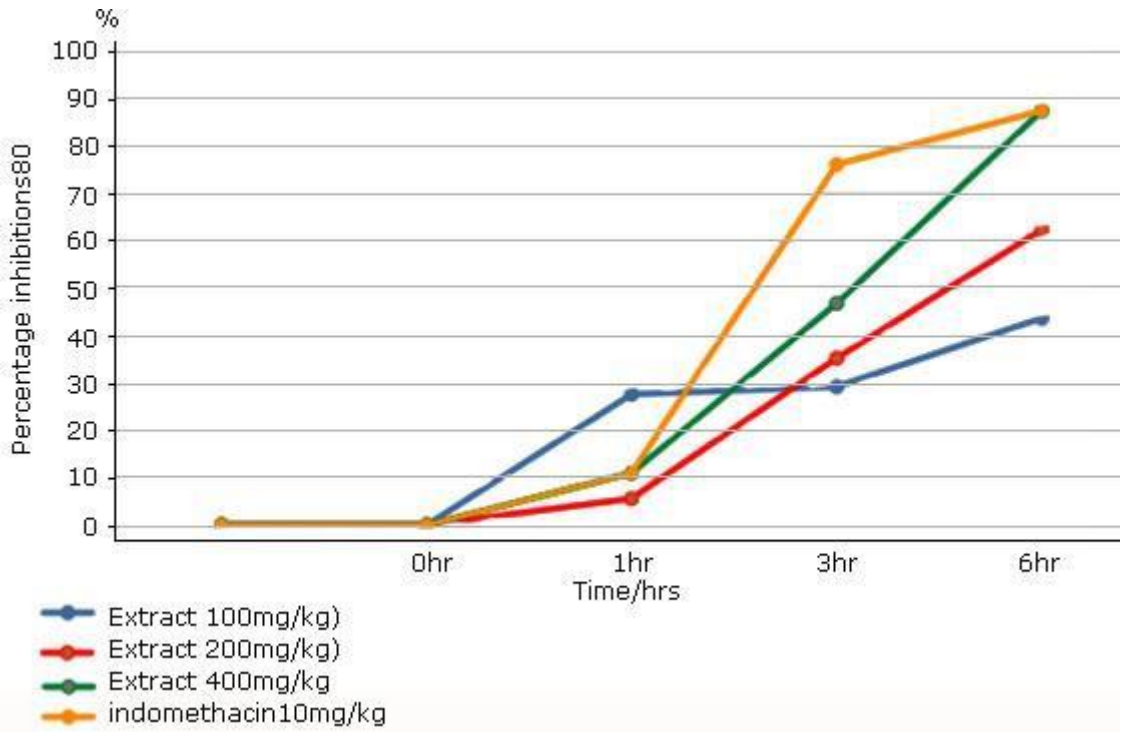
Was observed a mean increase in paw size due to carrageenan injection and significant values were those of the extract 400mg/Kg at the sixth hour and indomethacin 10mg/kg after the third and sixth hour. (table 1)

Table 1. Mean increase in paw size due to carrageenan injection

Treatment	Dose/Kg	Mean increase in paw size ± SEM (mm)		
		1 hr	3 hr	6 hr
NaCl	10 mL	3.00±0.52	2.83±0.60	2.67±0.49
Extract	100 mg	2.17±0.79	2.00±0.86	1.50±0.50
	200 mg	2.83±0.60	1.83±0.40	1.00±0.26
	400 mg	2.67±0.67	1.50±0.50	0.33±0.21**
Indomethacin	10 mg	2.67±0.49	0.67±0.33**	0.33±0.21**

Values are expressed as Mean±SEM (n=6),
** $P<0.05$ significantly different from negative control

Graph 1 shows the percentage inhibition of inflammation of the different administered doses. They show dose dependence as inhibitory activity increases with increase in dose. After six hours the extract (400mg/kg) has the same inhibition as the positive control (indomethacin 10mg/kg).



DISCUSSION

The screened *Z. maiz* methanol husk extract contained polyphenols, tannins and saponins in high concentrations, which match with those components reported by Owoyele and

collaborators.[7] These phytochemicals have been reported to be linked to various degrees of anti-inflammatory effect.[8,9]

The effect of the methanol extracts on carrageenan-induced edema was time dependent being significant after sixth hours of edema induction and at the highest dose (400mg/Kg). Mean increase in paw size was inversely proportional to the anti-inflammatory activities.

From a study carried out by Owoyele and collaborators using the aqueous extract of the same plant part, percentage inhibition of the extract at doses of 200mg/Kg at the third and fifth hour was 31.43% and 78.13% respectively as compared to the 35.3% and 62.5% (at sixth hour not fifth as for the aqueous) of the methanol extract. The percentage inhibition at high doses (400mg/Kg) of the methanol extract was the same as that of the positive control (indomethacin 10mg/kg).[7]

The development of inflammation induced by carrangeenan is believed to be biphasic. The initial phase (0 -1hr) is due to release of serotonin, bradykinin and histamine. The second phase (1 -6hrs) is due to release prostaglandins and induction of COX-II. The inflammation is maximal after 6hrs and lasts for a total of 10 hours.[5] Because significant anti-inflammatory activity of the extract was only found in the second phase of induction, the probable mechanism of action of the metabolites identified is through inhibition of the second phase of carrageenan induced edema which is associated with prostaglandin release and cyclooxygenase II enzyme stimulation.

CONCLUSIONS

Taking into account our findings it can be concluded that *Zea maiz* cob husks methanol extract has anti-inflammatory activity which may be attributed to the presence of polyphenols, tannins and saponins. Percentage inhibition of inflammation by the extracts showed dose and time dependence. The anti-inflammatory activity was found significant for safe concentrations of the plant extract.

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