



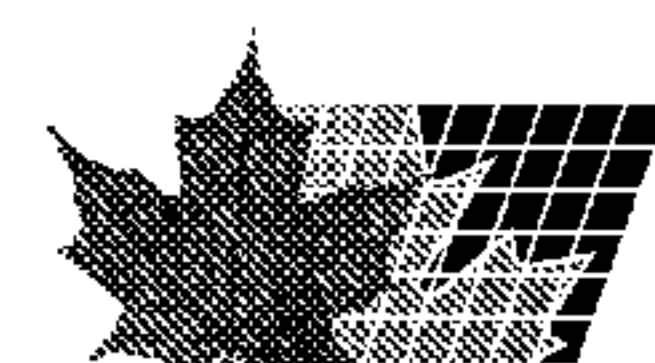
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(54) Titre : PROCEDE D'OBTENTION D'UN EXTRAIT DE GINGEMBRE

(54) Title: METHOD OF OBTAINING AN EXTRACT OF GINGER

(57) **Abrégé/Abstract:**

The invention relates to the pharmaceutical and food industry and, in particular, to a method of obtaining an extract of ginger by means of heat using butter as an extraction substance. The inventive method is used to produce a yellow ochre compound, of liquid and dense consistency at ambient temperature, which contains the principal fat-soluble components of ginger. Said compound has a therapeutic activity which has been demonstrated in laboratory animals in relation to acute, chronic inflammation and can be used as an antihistamine, an antiserotoninic agent and an inhibitor of passive cutaneous anaphylaxis (type I hypersensitivity reaction), induced by IgE, in order to treat, in particular, allergic asthma.



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(54) Title: METHOD OF OBTAINING AN EXTRACT OF GINGER

(54) Título: PROCEDIMIENTO DE OBTENCIÓN DE UN EXTRACTO DE JENGIBRE

(57) Abstract: The invention relates to the pharmaceutical and food industry and, in particular, to a method of obtaining an extract of ginger by means of heat using butter as an extraction substance. The inventive method is used to produce a yellow ochre compound, of liquid and dense consistency at ambient temperature, which contains the principal fat-soluble components of ginger. Said compound has a therapeutic activity which has been demonstrated in laboratory animals in relation to acute, chronic inflammation and can be used as an antihistamine, an antiserotoninic agent and an inhibitor of passive cutaneous anaphylaxis (type I hypersensitivity reaction), induced by IgE, in order to treat, in particular, allergic asthma.

(57) Resumen: La presente invención se relaciona con la Industria Farmacéutica y la Alimenticia, y en particular se refiere a un procedimiento de obtención de un extracto de jengibre mediante calor que utiliza como sustancia extractora la mantequilla. Dicho procedimiento proporciona un compuesto de color amarillo ocre, de consistencia líquida y densa a temperatura ambiente, en cuya composición se encuentran los principales compuestos liposolubles del jengibre, con actividad terapéutica demostrada en animales de experimentación, en la inflamación aguda y crónica, como antihistamínico, antiserotonínico e inhibidor de la anafilaxia pasiva cutánea (reacción de hipersensibilidad tipo I), mediada por IgE, entre las que se encuentra el asma alérgica.



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PROCEDURE FOR OBTAINING LIPID-SOLUBLE COMPONENTS OF GINGER

DESCRIPTION

Technical field

The present invention refers to a process for the natural heat-aided extraction of lipid-soluble components of ginger, using butter as an extracting agent. An extract with proven therapeutic activity in experimentation animals is obtained. It is related with the chemistry and pharmacy fields.

Previous Art

Ginger is a plant native from Asia. Among its more significant applications it is used to treat diarrhea, vomits, gastralgia, indigestion, whooping cough, fever, flu, cold, bronchitis, rheumatism, ulcer, cancer, as well as antibacterial and antidiuretic.

Butter is a natural dairy product, composed of 80% fat, (50% of which is saturated, 25% is unsaturated, 3% is polyunsaturated) and 20% of water, forming dispersed droplets containing 2% of proteins and salts). Clarified butter is known as Ghee, an ancient food registered in the old Veda religious books. It is considered a superior food with therapeutic properties. Ghee doesn't decompose over time, keeping its properties, such as the ability to increase memory, creativity and intelligence, life expectancy by retarding the aging process, improvement of circulation and psychological and organic depression. It also strengthens the immune system, repairs damaged tissues, controls excess weight of people with high cholesterol and fights bacterial and viral infections. Patent US5693327 refers to its use in the preparation of creams and ointments for the treatment of skin diseases.

The procedures and the equipment used for the extraction of essential oils of ginger are described in patents US4257945 and US4406745. Its application in food preparation has also been an object of patent protection in Patents US4206241, US5447735, US5468493 and US5525340 which describe its use for manufacturing spices and food components, as well as for flavoring low-cholesterol and low-fat candies, and sweet desserts according to patent US6235334. More recently, US6051235 patent has been promoting its use in the manufacture of foods. Moreover, it is shown as dietary supplement in combination with other plants in patent US5248503. In this case it is regarded as useful for the treatment of gingivitis, acne, colds and herpes simplex, among others. Several forms and routes of administration are recommended.

The antioxidant activity of ginger extracts and other plants caused by their action on the enzyme 5-lipoxygenase, is reported in patents US6261607 and US6242012. The action of ginger on

inflammation of bones and joints by inhibition of the enzyme cyclooxygenase and also its action preventing the synthesis of the inflammatory mediators such as prostaglandins and leukotienes, is disclosed in patent US6264995,. Its actions as anti-oxidant that decreases free radicals formation is also quoted.

Patent US5494668 describes a composite of ginger and other compounds, for the treatment of musculoskeletal diseases, since it has known histamine release inhibitory action, by inhibiting both lipoxigenase, and cyclooxygenase enzyme systems.

The ginger extraction process with organic 'solvents shows anti-inflammatory and anti-platelet aggregation activity in patent US627 4177.

Disclosure of the invention

The present invention describes the natural process of ginger extraction using a fatty dairy product known as clarified butter as extracting agent. The resulting extract is yellow ocher with a liquid and dense consistency at room temperature. In its composition there are the main lipid-soluble compounds of ginger that keep the main characteristics of the plant. The extraction process consists of three fundamental steps: butter clarification, ginger extraction and filtration, using heat in the first two steps.

Butter clarification is carried out to remove water and solid residues. Foam is generated during the process, it should be continually removed by means of skimmer until it is no longer generated. Butter is placed in a container and is heated at 80-100° e for 10 to 15 minutes. It is finally filtered to remove the remaining solid residues.

Extraction is performed by placing the clarified butter in a container and adding clean and fresh ginger cut in thin 2-3 mm slices at a 0.8:1 (ginger:butter) ratio, and boiling it during 20-30 minutes until ginger slices are dry. The extract is filtered through a 2-mm mesh filter. If necessary it should be filtered again to remove any residue. A yellow ocher and dense liquid is obtained.

The preferred extraction procedure includes:

Butter clarification

Place 1 Kg of butter in a heat resistant container. Heat butter until it boils during 15 minutes. Remove the foam generated. Let it cool to 60 .e and filter.

Extraction of ginger lipid-soluble compounds from clarified butter.

800 g of Ginger. Thin slices of fresh and clean ginger (2,5 mm). Weigh the slices, and add them to the clarified and filtered butter. Heat up to 1000 e during 30 minutes until slices are dry.

Filtration process:

Filter the extract through a 2-mm mesh filter to remove solid residues.

The product obtained by means of this process is an oleaginous extract of *Zingiber officinale* Roscoe (Zingiberaceae) prepared from the rhizome of the fresh plant, in a 1 :08 solvent/vegetable material ratio.

The extract shows anti-inflammatory, antihistaminic and antiserotoninic effects by blocking the inflammatory action induced by carragenin, histamine and serotonin in the rat paw (figure 1). We may state that the extract has a remarkable antiserotoninic and antihistaminic effect, since it caused more than 50% reduction of serotonin induced inflammation, while showing diphenhydramine-like results used in reducing histamine-induced inflammatory response.

The ginger extract has the inhibitory effect of the IgE-mediated hypersensitivity (type I allergy) by inhibiting cutaneous passive anaphylaxis in rats (figure 2). It also displays effective antihistaminic activity in the prevention of the histamine-induced bronchospasm (figure 3). It shows as well proven antiarthritic effect by reducing the chronic inflammation induced by Freund's adjuvant (figure 4).

Example 1

Anti-inflammatory activity assay in acute edema induced by the phlogistic agents, carragenin, dextran, histamine and serotonin.

Phlogistic agents: histamine acid phosphate (BDH), serotonin sulphate (BDH), dextran (Cuba), carragenin (Sigma).

Test substances: ginger extract (MVZ), diphenhydramine (Medsol), cyproheptadine (Medsol), and indomethacin (Medsol).

Experimental animals. Female Wistar Rats with a body weight between 150 and 200 g were used. Animals were kept at a 22:12 °C controlled temperature, with shavings changed every 48 hours and

12 x 12 hours light-dark cycles.

Each group was made up by 6 animals individually identified for exact dosing, according to body weight. The products were administered by the oral route one hour before injecting 0.1 ml of the phlogistic agents in the right hind foot pad and 0.1 ml of 0.9% saline solution (NaCl) in the collateral paw. The treatment schedules are shown in table 1.

Table 1. Treatment schedules of edemas induced by different phlogistic agents

Treatment	Phlogistic Agent	Dose Administered	Time of edema measurement
Control	1 % dextran	-	1 hour
Cyproheptadine		5mg/kg	
Diphenhydramine		60mg/kg	
Ginger Extract	1 % histamine	6.5ml/kg	1 hour
Control		-	
Diphenhydramine		60mg/kg	
Extract	0.01 % serotonin	6.5ml/kg	1 hour
Control		-	
Cyproheptadine		5mg/kg	
Ginger Extract	1 % carragenin	6.5ml/kg	4 hours
Control		-	
Indomethacin		10 mg/kg	
Ginger Extract		6.5ml/kg	

The volume increment of the paw treated with the phlogistic agent as compared to the contralateral control paw was measured in a plethysmograph.

The results were statistically processed by means of a one-way analysis of variance and the Duncan test.

Figure 1 shows the anti-inflammatory effect of the ginger extract, administered by the oral route at a

dose of 6.5 mL/kg of body weight. The figure shows the percent of inhibition of the inflammation for each treated group. The action of the ginger extract is demonstrated when comparing its effects with those of different substances used as positive control, such as indomethacin, cyproheptadine and diphenhydramine, showing no significant differences.

Example 2.

Passive cutaneous anaphylaxis assay (PCA). Type I hypersensitivity reaction. One-milligram ketotiphen tablets (batch 020899043, from Eron Laboratories SA) were used as positive control for the passive cutaneous anaphylaxis assay. *Animals*

Male Wistar rats weighing 300 to 350 g were used for the passive cutaneous anaphylaxis and 18 to 22 g albino male Swiss mice to obtain ovalbumin antiserum.

All the animals were kept under controlled temperature at 22 ± 2°C and a 12 x 12 hours light-dark cycle.

Groups of three animals each were structured for the assay. A group received the ginger extract at a dose of 6.5 mL/Kg, the positive control group received 3 mg/Kg ketotiphen and a control group didn't receive any treatment.

The dorsum of rats were shaved one hour before the intradermal administration of 0.1 mL of an antiserum against egg albumin in six sites of the back. Twenty-four hours later an anaphylactic reaction was caused by means of the intravenous administration of 1 mL of a 0.1 % egg albumin solution dissolved in 1 % Evans blue stain. The animals were killed one hour after the anaphylaxis reaction took place and skin samples were taken, placed in test tubes containing 4 mL formamide and incubated at 37 °C during 48 h. The optical density of the extravasated stain was measured at $\lambda = 623$ nm. The micrograms (μ g) of extravasated Evans blue/skin site were obtained from a calibration curve. The results obtained were processed by means of a one-way ANOVA and the Duncan test with a significant level set at $p < 0.01$.

The results are shown in Figure 2. An inhibitory effect of the ginger extract on the passive cutaneous anaphylaxis, comparable to that of ketotiphen is observed. No significant differences exist between them. A decrease of the quantity of extravasated stain/skin site (μ g/site) is observed in these two

groups, as compared to the non-treated control group, demonstrating the inhibition of the anaphylactic reaction characteristic of type I hypersensitivity present in allergic asthma processes.

Example 3

In vivo antihistaminic activity assay. Inhibition of histamine-induced bronchospasm.

Male Hartley guinea pigs, weighing 400 - 450 g were used in the vivo antihistaminic activity assay.

Groups of six animals were set up, one treated with ginger extract (6.5 mL/Kg) administered orally during 7 days and an untreated control group.

Afterwards, all the animals were anesthetized with sodium pentobarbital (40 mg/Kg) to perform a tracheotomy and ventilate them with a respirator. The jugular vein was catheterized and 15 mg/Kg of sodium pentobarbital were administered to produce respiratory arrest, and following 15 g/Kg of histamine were administered intravenously three times to both groups, and the bronchial pressures were recorded after each administration. The result is considered positive when the mean value of the individual bronchial pressure difference caused by histamine administration in the animals treated with ginger extract is smaller than 50% of the mean value of the bronchial pressure difference of control animals.

Oral administration of ginger extract in butter for 7 days to animals, proved to be 100% effective for the prevention of histamine-induced bronchospasm, since it fulfilled the criteria to be considered positive. Furthermore, there are statistically significant differences between the values of the control group and those of the treated group. This effect is mediated by the inhibitory action of the ginger extract on the bronchial effect of histamine (figure 3).

Example 4

Antiarthritic activity assay. Chronic Inflammation Induced by Freund's adjuvant.

Ginger extract on the arthritis induced by Freund's adjuvant.

Rats grouped into three treatment groups for the study were used. A first positive control group, treated with 2.5 mg/Kg tenoxicam, another group treated with 6.5 mL/Kg of ginger extract and a third non-treated control group. All the animals were administered 0.05 mL of complete Freund's adjuvant in the right hind paw. Oral administration of the ginger extract and tenoxicam began 18 days later during a period of 12 days. When the administration was completed, the volume increment of the paw treated with complete Freund's adjuvant was determined as compared to the contralateral

control paw, with the aid of a plethysmograph.

The results are shown in Figure 4. It may be observed that the ginger extract significantly inhibits Freund's adjuvant-induced inflammation. Its effects are comparable to those achieved by a dose of 2.5 mg/Kg of Tenoxicam. No significant differences were found between them.

CLAIMS

1. Procedure for obtaining a ginger extract with pharmacological activity wherein said procedure comprises the use of butter for extracting liposoluble compounds.
2. Procedure according to claim 1 wherein for the extraction of the liposoluble compounds, ginger roots cut in fine slices are mixed with clarified and filtered butter in a rate of 0.5-8:1-1.5 (ginger/butter) and boiling said mixture for 20-30 min and finally the extracts is filtered.
3. Ginger extract obtained by the procedure of claims 1-2
4. Ginger extract obtained by using butter for extraction liposoluble compounds.
5. Ginger extract according 3 and 4 wherein said extract is a yellow ocher oleaginous extract with a liquid and dense consistency at room temperature.
6. Farmaceutical composition comprising as active principle a ginger extract in butter and an appropriate excipient.
7. Farmaceutical composition according to claim 6 comprising liquid formulation, emulsion and semisolids in soft gelatine capsule.
8. Use of the ginger extract according to claims 3-5 as anti-histaminic.
9. Use of the ginger extract according to claims 3-5 as anti-serotonininc.
10. Use of the ginger extract according to claims 3-5 as anti-inflammatory.
11. Use of the ginger extract according to claims 3-5 as anti-arthritic.
12. Use of the ginger extract according to claims 3-5 for the prophylaxis of broncoespasmo induced by histamines.
13. Use of the ginger extract according to claims 3-5 for the prophylaxis an treatment of the reaction of the hypersensitivity mediated or conditioned by IgE
14. Use of the ginger extract according to claims 3-5 for the treatment of allergy type I.
15. Use of the ginger extract according to claims 3-5 for the prophylaxis an treatment of allergic asthma.
16. Use of the ginger extract according to claims 3-5 for the prophylaxis an treatment of allergic induced by powders, fungus, bites, hair and skins of animals
17. Use of the ginger extract according to claims 3-5 for the prophylaxis an treatment of a topic dermatitis.
18. Use of the ginger extract according to claims 3-5 for the prophylaxis an treatment of allergic rinitis.
19. Use of the ginger extract according to claims 3-5 as nutritional supplement.

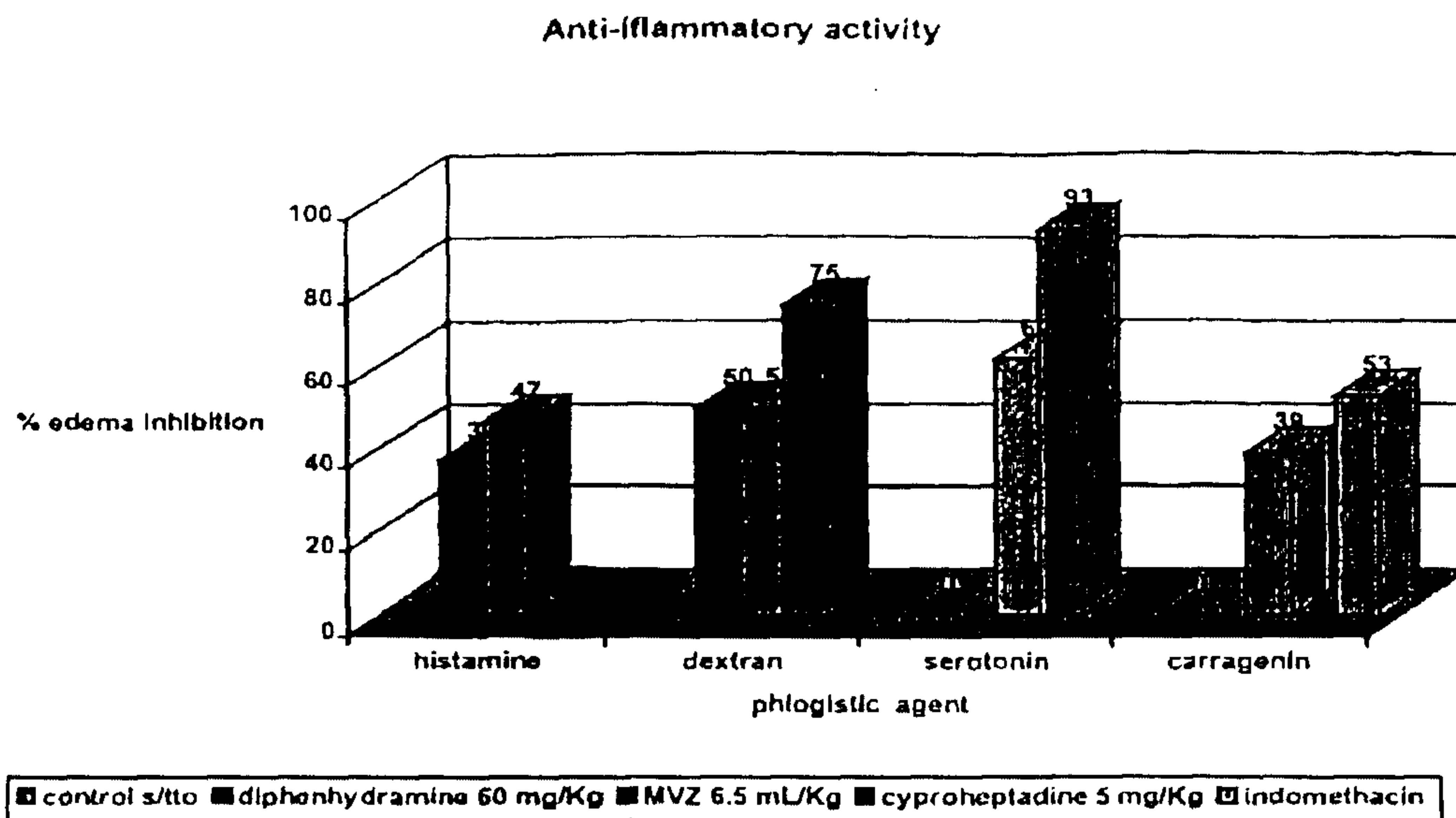


Fig. 1. Effect of the ginger extract (MVZ 6.5 mL/Kg) on the edema induced by different phlogistic agents.

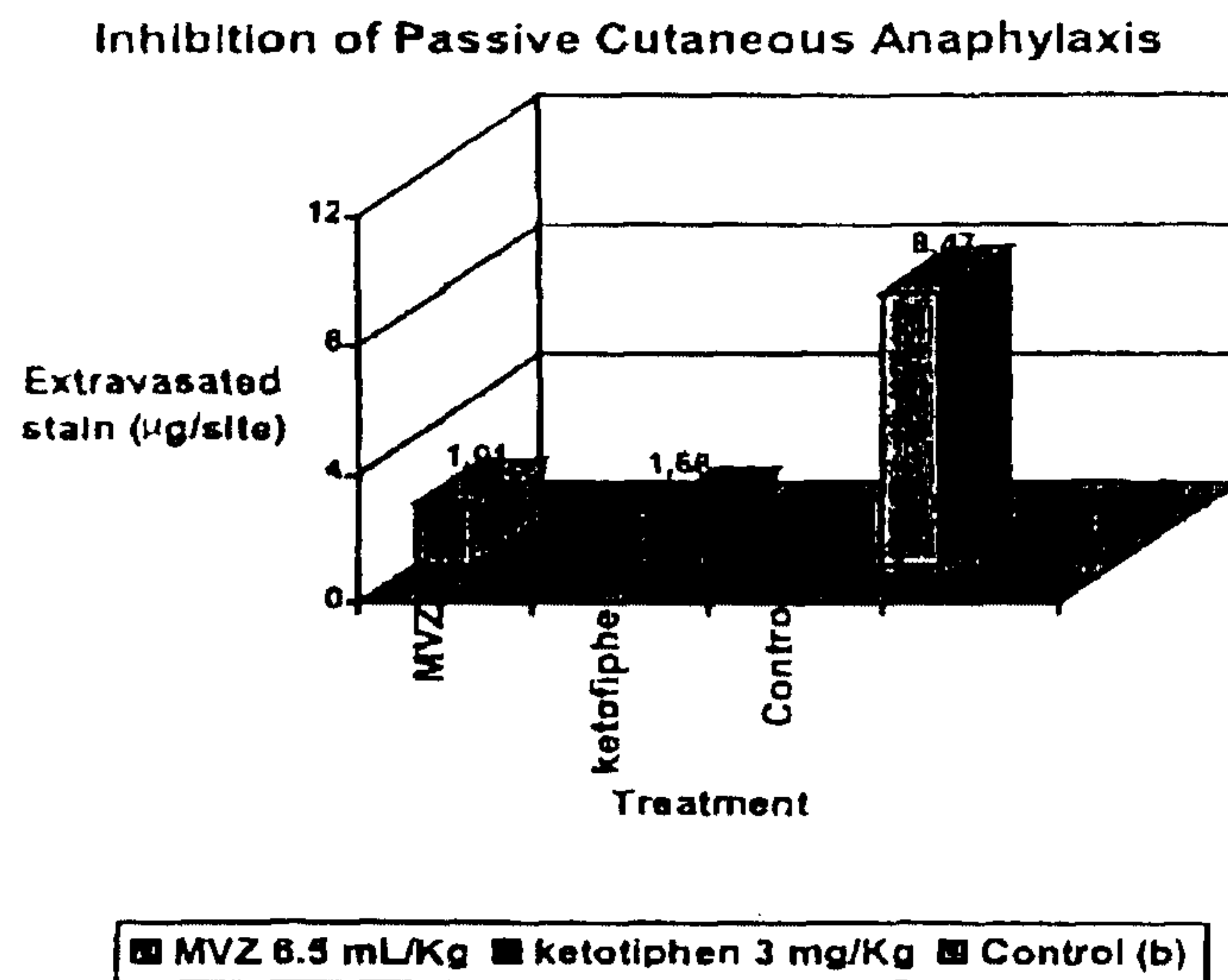


Fig. No. 2. Effect of the compound on passive cutaneous anaphylaxis in rats.

* Different letters stand for significant differences ($p < 0.01$)

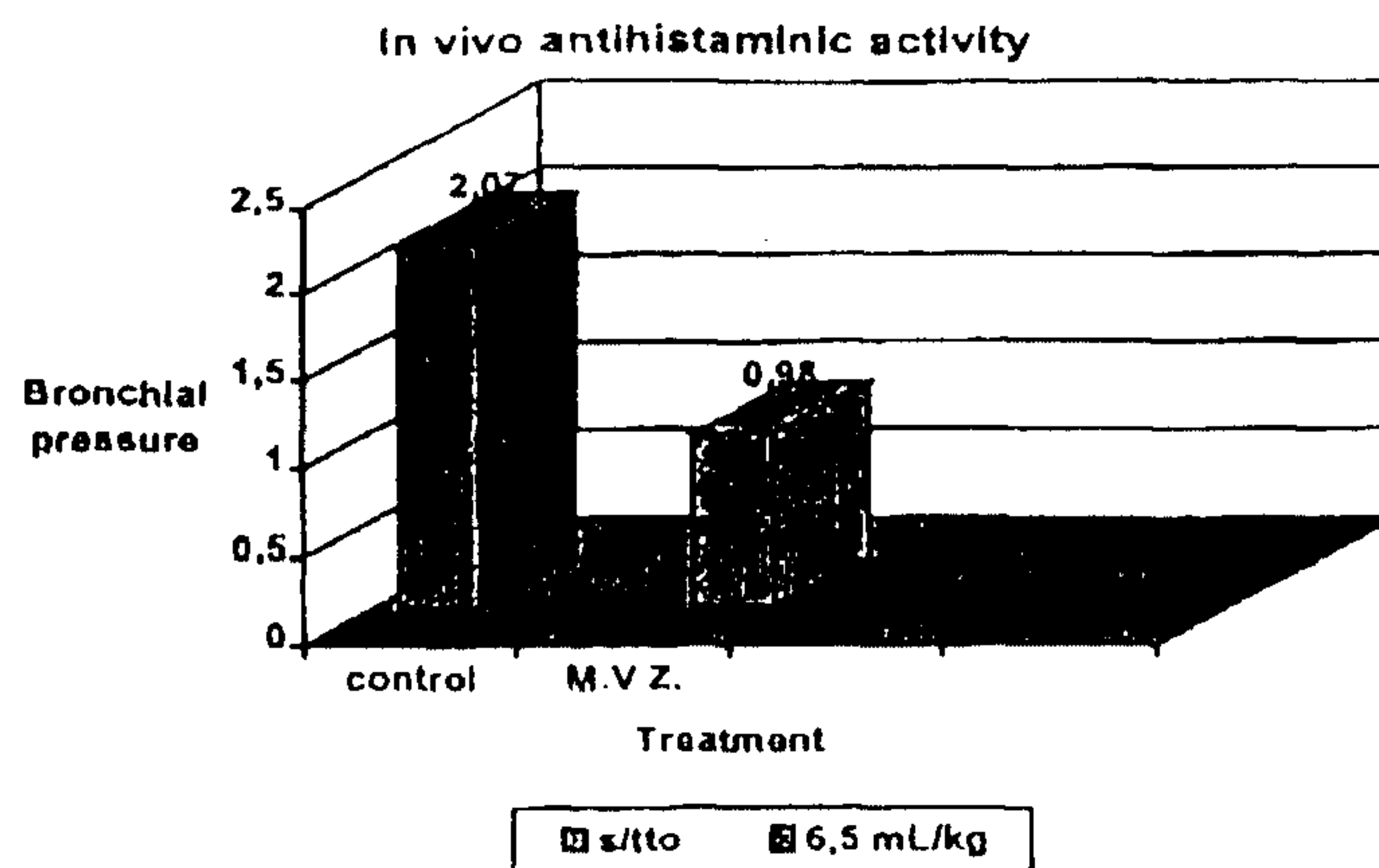


Fig 3. Effect of the compound on the histamine-induced bronchospasm
The difference between them is statistically significant ($p > 0.01$)

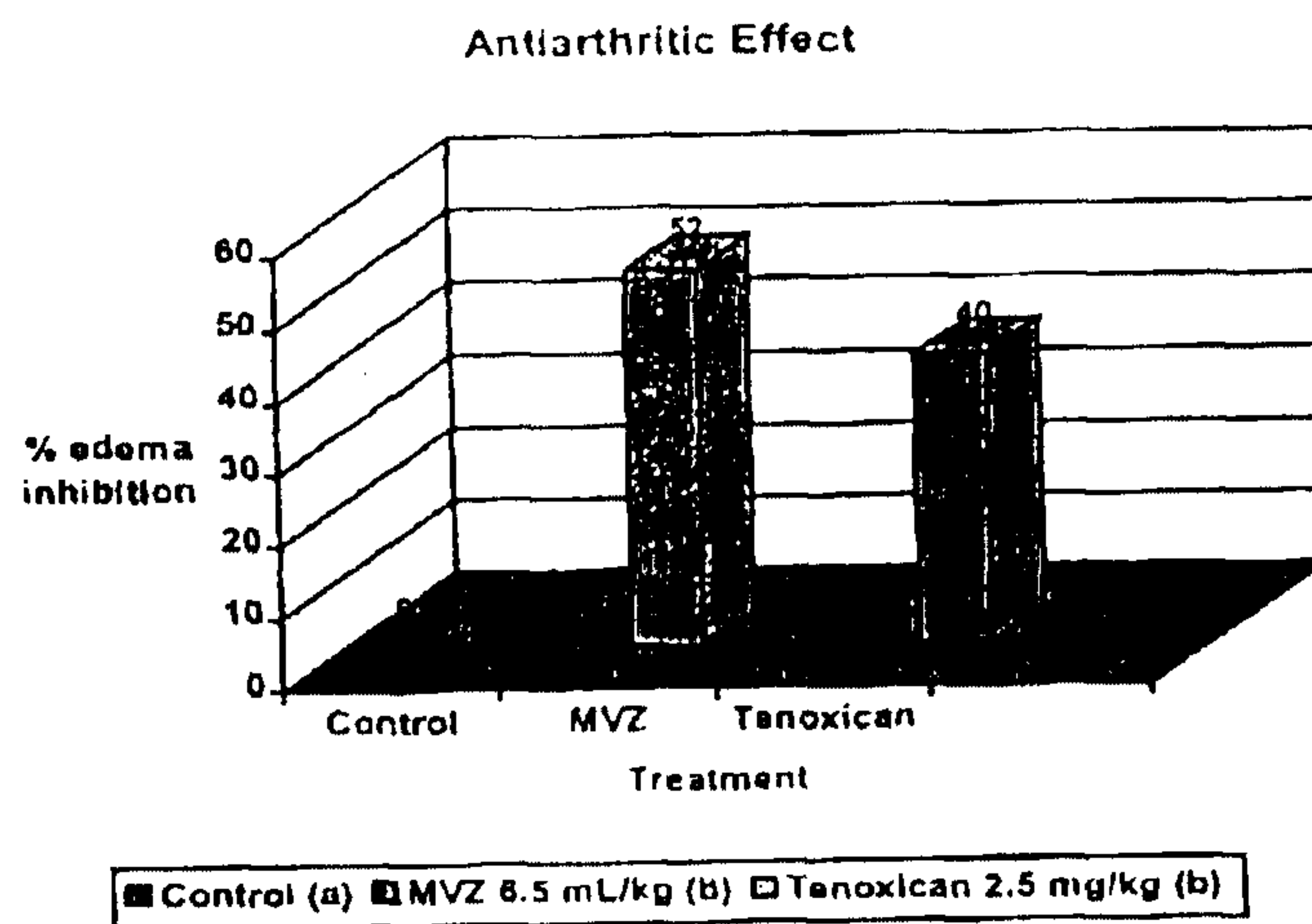


Figure 4. Effect of M.V.Z. on chronic edema induced by Freund's adjuvant
Unequal letters stand for significant differences ($p < 0.01$)