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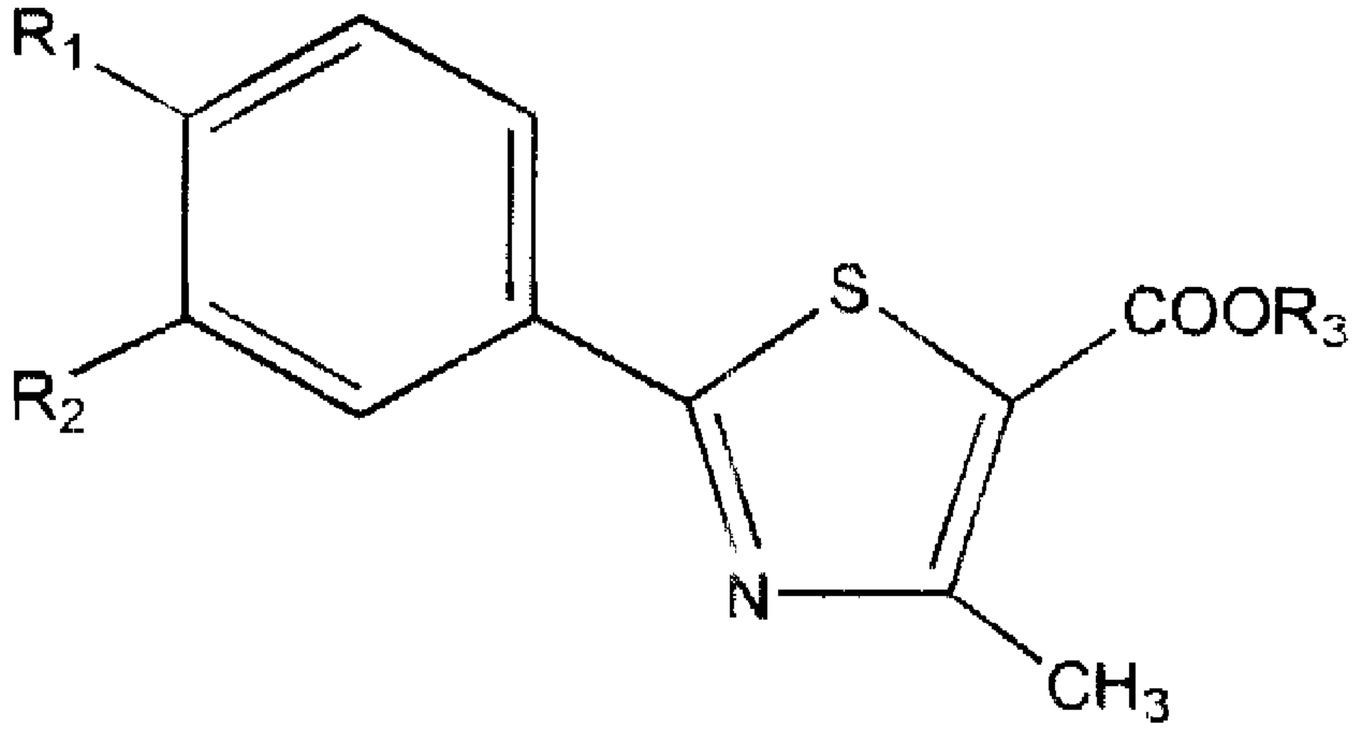

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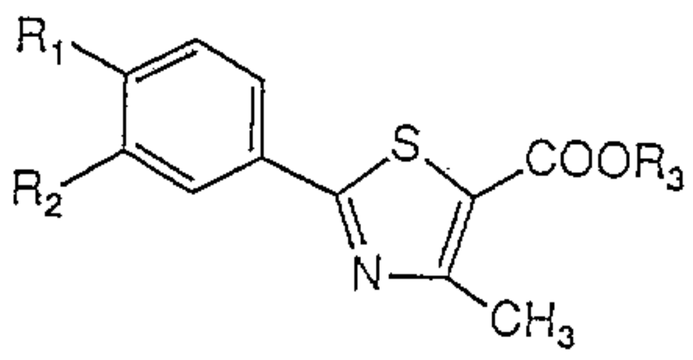
(57) Abrégé/Abstract:
An agent composition for the therapy or prevention of the injury of an organ or a tissue containing a phenylthiazole derivative expressed by the following formula (1) : (see formula 1) (R_1 is a C_1-C_8 alkoxy group or a cyclic amino group; R_2 is a cyano group or a nitro group; R_3 is a hydrogen atom or a C_1-C_4 alkyl group), and/or its salt as an active ingredient.

ABSTRACT

An agent composition for the therapy or prevention of the injury of an organ or a tissue containing a phenylthiazole derivative expressed by the following formula (1) :



(1)

(R_1 is a C_1-C_8 alkoxy group or a cyclic amino group; R_2 is a cyano group or a nitro group; R_3 is a hydrogen atom or a C_1-C_4 alkyl group), and/or its salt as an active ingredient.

PROTECTING AGENT FOR ORGAN OR TISSUE

This invention relates to a compound for the treatment or prophylaxis of various kinds of diseases associated with an injury caused by active oxygen generated after ischemia-reperfusion.

In recent years, it has been found that a large amount of active oxygen is generated in an ischemic state and subsequent reperfusion of blood in various organs including the heart. This is one of the factors which induces various kinds of diseases. That is, when blood flow is blocked in a blood vessel by a thrombus, etc., the ischemic state occurs in the controlling region of the blood vessel. In this case, the oxygen supply to the tissue in the region is intercepted and accordingly the tissue falls in a dangerous state. When this state lasts for a certain length, the tissue runs into a necrotic state. Once this occurs, recovery is virtually impossible. Therefore, it is important that reperfusion of blood is achieved at the earliest opportunity to save the tissue before it suffers permanent damage.

However, even though reperfusion is started before necrosis of the tissue, reperfusion itself can have harmful effects on the tissue in a hypoxia state. This phenomenon is called an ischemia-reperfusion injury.

In a normal state, xanthine and hypoxanthine, metabolites of nucleic acids, exist in cells at a low concentration and are converted into nucleic acid with xanthine hydrogenase. However, in an ischemic state, the synthesis of ATP is reduced. Accordingly, ADP and AMP accumulate and, the concentrations of xanthine and hypoxanthine increase significantly. Further, when reperfusion of blood is initiated, xanthine hydrogenase is converted into xanthine oxidase. Xanthine oxidase in turn metabolizes xanthine and hypoxanthine to uric acid with oxygen which acts as an electron receptor. This process produces a large amount of oxygen-anion radicals (O^{2-}) as a by-product. The oxygen-anion radical is the active oxygen which is a factor causing ischemia-reperfusion injury. Further, it is believed that other active oxygen species, such as hydrogen peroxide and hydroxy radicals, are secondarily produced,

aggravating the injury.

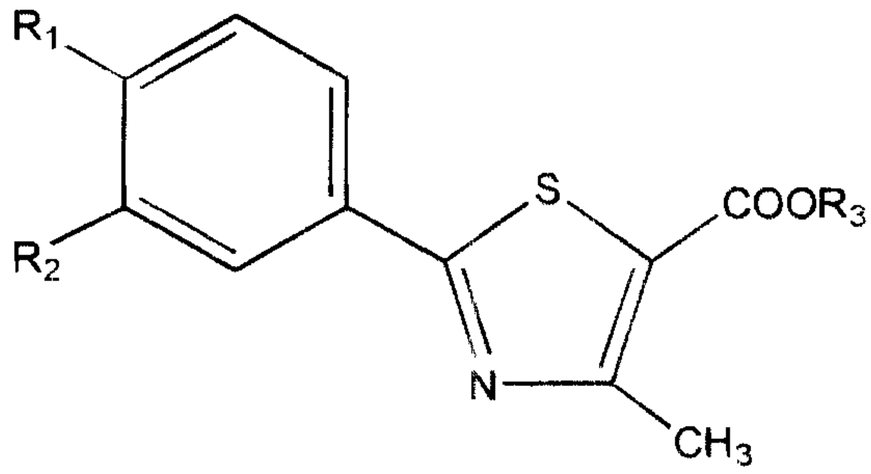
It is also known that active oxygen is generated from leukocytes through another path. That is, in an ischemic state, leukocytes are activated by a chemical mediator such as leukotriene, and accumulate at ischemic sites, generating active oxygen there. Accordingly, it plays an important role on the ischemia-reperfusion injury.

The generation of active oxygen is believed to aggravate prognosis of various ischemic diseases including myocardial infarction, cerebral infarction, pulmonary thromboembolism, thrombosis in other organs, etc. Similarly, the generation of active oxygen aggravates prognosis in various kinds of operations such as coronary artery bypass operation in which blood flow is blocked temporarily, percutaneous transluminal coronary angioplasty, injuries during the application of a thrombolytic agent, operations and treatments during organ transplantation, etc. Therefore, a substance which suppresses the generation of active oxygen is considered to be useful for the prophylaxis of these injuries.

Allopurinol, which is an inhibitor of xanthine oxidase and used as a therapeutic agent for gout, and a xanthine oxidase inhibitor disclosed in Japanese Unexamined Patent Publication (Kokai) No. 3-157385 have been studied to determine the effectiveness in suppressing the generation of active oxygen to minimize organ injuries caused by ischemia-reperfusion. However, the results of the animal experiments using an ischemia-reperfusion injury model are divided into two groups: an effective group (Journal of Clinal Investigation 74:1156-1164; 1984 and European Journal of Pharmacology 140:203-207; 1987) and a group in which the effect is not clear (Hepatology 8:3:580-584; 1988 and Research in Experimental Medicine 193:275-283; 1993). Accordingly, no clear conclusion has been obtained yet. In fact, so far, they have practically not been used.

Besides these compounds, SOD and the like, are being studied as an agent for depleting the generated active oxygen. To date, none of these have been used.

A phenylthiazole derivative expressed by the following general formula (1):



(1)

wherein R_1 is a C_1-C_8 alkoxy group or a cyclic amino group, R_2 is a cyano group or a nitro group, R_3 is hydrogen or a C_1-C_4 alkyl group,

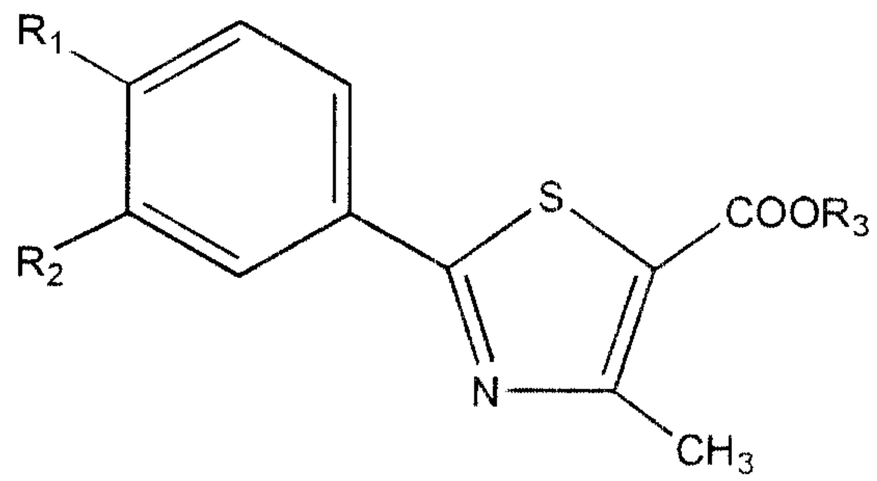
has exhibited strong xanthine oxidase inhibiting activity and is used as a therapeutic agent for hyperuricemia and gout (International Publication WO92/09279). However, the effect of the compound of formula (1) on ischemia-reperfusion injury of organs caused by the generation of active oxygen is not known.

Therefore, the issue that the present invention intends to solve is to provide a compound for the treatment or prophylaxis of diseases involved in injuries caused by the active oxygen generated after the ischemia-reperfusion, etc.

The present inventors researched the effectiveness of compounds of formula (1) on ischemia-reperfusion injury and found that they have suppressing activity against ischemia-reperfusion injury of an organ or a tissue and further have suppressing activity against the production of chemical mediators activating leukocytes which play a part in the ischemia-reperfusion injury.

Therefore, the present invention provides a therapeutic or a preventing agent composition against the injury of an organ or a tissue, which contains a phenylthiazole

derivative expressed by the following general formula (1):



(1)

wherein R_1 is a C_1-C_8 alkoxy group or a cyclic amino group, R_2 is a cyano group or a nitro group, R_3 is hydrogen or a C_1-C_4 alkyl group, and/or a salt thereof, as an active component.

Further, the present invention provides a method for the treatment or prophylaxis of injuries of an organ or a tissue based on the use of the phenylthiazole derivative expressed by the above formula (1).

Furthermore, the present invention provides the use of the phenylthiazole derivative expressed by the above formula (1) for the treatment or prophylaxis of injuries to an organ or a tissue.

In the accompanying drawings which illustrate embodiments of the present invention,

Figure 1 is a graphical representation of the effect of administration of the compound of the present invention on serum Cre value in a renal ischemia-reperfusion injury model in rats;

Figure 2 is a graphical representation of the effect of administration of the compound of the present invention on serum BUN (blood urea nitrogen) value in a renal ischemia-reperfusion injury model in rats;

Figure 3 is a graphical representation of the effect of administration of the compound of the present invention on leukotriene-B_4 production in cultured cells;

Figure 4 is a graphical representation of the effect of administration of the compound of the present invention on survival rate in DNFB-sensitized mice;

Figure 5 is a graphical representation of the effect of administration of the compound of the present invention on body weight in DNFB-sensitized mice; and

Figure 6 is a graphical representation of the effect of administration of the compound of the present invention on variation of ear thickness with time after the elicitation of an immunological reaction in DNFB-sensitized mice.

In the above formula (1), R_1 is a C_1-C_8 alkoxy group or a cyclic amino group. Examples of the C_1-C_8 alkoxy group include methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, tert-butoxy, pentyloxy, isopentyloxy, hexyloxy and octyloxy groups. Examples of the cyclic amino group include pyrrolidino, piperidino, morpholino and piperazinyl groups. Preferably R_1 is a C_2-C_6 alkoxy group, more preferably R_1 is an isobutoxy group.

R_2 is a cyano group or a nitro group, with the cyano group being preferable.

R_3 is hydrogen or a C_1-C_4 alkyl group. Examples of the C_1-C_4 alkyl group include methyl, ethyl, propyl, isopropyl, butyl and isobutyl groups. A methyl group is the preferred group for R_3.

The compound of the present invention can be administered in an oral preparation such as a soft capsule, a hard capsule, a tablet or syrup, by injection or as an external preparation in a conventional manner using an appropriate diluent, etc.

Examples of suitable diluents include vegetable oil (for example, corn oil, cottonseed oil, coconut oil, almond oil or peanut oil), oily esters such as a middle-chain fatty-acid glyceride, mineral oil, vaseline, animal oil or fat, a cellulose derivative (for example, crystalline cellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose or methylcellulose), polyvinyl pyrrolidone, dextrin, lactose, mannitol, sorbitol and starch.

Dosage of the compound of the present invention is usually about 0.1-300 mg/day, preferably 1-50 mg/day, and can be administered 1-3 times/day. The preparation is preferably formulated so that it can satisfy these conditions.

To evaluate the safety of the compound of the present invention, an acute toxicity test was carried out with rats with 2-(3-cyano-4-isobutyloxyphenyl)-4-methyl-5-thiazolecarboxylic acid as the active component. None of the rats died by a single oral

administration up to 300 mg/kg.

The compound of the present invention expressed by the formula (1) can be synthesized, for example, by the method described in the International Publication WO92/09279.

The compound of the present invention can be used for the treatment or prophylaxis of an injury of an organ or a tissue. Examples of injuries of an organ or a tissue include diseases associated with the ischemia-reperfusion injury of an organ. For instance, the agent can be used against the disease associated with the ischemia-reperfusion injury of an organ caused by the generation of activated oxygen. In particular, examples of the diseases include diseases associated with the ischemia-reperfusion injury in heart, liver, brain, kidney, digestive canals and lung caused by the generation of active oxygen.

The following Examples illustrate the present invention.

Example 1

Tablets were prepared with the following composition:

2-(3-Cyano-4-isobutyloxyphenyl)-4-methyl-5-thiazolecarboxylic acid	50 mg
Lactose	230 mg
Potato starch	80 mg
Polyvinyl pyrrolidone	11 mg
Magnesium stearate	5 mg
Total weight	376 mg

The phenylthiazole derivative, lactose and potato starch were well mixed, and the mixture was homogeneously moistened with a 20% polyvinyl pyrrolidone solution in ethanol. Subsequently, this was passed through a 20 mesh sieve, dried at 45°C, and then passed through a 15 mesh sieve. The obtained granules were mixed with magnesium stearate, and compressed into tablets.

Example 2

An ischemia-reperfusion injury model of a kidney for studying the effect on ischemia-reperfusion injury was prepared with a rat as described below, and the effect of the administration of the compound of the present invention was studied using the model animal.

The abdomen of an SD rat (male, 6 weeks of age) was opened under anesthesia with sodium pentobarbital, the separated left and right renal arteries were clamped with arterial clips to block blood flow completely, and the kidneys were brought into an ischemic state. After 60-minute ischemia, the arterial clamps were released, and blood flow was resumed to bring the kidneys to a reperfused state. After the abdomen was sutured, the animal was returned to a cage and allowed free access to food and water. After 24-hour reperfusion, the abdomen was opened under ether anesthesia and a blood sample was drawn from abdominal aorta.

Serum was separated from the blood sample by centrifugation. Blood urea nitrogen (BUN) level and serum creatinine (Cre) level, a parameter for kidney function injury, were determined using an automatic analyzer (Hitachi 7070™) by the urease-indophenol method and Jaffe method, respectively.

2-(3-Cyano-4-isobutyloxyphenyl)-4-methyl-5-thiazolecarboxylic acid was suspended in a 0.5% methylcellulose aqueous solution. The active component was orally administered to each ischemia-reperfusion injury model animal at a dose of 50 mg/kg in a 4-ml suspension 60 minutes prior to the ischemic state. At the same time, 4 ml of the 0.5% methylcellulose aqueous solution was orally administered to each of the animals of a control group and a sham-operation group. Numbers of animals in the sham-operation group, the control group and the compound of the present invention group were 7, 6 and 6, respectively. The results are expressed as the mean ± standard deviation. Tests of significant difference against the control group were performed using Welch's t-test.

The result of the serum Cre measurement is shown in Figure 1. The serum Cre level of the rats of the ischemia-reperfusion operation group (control group) was 3.6±0.4 mg/dl and this was clearly higher than that (0.4±0.0 mg/dl) of the rats of the sham-operation group. Compared with the control group, the increase of the serum Cre level in the rats administered with the compound of the present invention was significantly suppressed

(2.3±1.1 mg/dl, p<0.05).

The result of the serum BUN measurement is shown in Figure 2. The serum BUN level of the rats of the ischemia-reperfusion operation group (control group) was 151.3±16.8 mg/dl and this was clearly higher than that (19.3±1.7 mg/dl) of the rats subjected to the sham-operation. Compared with the control group, the rats administered with the compound of the present invention exhibited a tendency to suppress the increase of the serum BUN.

Example 3

The effect of the compound of the present invention on the production of leukotriene, which is one of the strong chemical mediators against leukocytes and is thought to be associated with the pathology of the ischemia-reperfusion injury, was investigated using cultured cells. RBL-1 cells (Dainippon Pharmaceutical Co.) were cultured in a Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum. The cultured cells were adjusted to $5x10^6$ cells/ml. The solution of 2-(3-cyano-4-isobutyloxyphenyl)-4-methyl-5-thiazolecarboxylic acid, a compound of the present invention, or allopurinol as a control in dimethyl sulfoxide was added into 1 ml of the cell suspension. After incubation at 37°C for 5 minutes, this was immersed in ice for 10 minutes. Subsequently, $2x10^{-5}$ mol of A23187, Ca-ionophore, was added to the chilled cell suspension to make the final concentration of 25 mM, and the cell suspension was incubated again at 37°C for 15 minutes. The suspension was then held in ice for 10 minutes. The suspension was centrifuged at 3000 rpm for 10 minutes and the cells were removed. The leukotriene B_4 (LTB_4) level in the supernatant was measured by enzyme immunoassay. The results were each expressed as the mean ± standard deviation. A significant difference test against the control group was carried out using Bonferroni's test of a multiple comparison.

The result of LTB_4 measurement is shown in Figure 3. As can be seen from the drawing, the addition of the phenylthiazole derivative of the present invention, in an amount of $1x10^{-8}$ mol (final concentration of 10 μM) suppresses the production of LTB_4 significantly ($p<0.001$). On the other hand, the addition of allopurinol in an amount of up to $1x10^{-8}$ mol (final concentration of up to 10 μM) had no effect on the production of LTB_4.

Example 4

Allopurinol, an inhibitor of xanthine oxidase and used as a therapeutic agent for gout, has known side effects including an allergic reaction during clinal application and occasionally the side effect is so severe that the patient dies (The Journal of Medicine 76:47-56; 1984; The Annals of Pharmacotherapy 27:337-343; 1993), and accordingly caution is required in clinical application. Therefore, the presence of such side effects was examined for the compound of the present invention using a mouse allergic dermatitis model.

Hair was removed from the abdomen to the breast of a BALB/c mouse (male, 8 weeks of age) with hair clippers and a shaver. A 0.5% dinitrofluorobenzene (DNFB) ethanol solution (100 μl/head/day) was applied on the shaved part on the first and second days to establish sensitization. 2-(3-Cyano-4-isobutyloxyphenyl)-4-methyl-5-thiazolecarboxylic acid, a compound of the present invention, and allopurinol, for comparison, were each suspended in a 0.5% methylcellulose aqueous solution. Each suspension (3, 10, 30 and 100 mg/kg/10 ml/day) was orally administered to an animal at the same time of each DNFB sensitization. The number of animals was 8 for each of a 3- and a 10-mg/kg/10 ml/day group, and 10 for each of a 30- and a 100-mg/kg/10 ml/day group in each of the compounds of the present invention and the allopurinol. 10 ml/kg of 0.5% methylcellulose aqueous solution was administered to each of 8 sensitized animals in a control group.

Survival rates and body weights of the mice administered with each preparation were determined and recorded for 6 days following the final sensitization. A solution of 0.3% DNFB acetone-olive oil (4:1) was then applied on the sixth day on one ear of each of the animals which survived (10 μl on each of the adverse and the reverse side of an ear) to elicit an immune reaction. An acetone-olive oil (4:1) solution was applied on the other ear in the same manner. The ear thicknesses were measured with a dial thickness gauge (Ozaki Seisakusho, G-1A) prior to application, and 24 hr and 48 hr after application.

Variations in body weights and ear thicknesses were expressed as the mean ± standard deviation for each group. A significant difference test against the control group was carried out using Dunnett's multiple comparison test.

Survival rates after administration of the preparations as a function of time are shown in Figure 4 (the day of the final sensitization is expressed as day zero). In the groups

administered with the compound of the present invention, all animals survived, excluding one animal in the 100 mg/kg/day group. In contrast, the survival rate decreased with increasing dosage from a low dose level in the allopurinol group.

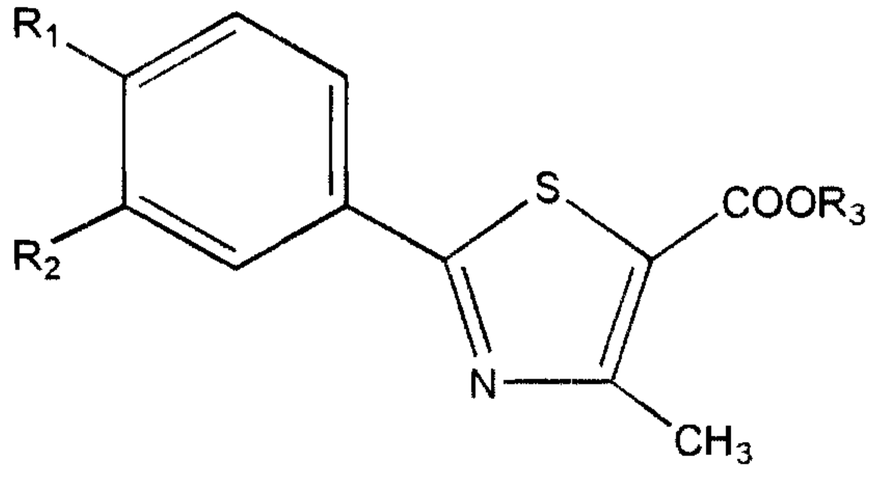
Variations of body weight of the animals which survived after administration of each preparation as a function of time are shown in Figure 5 (the day of the final sensitization is expressed as day zero). The phenylthiazole derivative of the present invention, did not produce a significant change on the body weights of the animals. In contrast, body weights decreased significantly at doses of 30 and 100 mg/kg allopurinol ($p<0.01$ and $p<0.05$, respectively).

Variations of ear thicknesses after elicitation of an immune reaction are shown in Figure 6 as a function of time (the hour of elicitation of the immune reaction is expressed as hour zero). In the groups administered with the phenylthiazole derivative of the present invention, no effect, in terms of an increase in ear thickness, was observed. In contrast, allopurinol stimulated an increase in ear thickness and an allergic reaction significantly at a dose of 30 mg/kg ($p<0.05$).

The results of Examples 2 to 4 show that the compound of the present invention is effective as a protecting agent for organs or tissue in various kinds of diseases caused by, for example, ischemia-reperfusion injury. Further, they indicate that the compound of the present invention is superior to the conventional xanthine oxidase inhibitor since it can suppress the generation of active oxygen from leukocytes through the inhibition of the production of a chemical mediator, such as LTB_4, in addition to the above protective activity in the course of its expression. Furthermore, the compound does not cause an allergic reaction and severe side effects including death, which are observed with allopurinol, a conventional xanthine oxidase inhibitor and a therapeutic agent for gout. Accordingly, it is concluded that the compound of the present invention is safer than allopurinol.

CLAIMS

1. A phenylthiazole derivative for the treatment of an organ or tissue injury caused by ischemia-reperfusion having the following general formula (1):



(1)

wherein R_1 is a C_1-C_8 alkoxy group or a cyclic amino group, R_2 is a cyano group or a nitro group, R_3 is hydrogen or a C_1-C_4 alkyl group, and salts thereof.

2. A phenylthiazole derivative for the treatment of a disease associated with an ischemia-reperfursion injury of an organ caused by the generation of active oxygen having the general formula (1) as defined in claim 1 or a salt thereof.

3. A phenylthiazole derivative for the treatment of a disease associated with an ischemia-reperfusion injury of heart, liver, brain, kidney, digestive canal or lung caused by the generation of active oxygen having the general formula (1) as defined in claim 1 or a salt thereof.

4. A phenylthiazole derivative for the treatment of an injury of an organ or a tissue having the general formula (1) as defined in claim 1 or a salt thereof.

5. A phenylthiazole derivative for the prophylaxis of diseases associated with an ischemia-reperfusion injury of an organ having the general formula (1) as defined in claim 1 or a salt thereof.

6. A phenylthiazole derivative for the prophylaxis of a disease associated with an ischemia-reperfusion injury of an organ caused by the generation of active oxygen having the general formula (1) as defined in claim 1 or a salt thereof.

7. A phenylthiazole derivative for the prophylaxis of a disease associated with an ischemia-reperfusion injury of heart, liver, brain, kidney, digestive canal or lung caused by the generation of active oxygen having the general formula (1) as defined in claim 1 or a salt thereof.

8. A compound according to any one of claims 1 to 7 wherein R^1 is C_2-C_6 alkoxy.

9. A compound according to any one of claims 1 to 8 wherein R^1 is isobutoxy.

10. A compound according to any one of claims 1 to 9 wherein R^2 is cyano.

11. A compound according to any one of claims 1 to 10 wherein R^3 is methyl.

12. A composition comprising a compound according to any one of claims 1 to 11 in admixture with a pharmaceutically acceptable diluent or carrier.

13. A composition according to claim 12 in the form of an oral composition.

14. A composition according to claim 13 in the form of a soft capsule, a hard capsule, a tablet or syrup.

15. A composition comprising a compound according to any one of claims 1 to 11 in admixture with a vegetable oil an oily ester, a cellulose derivative, polyvinyl pyrrolidone, dextrin, lactose, mannitol, sorbitol or starch.

16. A composition comprising a compound according to any one of claims 1 to 11 in admixture with corn oil, cottonseed oil, coconut oil, almond oil, peanut oil, a middle-chain fatty-acid glyceride, mineral oil, vaseline, animal oil, fat, crystalline cellulose, hydroxypropyl-cellulose, hydroxypropylmethylcellulose, methylcellulose, or starch.

17. A composition according to any one of claims 12 to 16 in dosage unit form.

18. A composition according to claim 17 wherein each dosage unit contains from 0.1 to 300 mg of the compound of the formula (I) or salt thereof.

19. A composition according to claim 17 wherein each dosage unit contains from 1 to 50 mg of the compound of formula (I) or salt thereof.

20. A use of a compound according to any one of claims 1 to 11 in the treatment or prophylaxis of an ischemia-reperfusion injury of an organ.

21. A use of a composition according to any one of claims 12 to 19 in the treatment or prophylaxis of an ischemia-reperfusion injury of an organ.

22. A use of a compound according to any one of claims 1 to 11 in the treatment or prophylaxis of an ischemia-reperfusion injury of an organ caused by the generation of active oxygen.

23. A use of a composition according to any one of claims 12 to 19 in the treatment or prophylaxis of an ischemia-reperfusion injury of an organ caused by the generation of active oxygen.

24. A use of a compound according to any one of claims 1 to 11 in the treatment or prophylaxis of an ischemia-reperfusion injury of an organ caused by the generation of active

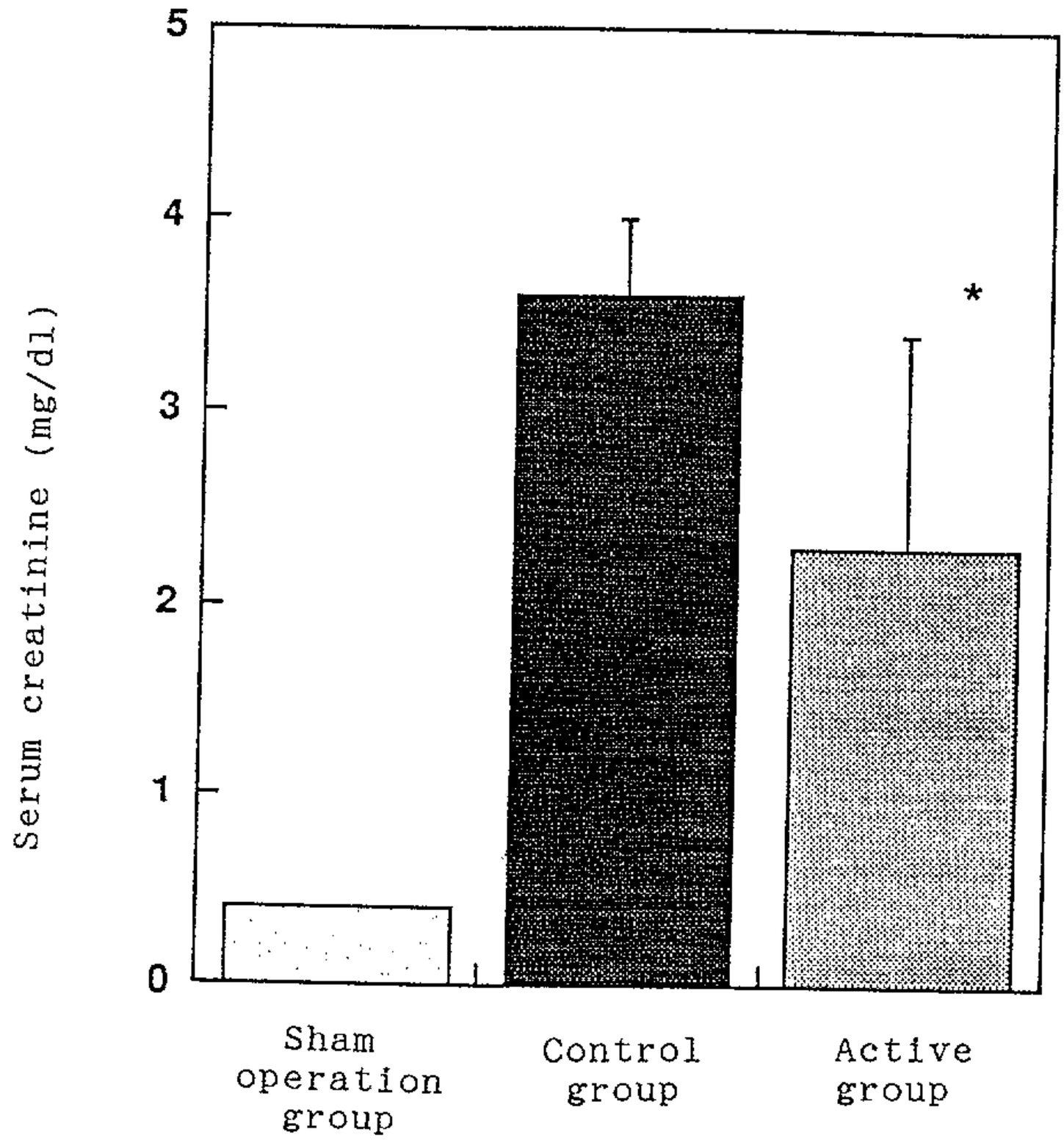
oxygen wherein the organ is selected from the group consisting of heart, liver, brain, kidney, digestive canal and lung.

25. A use of a composition according to any one of claims 12 to 19 in the treatment or prophylaxis of an ischemia-reperfusion injury of an organ caused by the generation of active oxygen consisting of heart, liver, brain, kidney, digestive canal and lung.

26. A commercial package containing a compound according to any one of claims 1 to 11 together with instructions for the use thereof in the treatment of an ischemia-reperfusion injury of an organ.

27. A commercial package containing a composition according to any one of claims 12 to 19 together with instructions for the use thereof in the treatment of an ischemia-reperfusion injury of an organ.

Fig. 1



*:p<0.05

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Fig. 2

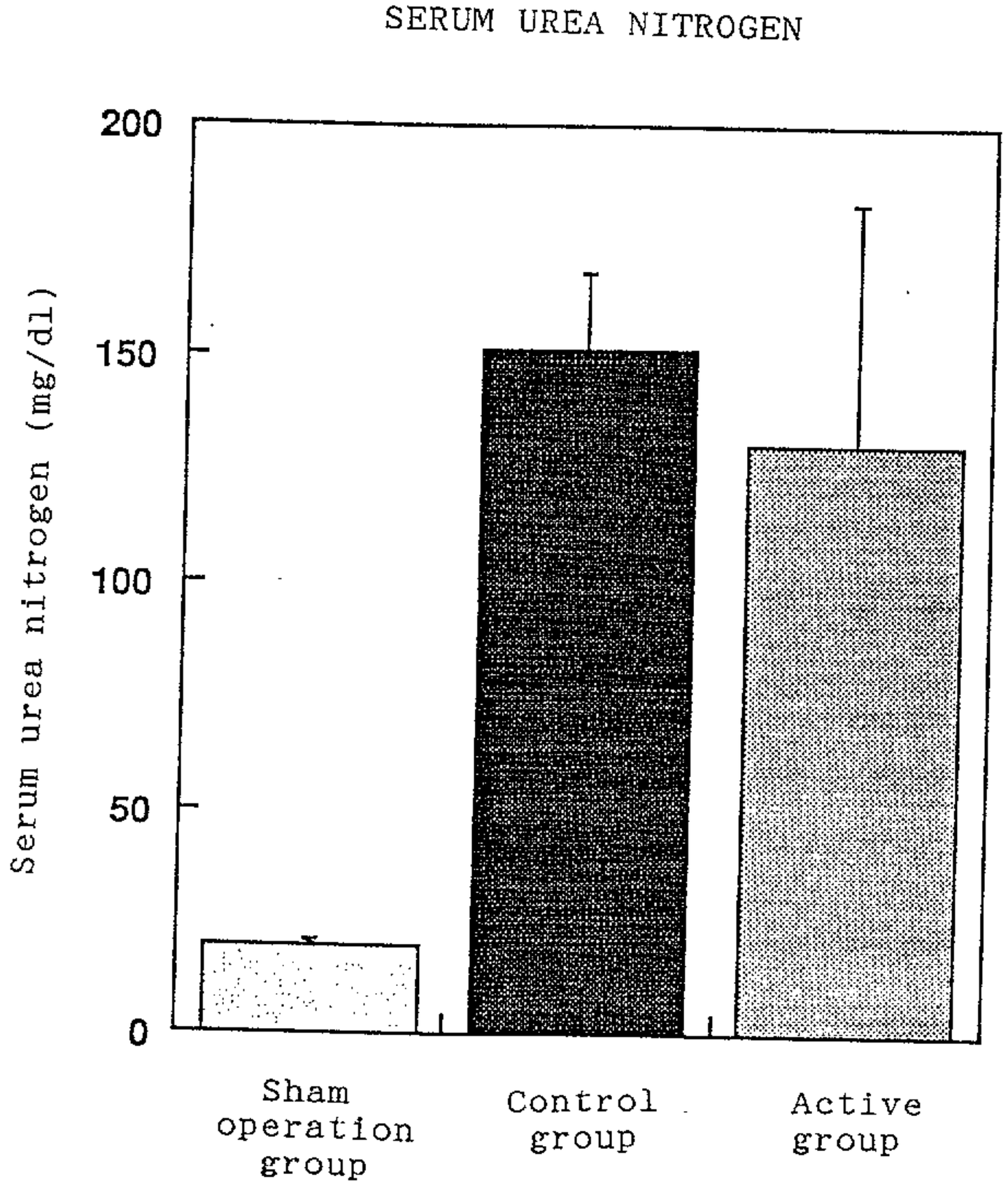

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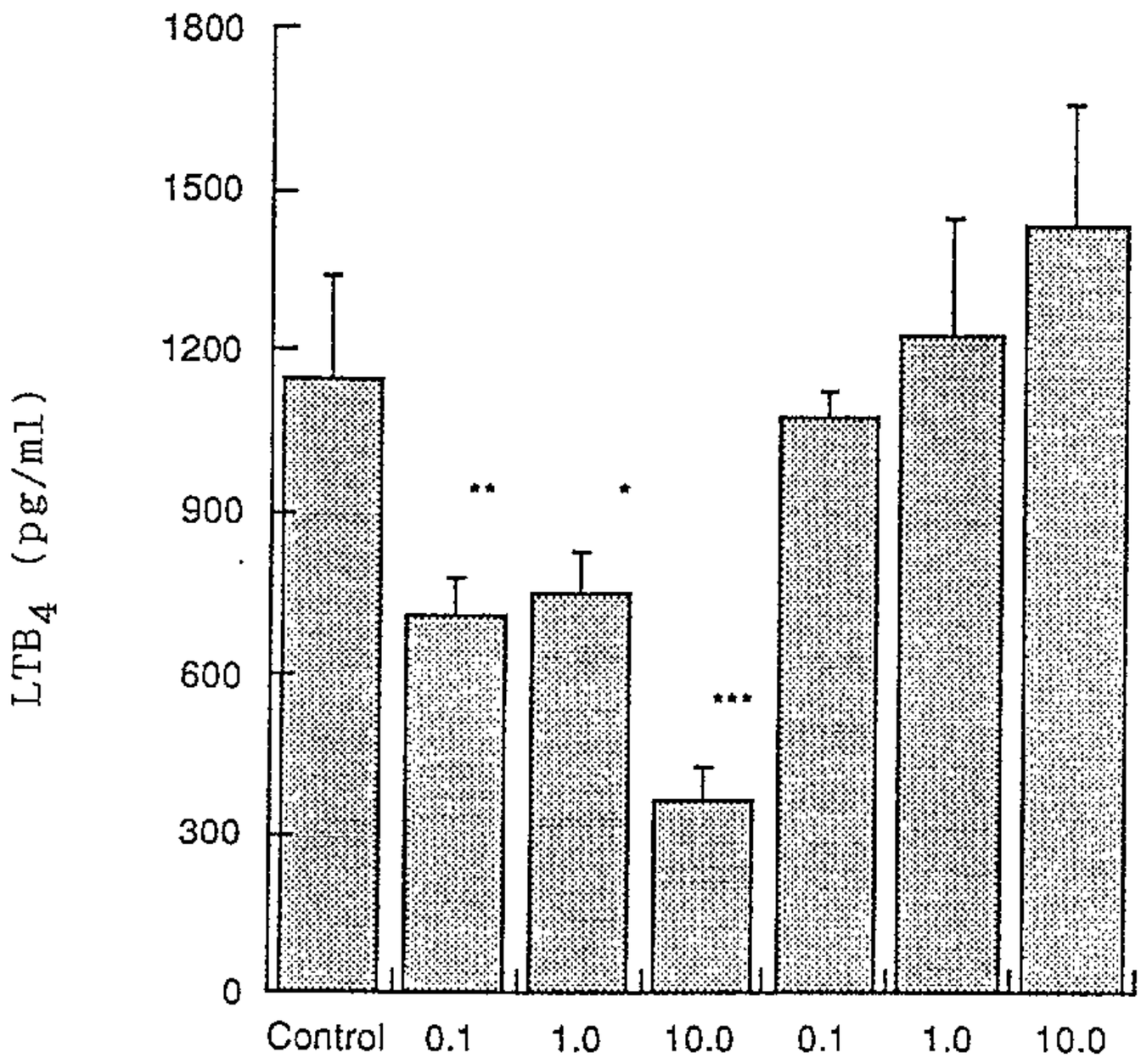
Fig. 3



* :p<0.05
** :p<0.01
*** :p<0.001

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Fig. 4

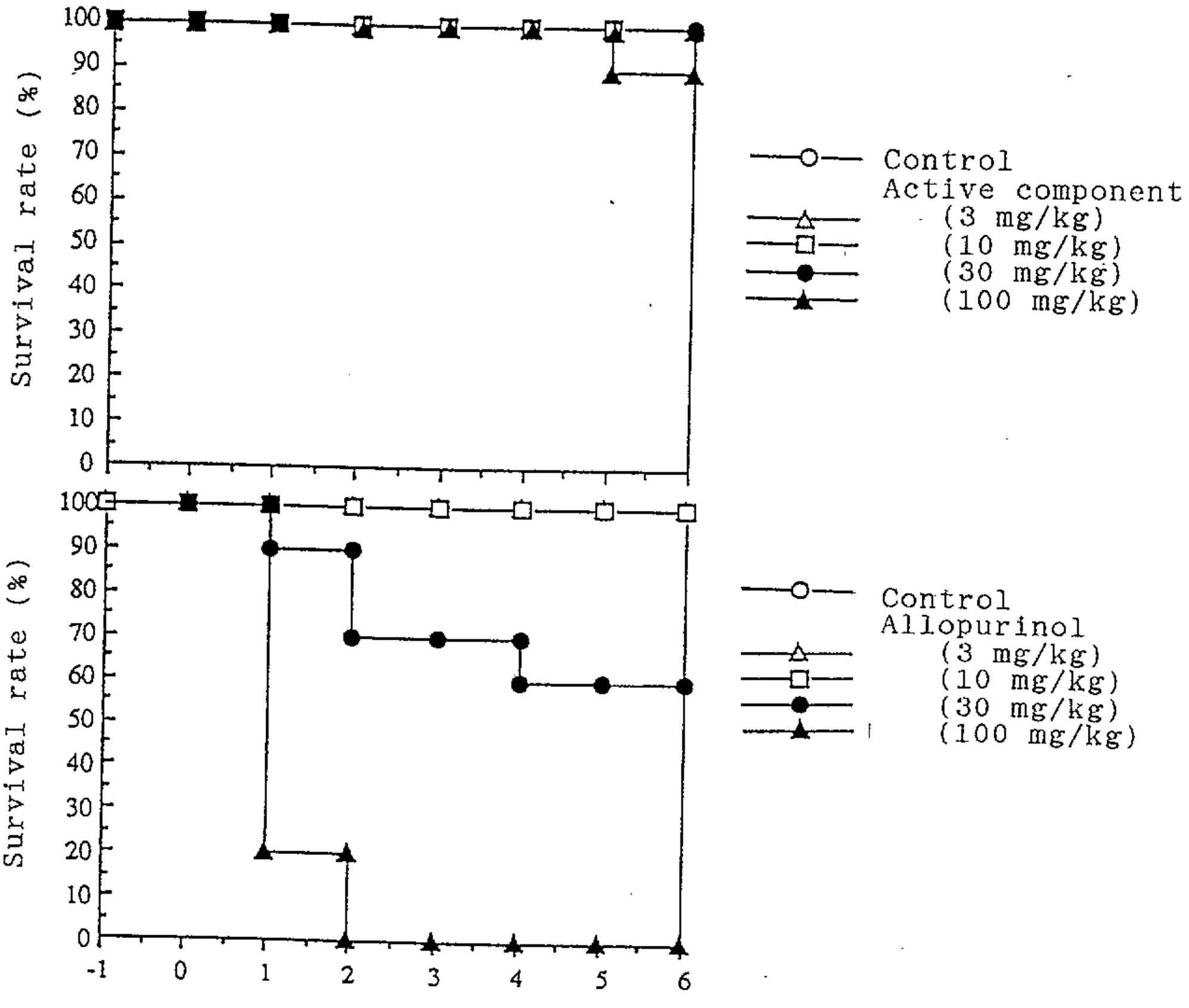

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Fig. 5

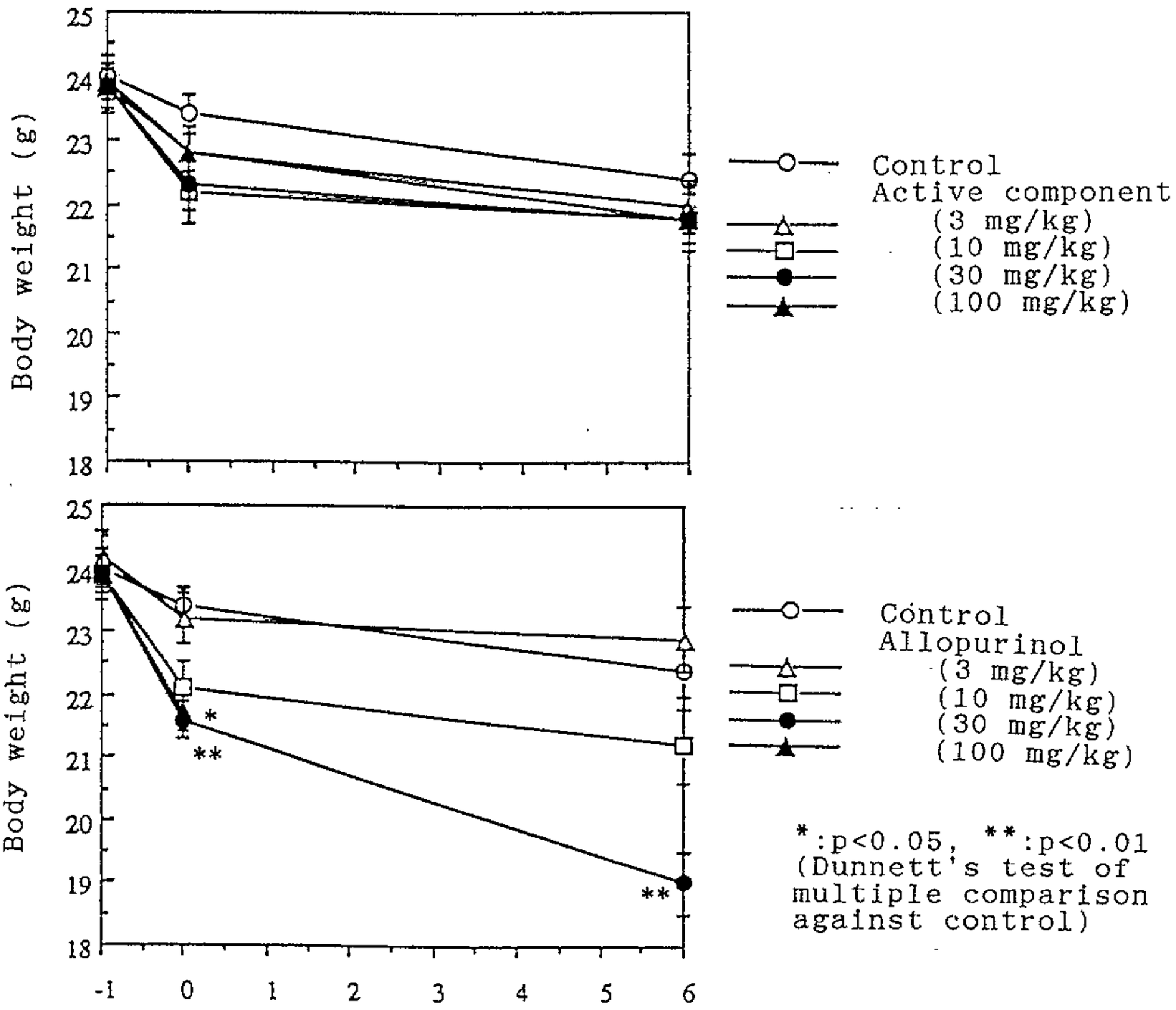

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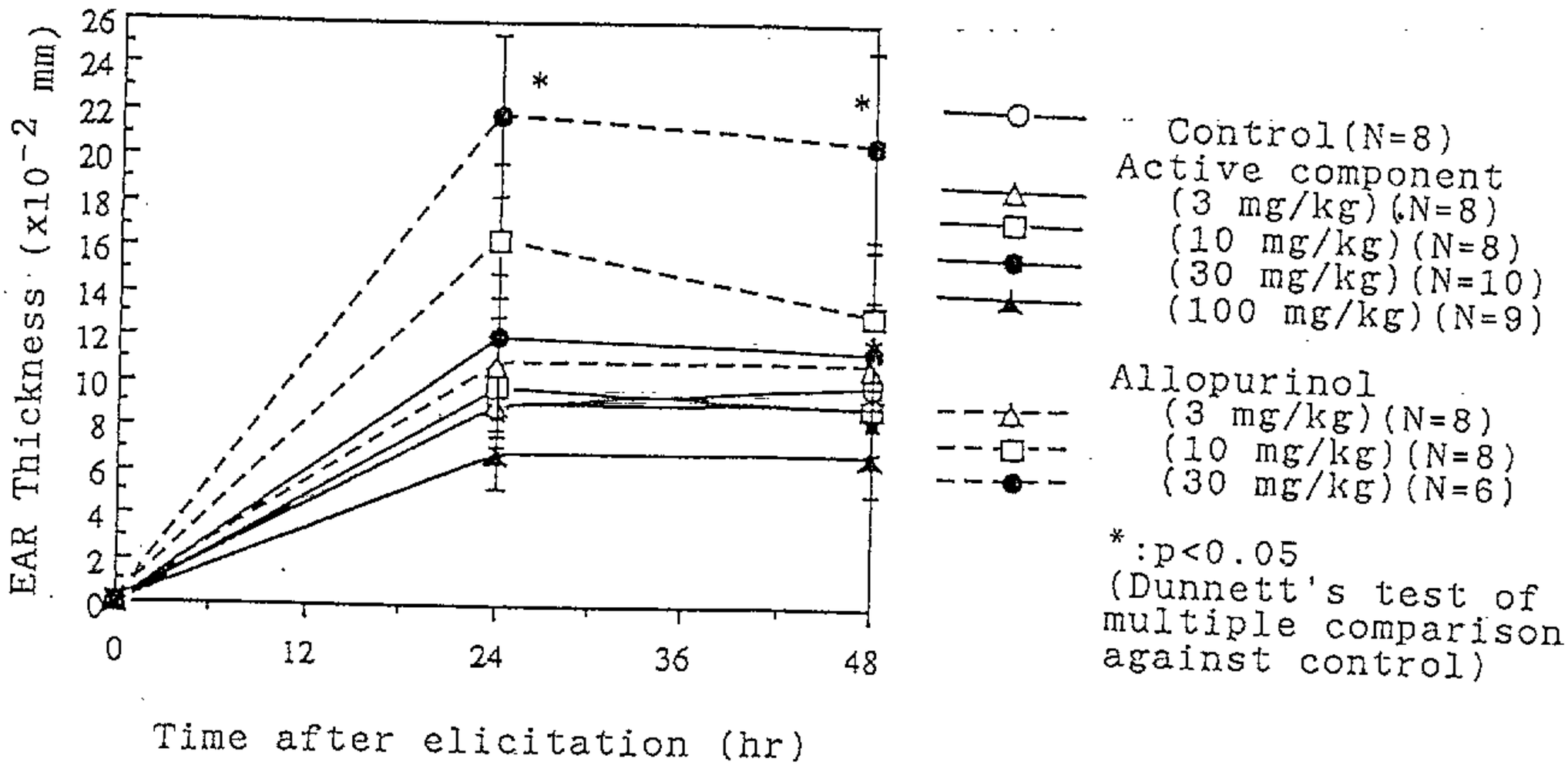
Fig. 6



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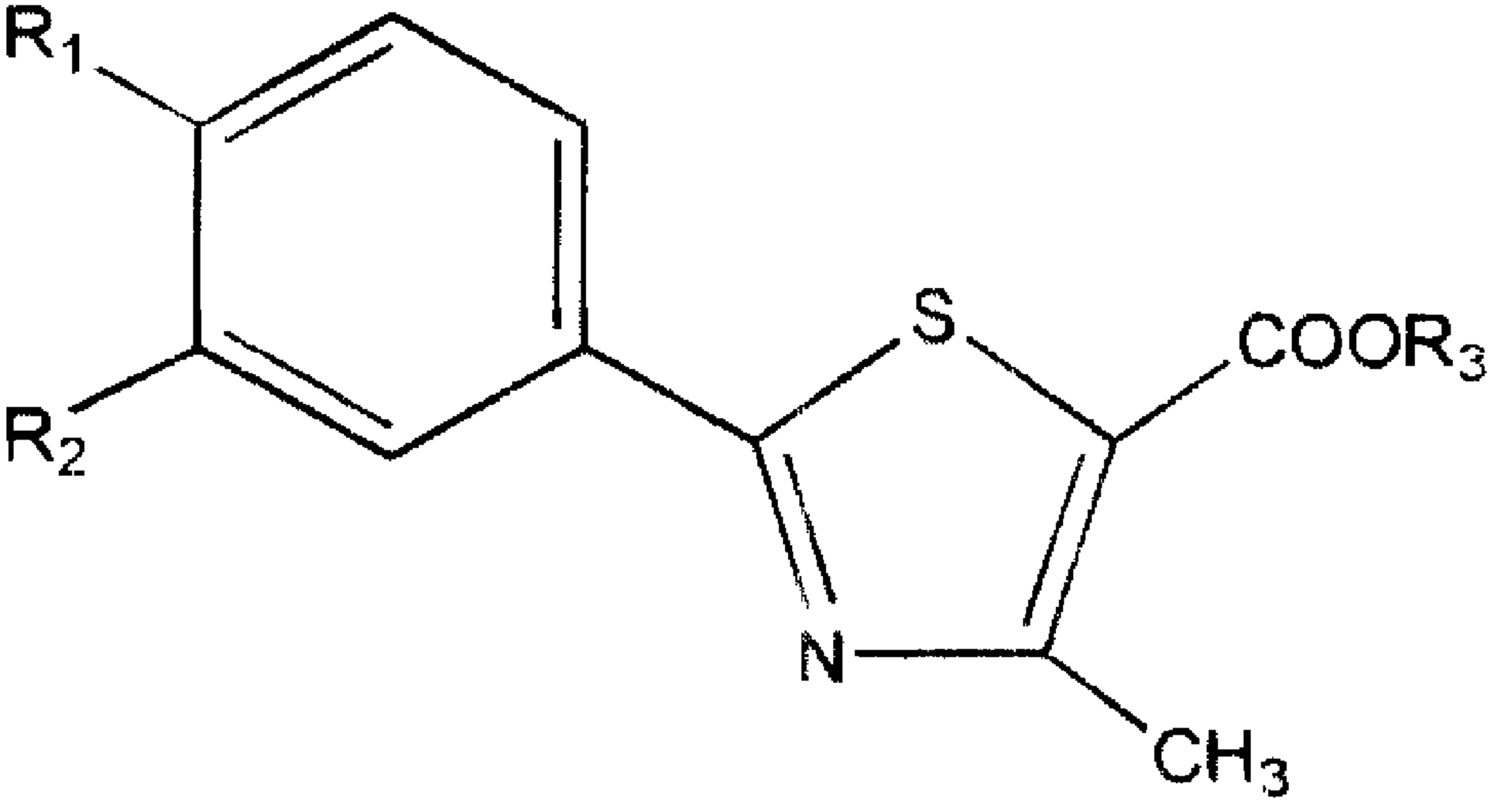

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