Some Toxic Effects of Griffonin in Mice

A.K. Abaitey, BPharm MPharm PhD MPSG
and
Christiana S. Atuobi, BPharm MPharm MPSG
Department of Pharmacology,
Faculty of Pharmacy,
University of Science and Technology, Kumasi,
Ghana

ABSTRACT

Acute and subacute toxicity tests show that griffonin has slight central nervous system depressant action, resulting in reduced locomotor activity in mice. The mice had increased urination and passed semi-solid stools, suggesting muscarinic activity. The LD_{50} of griffonin in mice was 4.31±0.58g/kg body weight.

In the subacute toxicity tests, mice were exposed to griffonin for six weeks. Griffonin caused an initial reduction in red blood cell count which later returned to normal. Microscopic examination of sections of the lung, heart, kidney, spleen and liver showed that griffonin had no effect on the lung and spleen but had dose- and time-dependent effects on the heart, liver and kidney.

Keywords: Griffonin, toxicity, erythrocyte, haemoglobin, leucopenia, haemopoiesis

INTRODUCTION

Griffonin, the main constituent of the roots of Griffonia simplicifolia (Baill), first isolated by Dwuma-Badu, Watson, Copalakirshina, Okarter, Knapp, Schiff and Slatkin in 1976 [7] has been shown to be a predominantly muscarinic drug devoid of nicotinic properties [1] and an antisickling agent [2, 3, 6].

Before a new drug is introduced into medical practice, there should be evidence that toxicity tests have been carried out on the drug. Toxicity tests are of great importance because it is essential to know the nature of the toxicity and side-effects that can be produced by drugs. Often, acute toxicity tests are performed and the data obtained is used to determine the LD_{50} and the therapeutic index of the drug.

Chronic or subacute toxicity tests become necessary if a drug is to be given over a long period. One of the aims of toxicity testing is to determine undesirable, harmful effects of a given substance and so high enough doses are administered to produce toxic effects in a reasonable time.

In chronic toxicity testing the duration of administration of the drug under investigation is a subject of

controversy. Various terms such as subacute, subchronic or chronic studies have been given different definitions by various workers. The WHO [12] defines a short term study of less than three months as subacute, from three to six months as long term or chronic study.

During the subacute studies, the general clinical condition of animals, changes in general behaviour, locomotor activity, food and water intake and changes in weight are observed. The effect of the drug on the clinical chemistry and haematology is also studied. Histological and other post-mortem studies are also carried out after sacrificing the animals.

METHODS

Acute Toxicity Test

Ninety mice of either sex weighing between 15g and 30g were used. The mice were divided into six groups of fifteen animals each. One group served as control. Five different doses of griffonin were given intraperitoneally one to each group and the effects on the mice were observed. 10%w/v aqueous solution of griffonin was used. The control group was given normal saline intraperitoneally. Changes in the response of the mice to different stimuli within the first six hours of administration of griffonin were observed. The mice were again observed 24 and 48 hours later and the total number of dead mice in each group was noted. The LD_{50} was determined graphically and by the method of probits [8]. The experiment was repeated two times.

Subacute Toxicity Test

Three groups each containing six mice of either sex were used in this experiment. One group was treated with a high dose of griffonin (718mg/kg, i.e. 1/6th of the LD_{50}) the second group received a low dose (144mg/kg, i.e. 1/30th of the LD_{50}), whilst the third group which served as control was treated with normal saline. Daily injections of griffonin were given intraperitoneally for six weeks. The animals were observed daily and they were weighed weekly. Two mice in each group were sacrificed every two weeks and the lungs, heart, liver, kidney and spleen were removed and preserved in 20% formaldehyde in normal saline. The blood was collected and used for haematological tests. The organs were used to prepare slides and the histology studied. The experiment was repeated two times.

Dr. A.K. Abaitey
Department of Pharmacology
UST, Kumasi, Ghana

Christiana S. Atuobi (Mrs.)
Krisam Chemists
Cantonments, Accra, Ghana

Haematological Tests

Mice used in the subacute toxicity tests were used for the haematological studies. Two mice in each group were killed every two weeks by fluorothane inhalation. The thoracic cavity was opened and blood was collected by cardiac puncture into tubes containing 0.1ml of a 4% aqueous solution of the di-potassium salt of ethylenediamine tetra-acetic acid (EDTA) which had been dried at 80°C.

The number of red and white blood cells were obtained by direct counting using a haemocytometer after suitable dilution of the blood in a Thoma pipette. Haemoglobin content was determined by colorimetric method after diluting the blood with Drabkin's solution. The colour intensity was measured with a photometer set at 540mμ band. Differential leucocyte count was carried out after staining the blood smear with Leishman's stain.

RESULTS

Acute Toxicity Test

There was a dose-dependent reduction in locomotor activity in all the five groups within the first six hours after administration of griffonin. The animals that received griffonin 1.25g/kg (Group A) became active after 24 hours but the surviving animals in the groups that received griffonin 2.50g/kg (Group B), 5.00g/kg (Group C), 7.50g/kg (Group D) and 10.00g/kg (Group E) remained sluggish and became active only after 48 hours. In all the test groups, the animals became sensitive to light and this was manifested by the tendency for the animals to hide in dark corners. They were however still alert to sound stimulus by trying to run away from the sound of snapping fingers. No animal lost its righting reflex within the first six hours, neither was there a decrease in grip strength of any animal. Animals in groups B, C, D and E had semi-solid stools and increased urination. However, no diarrhoea and lacrimation were observed in any of the animals. Animals in groups C, D and E went off their food within the first six hours and surviving members ate again after 24 hours. The LD_{50} obtained graphically was 4.29g/kg and that from the method of probits [8] was 4.31± 0.58g/kg. Table 1 shows the mortality and probit in the groups 48 hours after administration of griffonin.

Subacute Toxicity Test

Immediately after administration of the drug, stretching of the body was observed in the mice that received the high dose of griffonin (718mg/kg). The stretching continued for one hour after which normal movements were resumed. Stretching was not observed in the animals that received the low dose (Griffonin, 144mg/kg). There was reduced locomotor activity in both groups without loss of righting reflex and grip strength. There was the tendency to hide in dark corners in both groups after drug administration indicating photosensitivity. Food intake was reduced for the first two hours after injection in the group that received the high dose of griffonin. Animals that received the high dose of griffonin passed soft stools and had increased urination, but no diarrhoea was observed. Lacrimation was not observed in either group. There were no significant changes in body weight in both groups over the period of drug administration.

Haematological Tests

The haematological values obtained in the mice after two, four and six weeks of administration of the low and high doses of griffonin are shown in Table 2. There was severe leucopenia in the mice that received the high dose of griffonin for six weeks. The few leucocytes obtained in these mice were mostly lymphocytes. Very few eosinophils (0.25×10^3/ml) were observed only in mice that received the low dose of griffonin for two weeks. There was an initial reduction in haemoglobin content of the blood which returned to normal by the sixth week of administration of both the low and high doses of griffonin.

Histological Studies

Microscopic examination of sections of the organs showed that griffonin had no toxic effects on the lungs and the spleen.

The high dose of griffonin (718mg/kg) produced degeneration of the kidney cells after exposing the animals to the drug for six weeks (Fig. 1). The low dose of griffonin (144mg/kg) had no toxic effects on the kidney throughout the six week period of drug treatment.

Griffonin produced dose- and time-dependent changes in the liver cells. The low dose of griffonin produced few damaged cells in the liver after four weeks and more damaged cells after six weeks of treatment. The high dose of griffonin produced changes in the liver cells in the second week which were comparable to those produced by the low dose in the fourth week. The damage produced by the high dose of griffonin was more pronounced in the fourth week and most pronounced in the sixth week of treatment. Disintegration of the liver cells was observed in the sixth week (Fig. 2).

Griffonin produced dose- and time-dependent changes in the heart cells of the mice. The low dose of griffonin produced slight degeneration of the heart cells after six weeks of drug treatment. The high dose produced slight proliferation of the heart cells after the second week, separation of the muscle fibres and a more pronounced proliferation after the fourth week, and most proliferation after the sixth week of treatment. Numerous nuclei were observed (Fig. 3).

DISCUSSION

Griffonin produced slight reduction in locomotor activity which could be due to depressant action of the drug on the central nervous system which was not severe enough to cause loss of righting reflex or decrease in grip strength. The increased urination and semi-solid stools

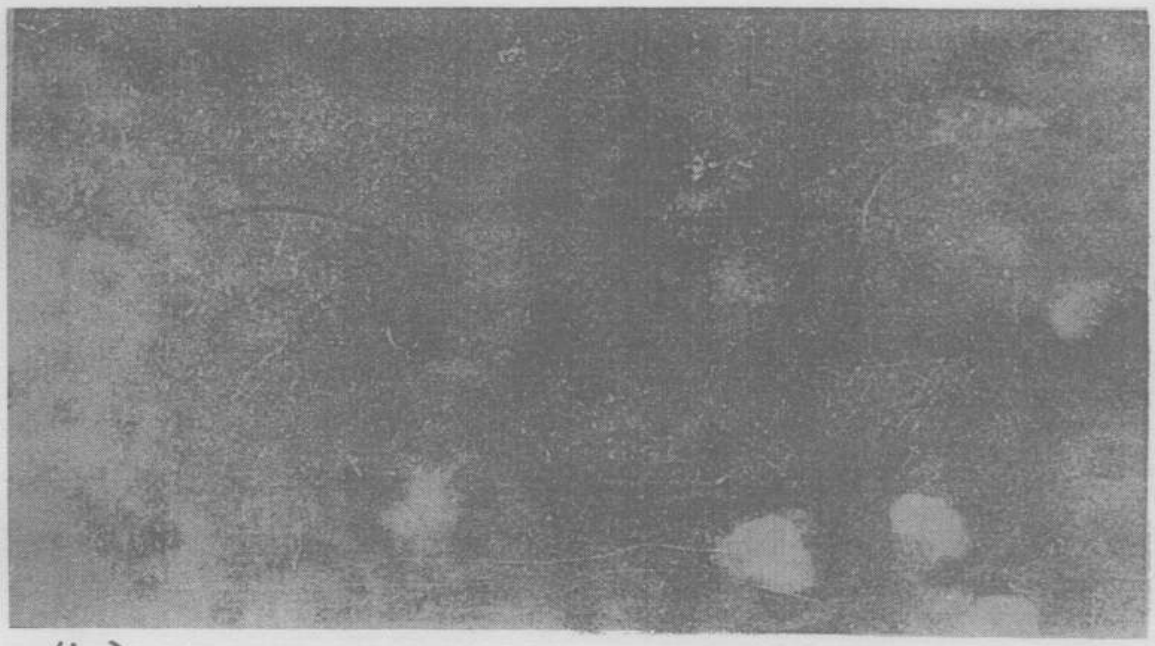
Table 1: Mortality and probit in groups of mice 48 hours after administration of griffonin.

Dose of griffonin (g/kg)	No. of Animals (n)	No. of Deaths	% Mortality	Probit
0 (Control)	15	0	0	-
1.25 (Group A)	15	0	0	-
2.50 (Group B)	15	3	20	4.16
5.00 (Group C)	15	9	60	5.25
7.50 (Group D)	15	12	80	5.84
10.00 (Group E)	15	15	100	-

Table 2: Haematological values obtained in mice after two, four and six weeks of griffonin administration. The control mice were injected with normal saline. The values are the means of six experiments ± s.e. of the mean.

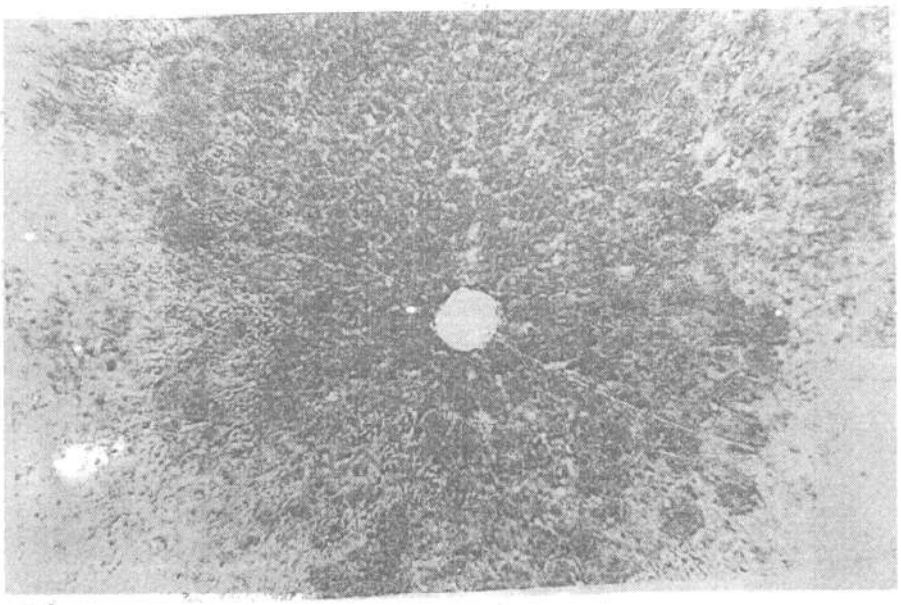
	Dose of Griffonin	RBC $x10^6$/ml	Hb g/100ml	WBC $x10^3$/ml	Neutrophils $x10^3$/ml	Lymphocytes $x10^3$/ml	Eosinophyils $x10^3$/ml	Monocytes $x10^3$/ml
2nd Week	Control	8.3±0.3	12.2±0.4	4.6±0.2	0.87±0.4	3.82±0.3	—	0.37
	144mg/kg	6.3±0.4	8.8±0.3	5.0±0.8	2.30±0.2	2.05±0.5	0.25	0.40±0.2
	718mg/kg	6.0±0.2	9.3±0.6	8.4±0.6	1.18±0.4	6.97±0.3	—	0.25±0.1
4th Week	Control	8.2±0.3	12.6±0.9	5.2±0.7	1.30±0.5	4.58±0.5	—	0.36±0.1
	144mg/kg	9.2±0.1	12.8±0.4	10.0±1.5	2.60±0.4	7.40±0.6	—	-
	718mg/kg	6.2±0.3	9.7±0.4	4.3±0.8	2.11±0.3	2.02±0.4	—	0.17
6th Week	Control	10.5±0.5	15.4±0.3	5.4±0.4	1.20±0.3	5.75±0.8	—	-
	144mg/kg	8.6±0.4	12.1±0.6	6.8±0.6	1.97±0.3	4.62±0.5	—	0.20
	718mg/kg	9.0±0.6	12.6±0.4	1.7±0.3	Leucopenia. Few cells mostly lymphocytes			

(a)

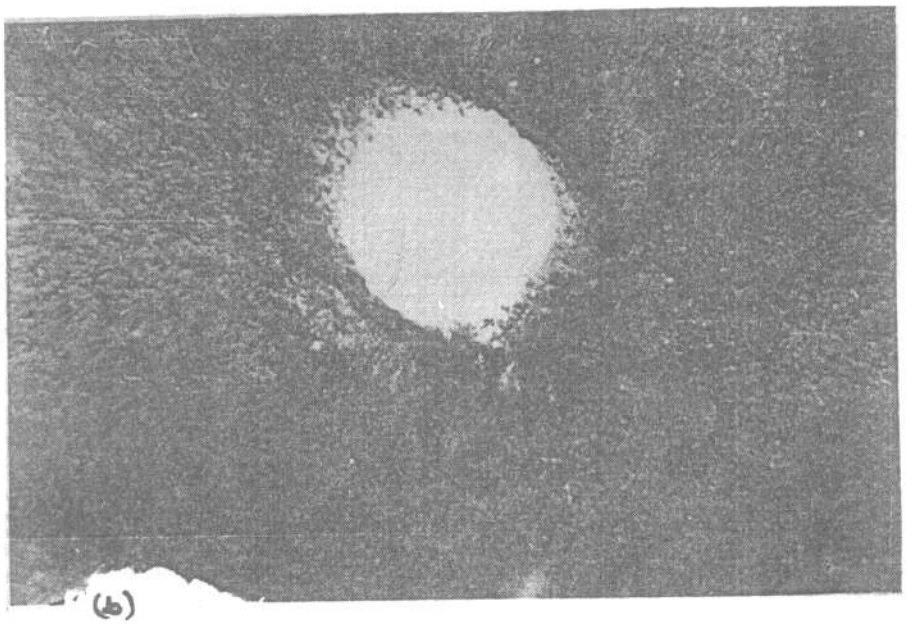


(b)

Fig. 1 Photomicrographs of sections of the kidney of mice (a) untreated (control) and (b) treated with griffonin 718 kg for six seeks. Mag. x 500. Note degeneration of the kidney cells of the treated animal.



(a)



(b)

Fig. 2: Photomicrographs of sections of the liver of mice (a) untreated (control) and (b) treated with griffonin 718 mg/kg for six weeks. Mag. x 500. Note the damage and disintegration of the liver cells of the treated mouse.

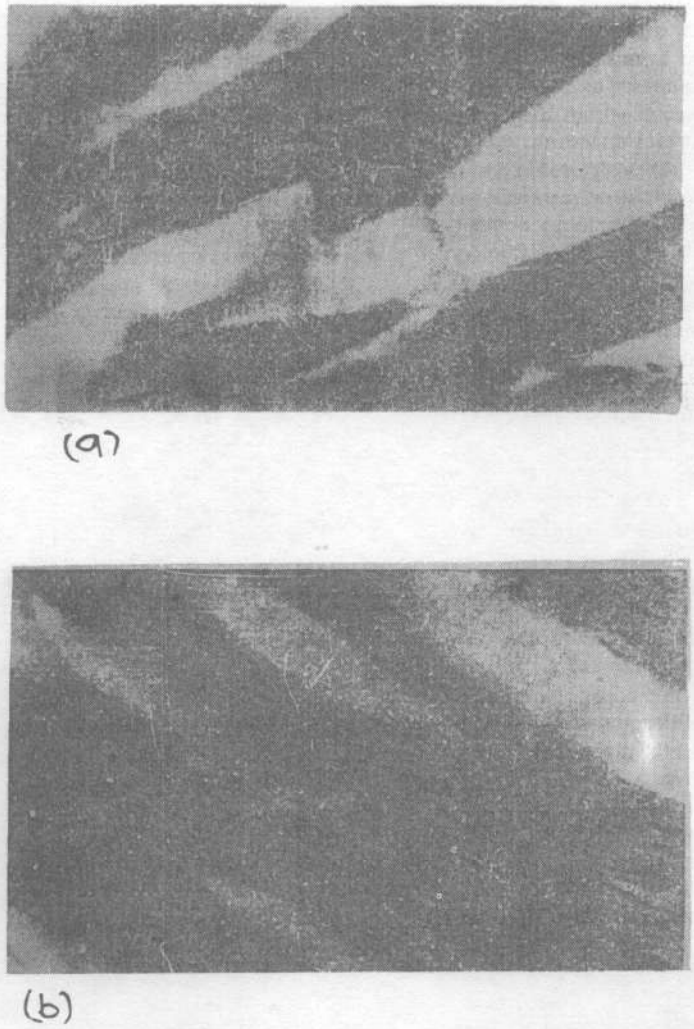


Fig. 3: Photomicrographs of sections of the heart of mice (a) untreated (control) and (b) treated with griffonin 718 mg/kg for six weeks. Mag. x 500. Note separation of the muscle fibres and the proliferation of the heart cells of the treated mouse.

observed may be due to the muscarinic effect of griffonin reported by Abaitey and Atuobi [1]. The stretching of the body which occurred in the group of mice that received the high dose of griffonin during the subacute test may be due to increased peristaltic movements of the gastrointestinal tract which gave rise to colicky pain as was observed with acetylcholine-like drugs by Rang and Dale [9].

The LD_{50} calculated by the method of probits was 4.31±0.58g/kg body weight and that from the graph was 4.29g/kg. When the LD_{50} is extrapolated to man, the LD_{50} for a 60kg human being would be 258.6g. This figure shows that griffonin is of low toxicity. Extending these results to man may be highly presumptuous, because of species variation. For example, the rate and pathway of drug metabolism vary from species to species. In man, pethidine is metabolised at a rate of 17% of the amount present per hour, with a single dose being effective for 3 to 4 hours. In dogs, 70% to 90% of a dose of pethidine is metabolised per hour and the drug is therefore short acting and without addictive properties in dogs. Furthermore in some laboratory animals, the rate of metabolism of drugs is sex related. For example, hexobarbitone is metabolised four times faster in male than in female rats.

The normal red blood cell count of the mouse is between 7.7 and 12.5 x 10^6/ml with a mean value of 9.3 x 10^6/ml [5]. Results obtained from the haematological studies indicate that griffonin causes an initial suppression of red blood cell formation within the first two weeks of administration but as administration of the drug is prolonged production of erythrocytes returns to normal. Haemoglobin content of the blood also followed a similar pattern. The normal haemoglobin content of blood for mice is between 10.0g and 15.0g /100ml with a mean value of 14.8g /100ml [5]. The reduction in erythrocyte count may account for the anaemia that was initially observed. Apart from the low leucocyte count (1.7x10^3/ml) in the mice treated with the high dose of griffonin for six weeks, the total leucocyte counts for all the mice used were within the normal range of 4.0 to 12.0x10^3/ml.

There are various causes of leucopenia most of which are due to infections, such as typhoid, influenza and malaria. Haemopoietic disorders, for example, pernicious anaemia, aplastic anaemia and disorders involving the spleen are all some of the causes of leucopenia [11].

The cause of leucopenia in the group of mice that received the high dose of griffonin for six weeks is however not clear. There was no evidence of any type of infection during the period of drug administration. For example, the mice were healthy throughout the course of drug administration and there was no loss of weight in the animals. Again, microscopic examination of the spleen did not show any abnormalities in the organ. Probably the precursors to leucocyte production are more sensitive to the depressant effect of griffonin on haemopoiesis than the precursors to erythropoiesis. Moreover, the process of maturation of the erythrocytes takes about 4 to 5 days whereas with the leucocytes the process takes about 6.5 to 11 days [10].

The differential leucocyte count revealed that all the different types of cells were within the normal values except the lymphocytes in animals treated with the low dose of griffonin for two weeks and in those treated with the high dose for four weeks. The normal lymphocyte count of the mouse is 3.0 to 8.5x10^3/ml [5]. Lymphocytes are not normally affected by drug toxicity and the changes observed in the lymphocyte count in this study cannot be readily explained.

Griffonin had no significant effect on the body weight of the mice, showing that the drug had neither debilitating nor anabolic effects on the animals.

Microscopic examination of the lung and spleen showed that griffonin had no adverse effect on these organs.

Griffonin produced time- and dose-dependent toxic changes in the liver, heart and kidney. In the heart, there was proliferation of the cells, loss of integrity of the cells and an increase in the number of nuclei. The toxic effect of griffonin on the heart is supported by the irreversible negative chronotropic and ionotropic effects and fasystoles observed on the isolated rabbit heart preparation together with abnormal contractions and ultimate death of the preparation [2]. In the acute toxicity tests, death of the mice was probably due to cardiac arrest induced by griffonin.

The toxic effect of griffonin on the liver was also time- and dose-dependent. The liver is the main organ concerned with drug metabolism and it is possible that as the duration of exposure to griffonin was prolonged, there was accumulation of the drug in the liver which resulted in liver damage.

The kidney receives the peak plasma concentration of all substances in the blood, and so it is susceptible to direct drug-induced damage [4]. The toxic effect on the kidney was observed when griffonin (718mg/kg) was administered for six weeks. At this stage there was general toxicity of griffonin and most organs in the body were affected.

The LD_{50} (4.31g/kg) obtained from the acute toxicity tests was far in excess of the high dose of griffonin (718mg/kg) used in the subacute tests. This suggests that even in low doses griffonin is likely to be toxic when given over a long period. It must however be noted that observations of toxic effects in one species may be due to species variation.

The initial suppression of erythropoiesis produced by griffonin in the subacute toxicity tests is an unfavourable effect of the drug. Even though there was a compensatory increase in the production of red blood cells after some time, griffonin may not be safe for use in sickle-cell anaemia patients in whom there is an already low level of erythrocytes. However, the ability of griffonin to revert sickled red blood cells to the normal shape and size[2,3,6] and thus preventing sickle-cell crises, may weigh against its ability to suppress erythropoiesis. Furthermore routine administration of folic acid in sickle-cell anaemia patients may offset this unwanted effect. The toxic effects of griffonin on the liver, heart and kidney after prolonged administration indicate that griffonin may not be used for long term treatment. Griffonin may however be used for symptomatic treatment of sickle-cell crises but not for prophylactic treatment.

CONCLUSION

Griffonin has slight central nervous system depressant action which is manifested in reduction in locomotor activity but not in loss of righting reflex or reduction in grip strength.

The LD_{50} of griffonin by probit analysis is 4.31 ± 0.58g/kh body weight Griffonin induced increased urination, passing of semi-solid stools and body stretching in mice which may be due to the muscarinic effect of the drug.

The drug produced time - and dose-dependent toxic effects on the liver, heart and kidney. Griffonin may be recommended for symptomatic treatment of sickle-cell crises but not for use as a prophylactic agent.

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