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TOXIC EFFECTS OF LARGE DOSE OF QUASSIN IN NORMAL AND DEPRIVED MICROFLORA RAT GROUPS

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SUMMARY

Eighteen rats were divided into three equal groups. The first group was dosed orally with quassin, the second group was dosed with quassin after the gut flora were suppressed by different antibiotics, and the third group was served as a control. Food intake, water intake, and change in body weight were measured daily before dosing, during two weeks of dosing, and during one week after stopping dosing. Two eats from each group were killed at the end of each week, and stomach, liver, and kidney were collected for histopathologic examination.

The results show a significant decline in daily food intake and daily change in body weight, and a significant increase in daily water intake in both dosed groups during the dosing period. Microscopic lesions were seen in the kidneys of both dosed rats group killed at the end of first and second week.

INTRODUCTION

Quassin is a mixture of bitter principles isolated from quassia wood obtained from quassia amara and multiflora, and cedronia granatensis, [1,2,3,4].

picraena excella, brucea javanica, picrasma ailanthoides Quassin was found to be alactone, and neoquassin the corresponding hemiacetal. A third minor component of the mixture is dehydroneoquassin [5]. Grieco et al. (1980) succeeded in the synthesis of quassin. This achievmen can aid in the synthesis of quassinoids-relatives of quassin which appear to be potent antileukemia agents [6,7].

Little information is available in regarded to its metabolism in the animal body or its toxicity.

Quassin extract has been used as an antihelmentic and as a bitter in medical and veterinary practice. It has been used as food additives and as an alternative to quinine [9]. Its use in this way is allowed in most countries except Germany, the recommended maximum permitted level in the final product being 50 mg/kg [10].

Danders that may arise from the use of food additives have been recognized, chronic administration of some food additives was shown to produce alteration in the animal intestinal microflora which may lead to considerable metabolic and /or toxicological effects [8].

In general the upper parts of G.I.T. including the stomach, duodenum, jejunum and upper ileum are separsely populated with micro-organism while the rat which is a convenient source of experimental data has a flora in forestomach and upper small intestine [11].

The aim of present study to explore the metabolic and/or toxic effect of large oral dose of quassin in rats and the role of microflora in this effect to evaluate the risk of long use of quassin as food additive in animals and human being.

MATERIALS AND METHODS

Materials:

Quassin crystals, Bush Boake, London, was provided by Dr.James,S.P.Dept.,; of biochemistry, Univ. of Birmingham.

Neomycin sulfate, tetracycline hydrochloride and bacitracin sulfate were supplied by Samara Drug Industries S.D.I Pentobarbitone sodium"Nembutal ® " , Abbott. Balance.

Experiment Design:

Eighteen mature male rats, of the same age (4-6) week, weighing (205-225 gm) were obtained from the central medical laboratory. The rats were divided into three groups, each of six rats and each was housed individually in metabolic cage. Food and water were available in containers which protected them from contamination with feces and urine. After a period (5 days) of acclimatization, animal body weight, food and water intake were measured daily during the period of experimental regimen which consist of three days before dosing, first and second week of dosing with quassin and a third week without dosing with quassin.

The gut flora of animals in the first group were suppressed by giving an oral dose of neomycin sulfate (100 mg), tetracycline hydrochloride (50 mg) and bacitracin sulfate (50 mg), antibiotics were given twice daily for two days before dosing the rats and during the period of dosing with quassin suspension (150 mg/kg), treatment was stopped at the recovery period [12]. The rat of second group were given (150 mg/kg)

orally quassin as suspension in distilled water using syringe provided with stainless steel stomach tube for its administration. The rats of the third group act as a control and dosed orally with equal amount of distilled water. Two rats of each group were killed by intraperitoneal injection of pentabrbitone sodium (80) mg/kg) at the end of first, second and third week. Different organs were removed surgically and fixed in (10%) formalin for histological examination.

Statistic Analysis:

Analysis of variance f-test was used to compare the results of the three animals group statistically [13].

RESULTS

Body Weights:

The results showed a marked decline in daily body weight in both dosed during the dose period with significant difference from the second day after dosing ($P < 0.05$ and $P < 0.01$) (graph 3). The decline was more obvious in the quassin + antibiotics group but with no significant differences between them (Table. 1). The daily body weight change in both dosed groups restored after stopping dosing its control value as before the dosing period but the rats body weight still have significant decrease from the control group, for quassin ($P < 0.05$) and for quassin +antibiotic group ($P < 0.01$) (Table 1,graph 3).

Food Intake:

The results shows a significant decline in food intake in both dosed groups from the first day and during most days of the dosing period ($P<0.05$ and $P<0.01$)(graph 2). The decline was more obvious in the quassin + antibiotic then in the quassin group ($P<0.05$)(Table 1). The animals restore in the

recovery period its normal food intake with no significant difference between the three groups (Table1 , graph 2).

Water Intake:

The results of daily water intake shows a fluctuation characterized by increase in water intake in both dosed groups with a significant difference ($P<0.05$ and $P<0.01$) from the control group in some days of the dosing period (graph 1).

Microscopic Lesions:

No microscopic lesions were seen in the liver and stomach of all rats in the experiment. Same histological changes were only seen in the kidneys of both dosed rats group that killed in the first and second week dosing period. There were various degrees of intratubular congestion and epithelium of some tubule were degenerating. The lumen of some tubules contained eosinophlilic hyaline cast and/or free red blood cells (Fig. 1 & 2). Some of the hyaline cast-containing tubules were dilated. The glomeruli were not effected. No microscopic lesion were seen in kidney of rats killed at the end of 3rd of week (stopping dosing period).

DISCUSSION AND CONCLUSION

Quassin is regarded as xenobiotic, in the sense that it is neither metabolized by the body with the release of energy nor is used to build up body tissues [14]. Previous study showed that quassin, neoquassin and different unidentified metabolites were excreted in the urine of rats dosed orally with quassin + antibiotic [15]. It was concluded that gut microflora play a role in quassin metabolism. In the present study the marked decline in body weight of dosed rats in both dosed group may be due to loss of appetite since quassin consider as bitter which cause increase in stomach acid secretion, so large dose certainly cause

more acid secretion and irritation of stomach mucosa with the consequence loss of appetite [9]. This was showed by the marked decline in rats daily food consumption from the first day and through out all the dosing period and by the increase in their daily water intake in some days of the dosing period. The decline in body weights may be also because of the toxic effect of quassin or one or more of quassin metabolites that execrated in the urine of rats in both dose groups. This was illustrated by the microscopic lesions seen in kidney of both dosed rats killed during the dosing period and microscopic lesions were seen in kidney of dosed rats killed in the third week after stopping dosing. The microflora are known to play important role in the metabolism and utilization of carbohydrate, cellulose and important Vitamins [16], so the deprivation of this vital activity when the microflora killed by antibiotics lead to more decline in body weights of antibiotics dosed rats group than that of quassin group. We concluded that any increase in quassin dose by many folds from that of the recommended one either due to its accumulation in the body after its long use or accidentally due to a mistake in calculation of the dose may cause a converse effect on weight gain and/or toxic effect by quassin, one or more of its metabolites. The microflora do not seem to play a role in quassin toxicity as same microscopic lesion and metabolic changes were seen in rats of both dosed group. Further studies are required investigate the metabolic pattern of quassin, identified its metabolites and determine which of them may cause the toxic effect in rat kidney.

Fig.1 : Kidney from a rat treated with quassin only and killed at the end of the first week.

A group of tubules containing hyaline casts and red blood cells. H&E 125X

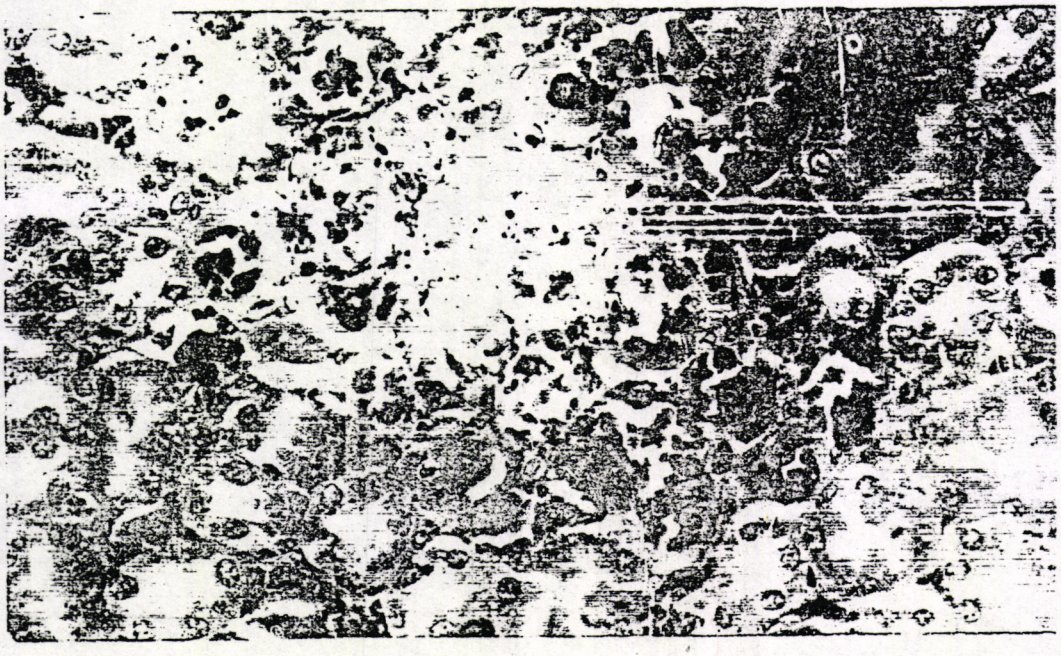
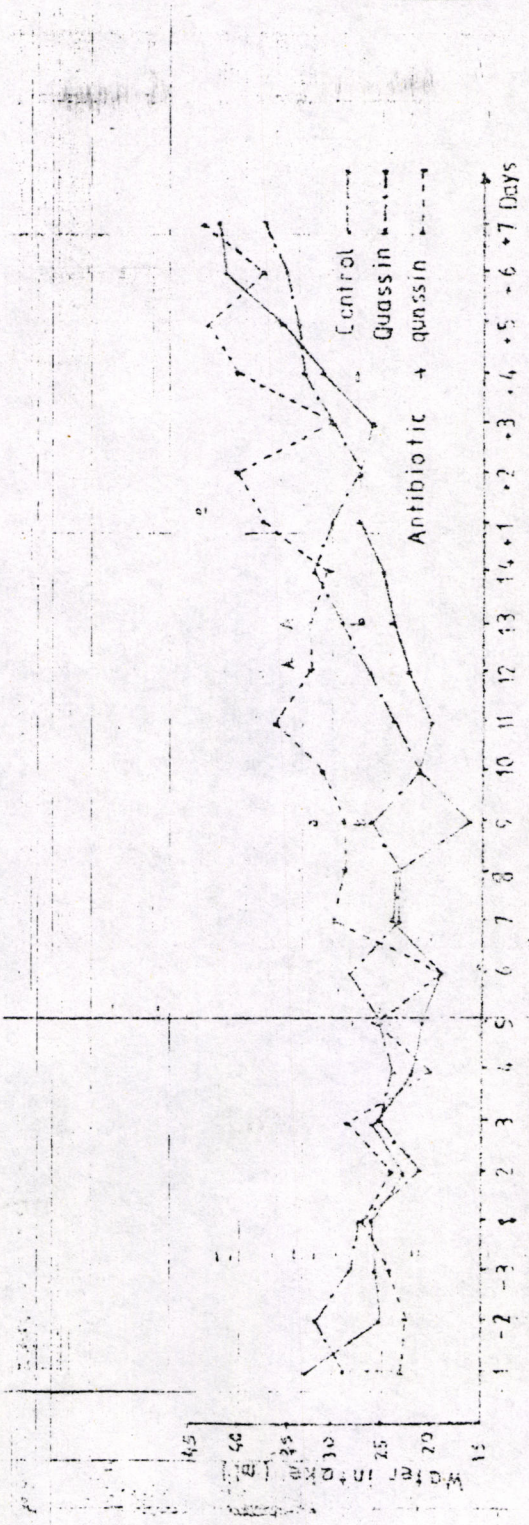
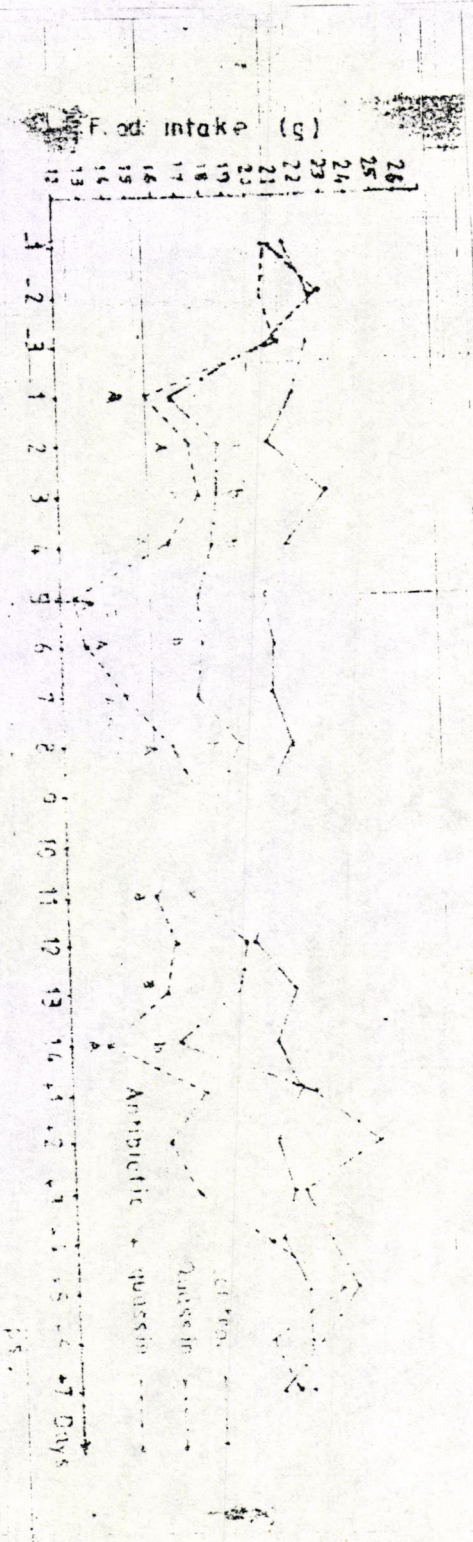


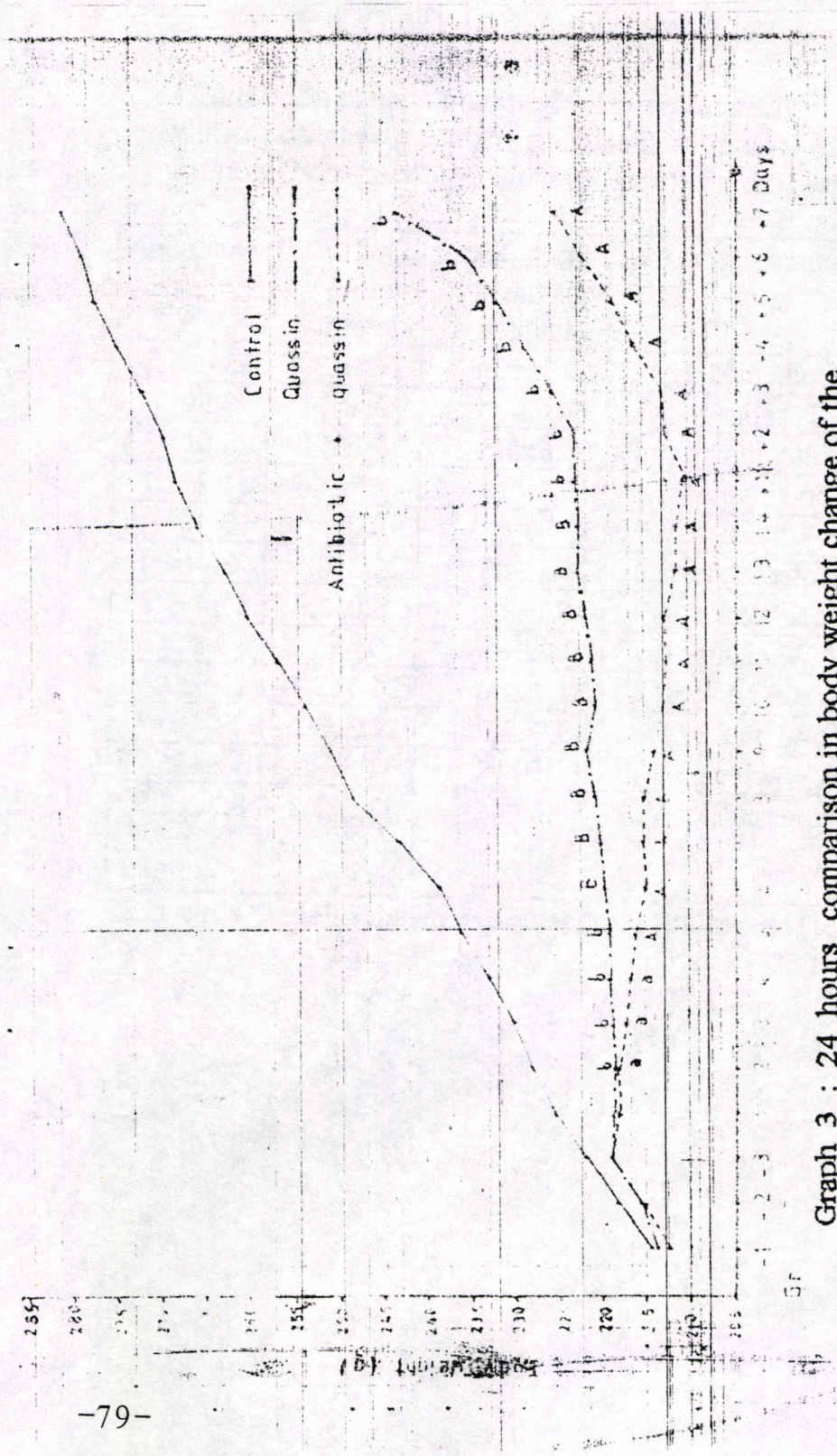
Fig.2 : Kidney from a rat treated with quassin only and killed at the end of the second week.
A group of tubules containing hyaline casts and red blood cells. H&E 125X



Graph 1 : 24 hours water intake comparison of the three animal groups.



Graph 2 : 24 hours comparison of food intake of the three animal groups.



Graph 3 : 24 hours comparison in body weight change of the three animal groups.

Table (1): The average daily body weight change, food intake and water intake comparison between the orally dosed antibiotic+quassin, quassin and control groups.

	Animals groups	Oral dosing quassin +antibiotics	Oral dosing quassin	Control
Week before dosing	Food intake (g)	20.7±0.8	21.4±.3.3	22.0±1.8
	B.Wt.change (g)	+3.60	+3.45	+3.90
	Water intake (ml)	23.6±5.5	30.1±5.8	28.0±5.1
First week of dosing*	Food intake (g)	15.3±1.9	17.8±3.6	21.2±1.7
	B.Wt.change (g)	-0.64	+0.06	+3.10
	Water intake (ml)	25.2±4.3	25.2±2.5	23.4±4.4
Second week of dosing**	Food intake (g)	16.1±2.4	16.0±3.8	21.4±0.8
	B.Wt.change (g)	-0.43	+0.43	+3.21
	Water intake (ml)	31.9±1.2	26.5±2.4	21.9±3.1
Third week, stopped or no dosing***	Food intake (g)	19.2±1.2	22.3±3.7	22.4±0.7
	B.Wt.change (g)	+1.90	+1.97	+2.11
	Water intake (ml)	38.6±4.5	32.5±3.2	32.8±2.8

Notes: The results represent the mean of six (*), four (**), and two (***) rats.

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التأثيرات السمية للجرع العالية للكواسين في مجاميع الجرذان الطبيعية والمحرومة المايكروفلورا

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الخلاصة

ثلاث مجاميع من ستة جرذان ، جرعت الأولى بمادة الكواسين وجرعت الثانية بنفس المادة بعد ان تم قتل المايكروفلورا بواسطة جرعات فموية من بعض المضادات الحيوية ، اما الثالثة فكانت مجموعة السيطرة. تم قياس معدل وزن الجرذان واستهلاك الماء يوميا للمدة قبل التجريع ولاسبوعي التجريع ولاسبوع آخر بعد توقف التجريع. تم قتل حيوانين من كل مجموعة في نهاية كل اسبوع وحفظت بعض الاعضاء الكبد ، الكلية ، المعدة في ١٠% فورمالين لغرض الفحص النسيجي. النتائج اظهرت وجود نقصان معنوي في استهلاك العلف اليومي وفي وزن الحيوان اليومي وبعض الزيادات المعنوية في استهلاك الماء اليومي لكلا مجموعتي العلاج خلال فترة التجريع. الفحص النسيجي اظهر وجود تغييرات نسيجية فقط في كلى حيوانات مجموعتي العلاج خلال الأسبوع الأول والثاني فقط للتجريع.