Interaction of C*itrus Maxima* Fruit Extract with CNS Agents Affecting Behavioral Patterns of Mice

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Abstract

The food drug interaction is a common yet crucial area of research where administration of a medicine and dose regimen is instructed with the type and time of food intake. The interaction of the food with drug is both pharmacokinetic and pharmacodynamic. The citrus fruit, *Citrus maxima,* commonly known as pomelo, when administered with the central nervous system acting drugs interfered with the activity of the drugs. The benzodiazepines which act as CNS inhibitory showed prolonged inhibitory action when taken with fruit extract of *Citrus maxima* as motor relaxation and antianxiety increased but the locomotor activity was reduced. The antidepressant, SSRI- amitriptyline, prolonged the mobility activity in the experimental animals indicating a potentiation of the drug action in the presence of the fruit extract in reducing the depression. Therefore, while prescribing a CNS drug the use of *Citrus maxima* needs to be restricted so that better efficacy with minimum adverse drug reactions can be achieved.

Keywords- *Citrus maxima, Diazepam, Amitriptyline, Antianxiety, Depression.*

INTRODUCTION

The study of drug interaction has become prominent in the present area of research as it is considered as the cross reaction between multiple drugs and also with food. This may modulate the potential activity of the drug and in certain cases may influence the adverse reactions of the drug. The interaction of food and drug may lead to newer unknown effect [1]. The drug interaction is usually a area which is considered critical because of the practice of polypharmacy and the administration of the drugs at different times of the day have the chances of interacting with foods. As pharmacokinetic and pharmacodynamic are both affected by the interaction, so dose and administration pattern of the drug are studied thoroughly. The drug interaction shows various results as the mode of action of the drug are interfered in the presence of other substances[2]. The various physicochemical characteristics of the body gets altered in the presence of many substances as a result of which the drug activity changes. As found the alkaline pH prevents the absorption of acidic drug and vice versa. Similarly, the pH causes the ionization of the drugs which results in reduced absorption and further it reduces the efficacy of the drug. For an example if ketoconazole, itraconazole and dapsone which requires acidic environment show reduced absorption when induced concomitantly [3]. In another condition, if the drug and food increases the protein binding of the drug the retention of the drug increases in the body which results in the toxic effects of the drug due to deposition. Similarly there are multiple foods which form chelate with the drug molecules preventing the absorption through the gastrointestinal tract. The

quinolone antibiotics when administered with magnesium and aluminum containing antacids results in chelation. The high fat containing meals result in significant increase in the absorption of fat soluble compounds such as griseofulvin, cefpodoxime, and cefuroxime axetil whose increased absorption may lead to toxicity. The vasoactive agents that increase the intestinal blood flow increases the drug absorption from the GIT.

When interactions of food with drug have been studied similar changes in the activity of the drug is observed. The food, beverages and dietary supplements interact with the efficacy and safety of drug. The food interferes mainly with the absorption of drugs in the presence of high fat, high protein and fiber diets. The bioavailability of the drug reduces in the presence of most of the food. The metabolism among the pharmacokinetic parameters is widely affected by the presence of citrus fruit. The various citrus fruits and grape fruit (*Citrus maxima*) affect the pharmacokinetic profile of multiple drugs.

Citrus maxima, also known in the different names like pomelo, shaddock or grape fruit is a citrus fruit which is native to Southeast Asia. This fruit belongs to the family of *Rutaceae*[4]. Grape fruit juice interacts with number of drugs increasing the adverse drug effects[5][6][7]. The basic and one of the most important constituent is furanocoumarins[8]. Furanocoumarins derivatives has been reported to cause alterations in the hepatic and intestinal CYP 450 especially CYP3A4. The metabolism of benzodiazepines [9], antihyperlipidemic drugs [10], antiarrhythmics. calcium channel antagonists and HMG Co-A reductase inhibitors are commonly affected[11].

In the present work *Citrus maxima* extract was chosen to observe its effect on the CNS drugs of two different categories-the CNS inhibitory like Diazepam and amitriptyline selected as CNS stimulant. These are the two common drugs often administered for multiple CNS related disorders. The fruit *Citrus maxima* is frequently taken by the people of India and especially in the eastern and southern India where it is common fruit. The necessity of the study is to find the interacting effect of the fruit with the CNS drugs in modulating the drug effect.

MATERIALS AND METHODS-

Materials: The extract from the pulp of *Citrus maxima*, diazepam (East India Pharmaceutical Works Ltd,, Kolkata, India), amitriptyline from Sigma-Aldrich, USA and sodium chloride from Loba chemie, Mumbai, India.

Collection of extract from Citrus maxima: The fruit was collected from the Durgapur region of Barddhaman district, West Bengal, India. The pulp was separated from the fruit and by mechanical process with the fruit mixer the juice was extracted. The juice was freshly prepared for every use and separated with the help of a sieve. The extracted juice was kept in clean container.

Study of extract on behavioral profile in mice

The study was done with adult Swiss albino mice of either sex weighing 18-22 g obtained from CPCSEA approved animal house of Dr. B. C. Roy College of Pharmacy and AHS, Durgapur that were kept in a group of 5 maintaining the diurnal/ nocturnal. The temperature was maintained at 22±1°C. The mice were kept in laboratory conditions a week prior to the study for acclimatization. Food was withdrawn 4 hours before dosing but water was available *ad libitum*. This study was performed according to CPCSEA guidelines with the approval number BCRCP/IAEC/2/2015.

Dosage preparation and administration

The extract of *Citrus maxima* was administered at a dose of 1 ml/kg body weight *p.o*. The diazepam was administered at the dose of 4mg/kg *i.p.* Amitriptyline was administered at the dose of 5mg/kg body weight *i.p.* The animals were randomly separated into five groups with five mice in each group. The treatment was done accordingly Group I- Untreated control, Group II- Pomelo extract treated, Group III- Diazepam treated, Group IV- Amitriptyline treated Group V- Treated with extract of *Citrus maxima* and Diazepam and Group VI-Treated with extract of *Citrus maxima* and Amitriptyline. The extract was administered thirty minutes before the treatment with diazepam and amitriptyline. The locomotor activity and mobility of the animals before and after the dose administrations were subsequently analyzed with Actophotometer (Manufacturer:

MVTEX, Ambala, Haryana, India) and Rotarod (Orchid Scientific and Innovative India Pvt. Ltd.) apparatus as described below.

Methods to analyze behavioral parameters

Actophotometer- The locomotor activity was assessed with actophotometer as per standard protocol [12]. The exposure time of animals to the instrument was 5 minutes. The alterations in total number of movements were considered. The score of all the individuals were recorded after 30, 60, 120 and 240 minutes after treatment with the drug.

Forced swim test or Porsolt swim test– The Forced Swim Test (FST) was carried out to assess the depression in animals [13]. The animals were exposed for six minutes but the mobility of last four minutes is counted. The immobility is used as the index for depression.

Rotarod- The motor coordination and the skeletal muscle relaxant property is determined with the rotarod [14] where time of fall from the rotating rod is counted. The speed of rotating rod was at 25 rpm and animals were checked for 30, 60, 120 and 240 minutes.

Elevated plus maze- The entry of the mice to the open arm and time spent in the open arm of the elevated plus maze measured the level of anxiolysis[14] .

RESULT ANALYSIS: The data were statistically analysed using ANOVA followed by Student T-Test. $p<0.05$ was considered significant and $p<0.001$ was considered as highly significant.

RESULTS AND DISCUSSIONS

The locomotor activity started reducing after 30 minutes of diazepam treatment and the reduced effect was observed till 120 minutes when compared with the untreated control group. The reduction was of high significance at 60 minutes of diazepam administration as given in Table 1. After 240 minutes of drug administration a significant recovery was observed for diazepam treated group and was similar to control. When animals were administered with pomelo (*Citrus maxima*) extract no change in locomotor activity was observed as compared to untreated control. But when the experimental animals were treated with pomelo *(Citrus maxima)* and diazepam it was observed that the reduction in the locomotor activity of continued till 240 minutes with a very high significance as compared with the untreated control (Table 1).

Table 1: Effect of *Citrus maxima* and diazepam on locomotor activity of mice

SNo	Groups	30 minutes	60 minutes	120 minutes	240 minutes
1	Control	105.30±15.5	120±10.25	155±14.5	180±10.5
2	Pomelo treated	115.25±4.5	122±6.25	130.50±4.0	136 ± 7.25
3	Diazepam (4mg/kg)	47.5±4.5*	17±3.5**	85±7.50*	147±5.50
4	Diazepam (4mg/kg) + Pomelo	36±5.5*	7.5±3.2**	16±6.5**	25±5.5**

*All values are Mean ± SD, n = 5, *P<0.05, **P<0.001 when compared with control*

With the change in the locomotor activity the muscle tone was evaluated which was found to get reduced in the diazepam treated animals as it is a skeletal muscle relaxant (Table 2).

Table2: Effect of *Citrus maxima* and diazepam on muscle relaxant activity of mice

SNo	Group	30 minutes	60 minutes	120 minutes	240 minutes
1	Control	25.5±5.5	42±5.8	63±6.5	124±6.8
2	Pomelo treated	35±4.25	28±2.75	101±7.50	112±4.35
3	Diazepam (4mg/kg)	14.02±2.5	5.5±2.5**	60±6.3	95±2.7
4	Diazepam (4mg/kg) + Pomelo	4.5±0.7**	4±1.5**	5±1.7**	17±4.6**

*All values are Mean ± SD, n = 5, *P<0.05, **P<0.001 when compared with control*

The reduction was observed after 30 minutes of diazepam treatment which was not significant but after 60 minutes the muscle relaxation was highly significant as compared with the untreated control group, which got recovered after 120 minutes. The group that received pomelo extract did not show any change in the muscle tone as compared with the untreated control. When pomelo (*Citrus maxima*) was administered along with the diazepam the skeletal muscle relaxant activity

reduced with high significance as compared with the untreated control from 30 minutes which continued for 240 minutes as presented in Table 2.

Similarly, in the elevated plus maze the antianxiety property was evaluated on the open arm entry and open arm time spent by the animals. The diazepam treated animals were found to have reduced anxiety at 60 and 120 minutes which were significant as compared with the untreated control as given in Table 3. The open arm stay for the pomelo (*Citrus maxima*) and diazepam treated group increased significantly 60 minutes and continued the significant stay upto 240 minutes as compared with the untreated control (Table 3). Whereas, in the study the open arm time spent by the pomelo (*Citrus maxima*) treated group did not much change as compared with the untreated control as shown in Table 3.

Table 3: Effect of *Citrus maxima* and diazepam on open arm activity in elevated plus maze of mice

SNo	GROUPS	30 minutes			60 minutes			120 minutes			240 minutes		
		Latency	Entries	Spent time(s)	Latency	Entries	Spent time(s)	Latency	Entries	Spent time(s)	Latency	Entries	Spent time(s)
1	Control	3.4±1.5	2	20±1.2	4±0.5	2	30±1.6	1.5±0.6	2	25±1.5	3.9±2.8	2	60±1.4
2	Pomelo treated	6.4±3.5	2	32±4.2	10±0.5	2	25±2.8	5±1.5	2	48±2.6	2±2.8	2	78±3.25
3	Diazepam (4mg/kg)	4.5±3.5	1	40±0.5	2.5±1.7	2	75±1.2*	1.5±0.9	3	98±1.2*	6.5±4.5	1	105±0.8
4	Diazepam (4mg/kg) + Pomelo	2.5±0.5	7	57±1.7	2.8±3.5	6	105±0.8*	1.3±4.6	9	116±0.8*	1.57±0.5	7	185±1.4*

*All values are Mean ± SD, n = 5, *P<0.05, **P<0.001 when compared with control*

In the study of depression model with amitriptyline the immobility time for animals got reduced at a dose of 5 mg/kg p.o., which was not significantly low as compared with the untreated control. But when pomelo (*Citrus maxima*) was administered along with amitriptyline at the same dose there was a high significance in the immobility time from 30 minutes which extended to 240 minutes as compared with the control given in Table 4.

Table 4: Effect of *Citrus maxima* and Amitriptyline on immobility of mice

SNo	Group	30 minutes	120 minutes	240 minutes
1	Control	90.56±4.4	90.6 ± 2.6	92.5 ± 4.6
2	Pomelo	75.35±2.5	82.6±4.25	68.22±1.6
3	Amitriptyline (5mg/kg)	52±1.4	74.6±2.7	66.33±0.9
4	Amitriptyline (5 mg/kg)+ pomelo	43.60±0.5**	40.6±2.7**	46.33±0.9**

*All values are Mean ± SD, n = 5, *P<0.05, **P<0.001 when compared with control*

The reduction in immobility is the index for reduced depression. But, pomelo extract itself did not reduce the immobility time as compared with the control.

From the study of different behavioral models of the animals with two different category of CNS agents it can be predicted that the fruit *Citrus maxima* itself did not show any change in the CNS activity and is therefore, not a responsible agent for increase or decrease in CNS activity as an individual. But when given along with the CNS agents it prolonged the activity of the drugs. Therefore, the food drug interaction of the fruit with the CNS agent was prominent. The *Citrus maxima* extract usually has been found to inhibit CYP3A4 present in small intestine that reduces the drug presystemic metabolism. The isoform CYP3A4, of CYP450 enzyme helps in first pass metabolism. As reported previously, the

furanocoumarins inhibit the cytochromic enzymes[15][16] which inturn reduce the breakdown of the benzodiazepine moiety. The benzodiazepines usually acts in the midbrain, ascending reticular formation and limbic system by activating the $GABA_A$ receptor chlorine ion channel complex and thereby increasing the frequency of the chlorine ion channels [17]. The inhibition of liver metabolic enzymes may be responsible for the retention of the drug in the body which may have shown the effect for a long period of time. The elimination of the drugs from the body got reduced thus enhancing the pharmacological activity of diazepam. Similarly, the psychotropic agent amitriptyline, is metabolized by the enzyme cytochrome P450 1A2 and cytochrome P450 3A4 and the pomelo has been reported to inhibit these two enzymes, therefore the metabolism amitriptyline may have got reduced [18]. The reduction in metabolism of these two drugs may have increased the bioavailability and thereby prolonged the pharmacological action of the drugs.

CONCLUSION

The study conducted to find the effect of *Citrus maxima* (pomelo) extract on the drugs on the behavioral changes showed that though the extract prolonged the CNS inhibitory activity of diazepam and CNS stimulatory effect of amitriptyline but itself did not alter any CNS activity in experimental animals. Therefore, it may be concluded that the fruit has high food interaction with the CNS agents which prolonged the pharmacological actions of the drugs. Moreover, as the pharmacological actions prolonged so during the treatment phase with these drugs the *Citrus maxima* (pomelo) should be avoided or dose should such be maintained so that the drug accumulation along with adverse reactions can be prevented.

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