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Research Article

# The *In-Vivo* Effects of Caffeine and Rutin Combination on Caffeine Intoxication in Rodent Model

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## Abstract

Caffeine, the principal component of coffee is a renowned psycho stimulant as well as possesses anti-asthmatic potential. Nevertheless the therapeutic use of caffeine is limited due to caffeinism i.e. caffeine dependence or withdrawal symptoms. In the present study Rutin (flavonoid) reported to exert inhibitory neuronal influence over anxiety, depression, epilepsy and nociception and therefore the current analysis aspires to inspect the in-vivo consequence of Rutin on the analeptic action of caffeine in combination. The models used in the study are locomotor activity, hole board test for exploratory behavior and test for motor co-ordination in mice (n = 6). It was found that administration of caffeine alone at a dose level of 200 mg/Kg b.w. showed significant CNS stimulation while that of Rutin at an analogous dose elicits into mild CNS depressant effect. However, treatment with caffeine: rutin combination in a ratio of 1:1 led to significant ($P < 0.05$) decline in the CNS stimulation. Thus, from the existing study it can be concluded that rutin on combi-nation with caffeine extinguish the analeptic potential of caffeine, thereby facilitating its use in prophylactic management of diseases like asthma.

**Keywords:** Caffeine Rutin; Actophotometer; Hole board test

## Introduction

Distinguished because of its presence in popular drinks, caffeine (Methylxanthines) is doubtlessly the most widely consumed of all behaviorally active drugs. Methylxanthines have been used to treat bronchial asthma, apnea of infants, as cardiac stimulants, as diuretics, as adjuncts with analgesics, in electroconvulsive therapy, and in combination with ergotamine for treatment of migraine (Orru., *et al*. 2013) [13].

Accordingly, it had hence been suggested that regular caffeine intake may reduce asthma symptoms in its potential role as an asthma treatment, since caffeine is a weak bronchodilator and it reduces respiratory muscle fatigue. Other potential therapeutic targets for caf-feine include diabetes, Parkinsonism, and even cancer. Caffeine has been used as a diagnostic tool for malignant hyperthermia (Schwarz-schild., *et al*. 2002) [15].

Caffeine intoxication is a syndrome involving psychological and physical distress caused by chronic or acute overconsumption of caf-feine. It is included in DSM-IV and ICD-10 and appeared in earlier editions of these nomenclatures as caffeinism. The syndrome is often manifested by such somatic complaints as diuresis, tachycardia, and tremulousness, headaches, irritation, lethargy, anxiety (DSM-IV and ICD-10) [7,8]. Caffeine has also been implicated in a number of other disorders, including depression, schizophrenia, bipolar disorder, eating disorders, ADHD in children, and restless legs syndrome (Watson., *et al*. 2002 and Peters, 2013) [17].

Plants that are the part of human diet and are often used as alternative medicines, as they provide numerous ingredients which might interact functionally with different organ systems in human body. Among these plant-derived compounds are flavonoids recently have attracted interest because of their biological activities to human health but also because of their influence on central ner-

vous system (Fernandez., *et al*. 2006) . The influence of flavonoids on anxiety (Joshi., *et al*. 2005) [9], depression (Bhutada., *et al*. 2010) [4], nociception, learning and memory processes (Azevedo., *et al*. 2013) [2] has been reported. Rutin is one of the widely occurring proved flavonoid obtained from plants used in varied pharmaceutical preparations and diet supplements with certain biological role on central nervous system in experimental and clinical studies on anxiety, depression, memory processes and convulsant activity (Nieoczym., *et al*. 2014) [11]. Thus aim of the present study was to detect the possible consequence of the flavonoid rutin on the central nervous system activity of caffeine.

## Materials and Methods

### Procurement of chemicals

Caffeine was purchased in powder form Central Drug House (P) Ltd (New Delhi); Rutin in the form of rutin hydrate was purchased from Sigma Company, USA. All drugs were dissolved in physiological saline and administered orally. At a dose level of 30 mg/kg b. w./day for 28 days.

### Qualitative analysis of the drug

The qualitative analysis of the given sample of caffeine and rutin was performed by analyzing the organoleptic features of the drug samples along with determining the solubility profile and partition coefficient of the same.

### Acute oral toxicity study and dose selection

Acute oral toxicity was performed as per OECD- 423 guidelines i.e. the starting dose level is selected from one of four fixed levels (5, 50, 300, and 2000) and it should be that which is most likely to produce mortality in some of the dosed animals. The animals were fasted overnight, provided only water after which the bioactive was administered to the groups orally at the dose level of 50 mg/kg body weight and the groups were observed for 3 days. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. Mortality was not observed then the procedure was repeated for further higher doses such as 100, 300 up to 2,000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsions and mortality for 72 hours (OECD Guidelines, 423) [12].

### Experimental Design

Animal experimentation was conducted only after getting approval from Sagar Institute of Pharmaceutical Sciences, Institutional Animal Ethical Committee, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No. SIPS/EC/2014/45). The Swiss Albino mice of either sex were randomly divided into postulated study groups (n = 6 mice per group) as described below:

**Group I (Normal control):** Composed of healthy animal treated with saline only.

**II (Rutin control):** Experimental animals were treated with Rutin (30 mg/kg).

**Group III (Caffeine control):** Experimental animals were treated with Caffeine (30 mg/kg).

**Group IV (Test group):** The experimental animals were treated with caffeine + rutin in the ratio (1:1) at a dose of 60 mg/kg.

### Locomotor activity in mice

The spontaneous locomotor activity score of each group was observed 30 mins after administration of the above treatment intervention for 10 mins using an Actophotometer. The locomotor activity was recorded as a count when a beam of light falling on the photocell is cut off by the animal. The equipment is turned on, the animals are placed individually in the activity cage for 10 mins, and the basal activity score of all animals is noted. Thirty minutes after the drug administration retest each animal for activity scores for 10mins. The difference in activity in each group was observed, and the percentage increase and decrease in motor activity was calculated (Kulkarni, 2012) [10].

### Hole-board test for exploratory behaviour in mice

Anxiety levels were also evaluated in mice by using a hole-board apparatus (35 cm × 35 cm × 15 cm), 1h after the oral treatment. Its walls were made of clear Plexiglas and the arena floor was constructed from black Plexiglas and divided in 16 equal squares with 16 holes (diameter 3.5 cm). The equipment is elevated 56 cm above the floor. Thirty minutes after receiving the prescribed treatment, each animal was placed on the central square of the arena and the number of crossed squares and holes poked were recorded for 5 min. An increase of the hole-poking response reveals a positive anxiolytic-like effect (Brown and Nemes, 2008) [6].

### Test for motor co-ordination (rota-rod test) in mice

The motor coordination and performance of each animal was evaluated thirty minutes after receiving the prescribed treatment, each mouse was placed on the horizontal rotating rod set at a rate of 16 revolutions per min for 180 sec, at intervals of 30 min, for 3h. This equipment has a 2.5-cm diameter bar divided in six parts and it is placed at a height of 25 cm, rotating at 12 rpm. Latency to fall from the rotating bar was noted. (Kulkarni, 2012) [10].

### Statistical analysis

All the data were presented as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) test for multiple comparisons followed by Bonferroni's test. The statistical significance was set accordingly.

## Result and Discussion

### Qualitative analysis of the drug

The organoleptic properties of caffeine claims it to be a white odorless powder with bitter taste while rutin (Table 1). Solubility profile states that the extracted compound is freely soluble in benzene and n-hexane, sparingly soluble in distill water and petroleum ether while insoluble in ethanol, methanol and chloroform (Table 2). The partition coefficient ($K_{ow}$) of the caffeine and rutin as calculated from mentioned formula was found to be 4.743 and 3.07 (Table 3) and since chemicals with $K_{o/w}$ of 2-6 are said to be lipophilic, it tends to exhibit that the bioactive is a lipophilic compound (Banker and Rhodes, 2000) [3].

| Parameters | Description of caffeine | Description of rutin |
|---|---|---|
| Color | White powder | White powder |
| Odor | Odorless | Odorless |
| Taste | Bitter | Bitter |

**Table 1:** Organoleptic features of the bioactive.

| Solvent | Solubility profile of caffeine | Solubility profile of rutin |
|---|---|---|
| Distilled water | ++ | ++ |
| Methanol | - | - |
| Ethanol | - | - |
| Chloroform | - | - |
| Benzene | ++++ | ++++ |
| n-Hexane | ++++ | ++++ |
| Petroleum ether | ++ | ++ |

**Keys:**

++ + +: Freely soluble = 1-10 part of solvent.

++: Sparingly soluble = 30-100 part of solvent.

- : Insoluble = < 10,000 part of solvent.

**Table 2:** Solubility profile of the bioactive.

| Bioactive | $C_{o\ (\mu g/ml)}$ | $C_{w\ (\mu g/ml)}$ | $K_{o/w}$ |
|---|---|---|---|
| Caffeine | 1.106 | 0.231 | 4.743 |
| Rutin | 1.01 | 0.328 | 3.07 |

**Table 3:** Partition coefficient of the bioactive.

### Acute oral toxicity study and dose selection

The acute toxicity studies performed for both drugs rutin and caffeine administered via oral route revealed no sign of toxicity, only up to a dose of 300 mg/kg body weight, accordingly $1/10^{th}$ of the safe dose was selected for executing the protocol.

### Locomotor activity in mice

Figure 1 depicts the locomotor activity score achieved in 10 minutes via different treatment groups measured using an Actophotom-eter. The locomotor activities observed in the animal models acts as an index of wakefulness of mental activity. Increase or decrease in the parameter is suggestive of stimulatory or sedative potential of the bioactive. From the currently performed experimental analysis it can be deduced that caffeine significantly ($P < 0.05$) increases locomotor activity but regular intake of the bioactive for 28 days signifi-cantly ($P < 0.05$) reduces the same depicting withdrawal effect of the drug. Also it was observed that of rutin alone had no significant effect on the spontaneous locomotor activity, however on administering it in a ratio of 1:1 with caffeine the locomotor activity after sig-nificantly increasing ($P < 0.05$) in the initial 14 days did not show any significant change further, thus indicating that that in combination with rutin the analeptic potential of caffeine can be maintained, thereby nullifying its withdrawal symptoms (Figure 1).

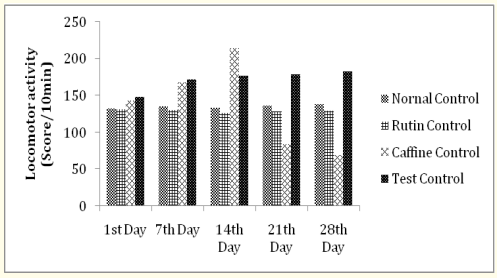


**Figure 1:** Graphical representation of Locomotor activity in mice.

### Hole-board test for exploratory behaviour in mice

The hole-board test is a measure of exploratory behaviour. An agent that decreases this parameter reveals a sedative nature. Anxiolytics have been shown to increase the number of head dips in the hole-board test (Brown and Nemes, 2008) [6]. The results of the exploratory behavior study in the experimental animals as depicted in (Figure 2) demonstrates a significant increase ($P < 0.05$) in the exploratory nature of the animals of the caffeine treated group for initial 14 days, but the parameter exhibited a significant declination trend for the next 14 days. Rutin administration in experimental animals did not have any significant change in the exploratory behavior. Although caffeine treatment accentuated the parameter but the exploratory behavior showed a declined trend in the next 14 days on the study, demonstrating caffeineism. The same study performed for the test group animals demonstrated that caffeine rutin combination increased head dips during the initial stages whereas in the latter half of the protocol no significant change was observed suggesting annul of caffeine withdrawal symptoms by the combination (Figure 2).

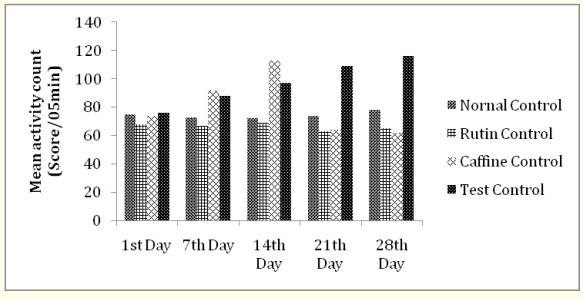


**Figure 2:** Graphical representation of Hole-board test for exploratory behaviour in mice.

### Test for motor co-ordination (Rota-rod test) in mice

Figure 3 data demonstrates the results of the motor-coordination test using a rota rod apparatus. The fall off time was found to vary non-significantly in the saline and rutin treated group animals while concomitant readings of the caffeine treated group depicted that caffeine treatment in the initial 14 days had no significant effect on the motor co-ordination in the experimental animals, however the skeletal muscle activity was observed to have declined in the subsequent 14 days, which may be attributed to rhabdomylosis or skeletal muscle degeneration as a consequence of caffeine intoxication. The test sample had no effect on the motor co-ordination, suggesting that the inhibitory effect of the test combination might be elicited via central mechanism, not by peripheral neuromuscular blockade, furthermore from the observation it can be deduced that the test combination also overcame the skeletal muscle degenerative action elicited as a consequence of caffeine intoxication (Figure 3).

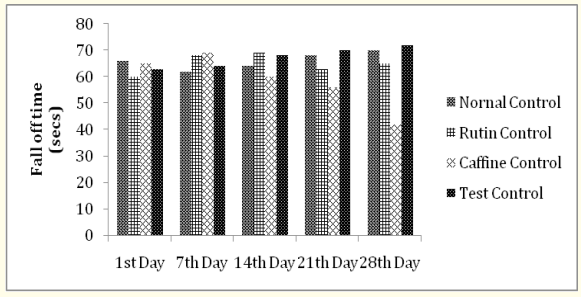


**Figure 3:** Graphical representation of Motor co-ordination (rota-rod test) in mice.

## Conclusion

German chemist Fridelieb Ferdinand d Runge was the first to isolate caffeine from coffee, he termed it as "Kafebase" (the base of coffee), since then caffeine is accredited to be world's most popularly consumed psychoactive drug, with global consumption estimated by USFDA at 12000 tonnes per annum. Apart from its renowned analeptic properties, caffeine consumption is associated with reduced overall risk of cancer. Caffeine is a weak bronchodialator and in clinical trial on asthamatic patients, the drug exhibited improvement in lung function at prophylactic doses. It can also be used as primary treatment for breathing disorders like apnea of prematurity. However the therapeutic use of caffeine is limited due to caffeineism or caffeine intoxication. Caffeine intoxi-

cation is a condition defined accord-ing to the diagnostic and statistical manual of mental disorders as demonstration of characteristic sign of distress or impaired neuronal functions associated with long term use of caffeine or caffeinated beverages.

Flavanoids have received much attention in the scientific literature owing to the variety of potential therapeutic benefits they have. Currently the intake of bevarages containing flavanoids is highly recommended (Agrawal, 2011). Rutin is classified as a polyphenolic flavanoid. Literature data advocates a moderate psycho-stimulant effect of hydroalcoholic plant extract containing both rutin and caffeine in animal model (Elizete., *et al*. 2014) [5]. The current study unveils the effect of the concerned flavonoid rutin on caffeine in-duced intoxication in animal model, justified by its potential to cause general inhibition of the neuronal depressant activity in the CNS, mediated by caffeineism, thereby facilitating its use in blend with caffeine as a prophylactic agent in the treatment of various diseases like bronchial asthma.

## Acknowledgement

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