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ORIGINAL RESEARCH article

# Propranolol effect on behaviour of mice in the presence of phenytoin using elevated plus maze

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**Abstract:** Drug-drug interaction is an important issue for the development of safe pharmaceutical drugs. Propranolol is a non-selective, competitive antagonist at beta-adrenergic receptors. Propranolol is used to control hypertension, pheochromocytoma, myocardial infarction, cardiac arrhythmias, angina pectoris and hypertrophic cardiomyopathy. Also, it is used to control symptoms of sympathetic overactivity in the management of hyperthyroidism, anxiety disorders and tremor. Phenytoin is a voltage-gated sodium channel blocker; it is a major anti-convulsant drug that is very effective in controlling a wide variety of seizure disorders. In this study, the elevated plus maze test was applied using five groups of male Albino mice, where each group consisting of six mice. The first group is control and given 1.0% tween 80 with a dose of 5.0 ml/kg, the second group received propranolol 10 mg/kg, the third group received phenytoin 20 mg/kg, the fourth group received a combination of propranolol and phenytoin and the fifth group received diazepam (1.0 mg/kg) as a standard. It was found that propranolol alone produces anti-anxiety effect which is abolished when administered with phenytoin. Thus, the combined treatment of propranolol and phenytoin showed no significant difference compared to phenytoin alone or propranolol alone. It can be concluded that propranolol has an anti-anxiety-like effect phenytoin antagonizes the propranolol anxiolytic effect when administered together. Propranolol, phenytoin and the combination of both decrease the spontaneous motor activity of mice. Propranolol and phenytoin partially antagonize each other in spontaneous motor activity.

## Introduction

Adverse drug effects are one of the important complications of medication therapy which could affect millions of patients each year and may lead to life-threatening consequences and heighten healthcare outlays [1]. Drug-drug interactions are an important sub-type of adverse drug events. Although damaging of the interactions has been recognized, the incidence of adverse drug events and hospitalization rate related to drug-drug interactions is still high [2]. One of the important aspects of drug safety is attention to drug-drug interactions because these interactions could decrease or increase the effectiveness of drug therapy and might lead to serious side effects [3]. Identifying drug-drug interaction is an important topic for the development of safe pharmaceutical drugs and for the optimization of multidrug regimens for complex diseases such as cancer and HIV [4]. Drug interaction is defined as a measurable modification in magnitude or duration of the action of one drug by prior or concomitant administration of another substance [5].

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Propranolol is a nonselective competitive antagonist at beta-adrenergic receptors which binds with high affinity to beta-1 and beta-2 receptor subtypes but with lower affinity at the beta-3 subtype [6]. Propranolol works by making the heart beat more slowly and reducing blood pressure; as blood pressure lowers, the heart pumps more efficiently [7]. Researchers have discovered that the physiological symptoms of stress could be alleviated with beta-blockers [8]. Beta-blockers are not specifically an anti-anxiety medication; their relief of physical symptoms may be sufficient to reduce anxiety levels in some performers. Propranolol has widely been used and for many years in the treatment of a variety of medical conditions including hypertension, tachycardia, tremors and migraines [9]. Propranolol crosses the membrane into the brain so that it is possible to experience effects relating to its action on the brain itself. These include light-headedness, mental depression manifested by insomnia, lassitude, weakness, fatigue, catatonia, visual disturbances, hallucinations and vivid dreams [10]. Phenytoin is a voltage-gated sodium channel blocker [11] which is an antiarrhythmic class I [12], local anaesthesia [13]. Sodium channel blockers are local anaesthetic agents with powerful analgesics when delivered at low concentrations [14]. Phenytoin is a major anticonvulsant drug that is very effective in controlling a wide variety of seizure disorders [15]. It is used in the treatment of epilepsy other than the petit mal type [16]. Phenytoin is widely prescribed as an anticonvulsant and anti-arrhythmic drug in the treatment of grand mal and psychomotor epilepsy [17, 18]. Phenytoin has been investigated to treat thought, mood and behaviour, cardiovascular disorders, neuromuscular disorders, gastrointestinal disorders and endocrine disorders [19]. Phenytoin showed an improvement in behaviour, well-being, cooperation alertness and general attitude. It is also found beneficial for irritability, hyperactive motility and variability on behaviour in epileptic children and showed a marked reduction in psychotic symptoms [19]. Thus, this research aimed to find out the effect of propranolol on behaviour in the presence of phenytoin using plus maze model of anxiety and motor behaviour in mice.

## Materials and methods

*Animals:* Male Albino mice (25-35 g) were used. The animals were housed in standard cages with free access to food and water. Mice were maintained under controlled standard environmental conditions (12 hrs. dark/light cycles with a constant room temperature of 24±2$^{o}$C). All manipulations were carried out between 8: 00 am and 16: 00 pm [20]. Ethical approval was obtained from the ethics committee (University of Tripoli, 2018) and the handling of animals and experiments were carried out according to International Guidelines for animal use.

*Design of the study:* Six groups of mice were used; each group consisted of six mice. Group 1, was administered 01.0% Tween 80 [21] at a dose of 05.0 ml/kg [22], group 2, received propranolol at a dose of 10 mg/kg [23], group 3, received phenytoin at 20 mg/kg [24], group 4, received a combination of propranolol and phenytoin and group 5 received diazepam at 01.0 mg/kg [25]. Diazepam was used as a standard reference for the plus maze test of exploratory behaviour. Subacute i.p. administration is applied in this study with 24, 5.0, and 1.0 hrs. before scoring.

*Elevated plus-maze:* The elevated plus-maze model was composed of two opposite open arms (30x5 cm) and two opposite closed arms (30x5x15cm) that extended from the common central platform (5x5 cm). The apparatus was elevated to a height of 45 cm above the floor level [26]. The test was conducted in a closed room with a low level of illumination. Each mouse was gently held by the right hand and placed on the centre square of the maze facing the closed arm [27]. Different parameters were scored to evaluate the anxiolytic–like effect and spontaneous motor activity of the mouse in the elevated plus-maze, which include time spent on each of the arms, lines crossed on closed and open arms, and the number of entries into closed or open arms. An arm entry was defined as the entry of all four paws into the arm [28]. The total lines crossed and a total number of entries were calculated. The total lines crossed and the total arm entries express spontaneous

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motor activity [21]. The anxiety measure was calculated by the time spent on the close arm by the total time of the test [29, 30]. The duration of the test was four minutes. The experiment was carried out on 14/10/2018 and 8/11/2018.

*Statistical analysis:* Descriptive analysis was performed using the computer program SPSS (version 20) to verify whether the data were parametric using the Kolmo grove-Simirnove test maximum deviation test for goodness of fit. If the parameters were parametric, treatments were compared by one-way analysis of variance (ANOVA), post-hoc test (LSD test) was applied. If the parameters were non-parametric, treatments were compared by the Mann-Whitney *U* test. The differences are considered significant at $P \leq 0.05$. All values are expressed as mean±standard error.

## Results

In **Table 1**, the anxiety measure was decreased significantly by propranolol (10.0 mg/kg) and diazepam (01.0 mg/kg) treated groups compared to the control group at p=0.004 and 0.025, respectively. While phenytoin with a dose of 20 mg/kg did not show any change in the anxiety measure compared to the control group (p= 0.628). The combined treatment of propranolol with phenytoin did not produce any significant change in anxiety measure compared to the control group at p=0.224. Administration of phenytoin with propranolol together did not change the anxiety measure compared to phenytoin administration alone or propranolol alone (p=0.629 and 0.077, respectively). Thus, the anti-anxiety effect of propranolol was abolished by co-administration with phenytoin (**Figure 1**).

**Table 1:** Effect of phenytoin on anti-anxiety action of propranolol in the plus maze

| Treatment groups | Anxiety measure | P Compared to control | P Compared to phenytoin | P compared to propranolol |
|---|---|---|---|---|
| Control (1.0% T80) | 0.971±0.0115 | | | |
| Diazepam (1.0 mg/kg) | 0.890±0.0273 | 0.025 | | |
| Phenytoin (20 mg/kg) | 0.922±0.0496 | 0.628 | | |
| Propranolol (10 mg/kg) | 0.754±0.0844 | 0.004 | | |
| Propranolol + Phenytoin | 0.928±0.0306 | 0.224 | 0.629 | 0.077 |

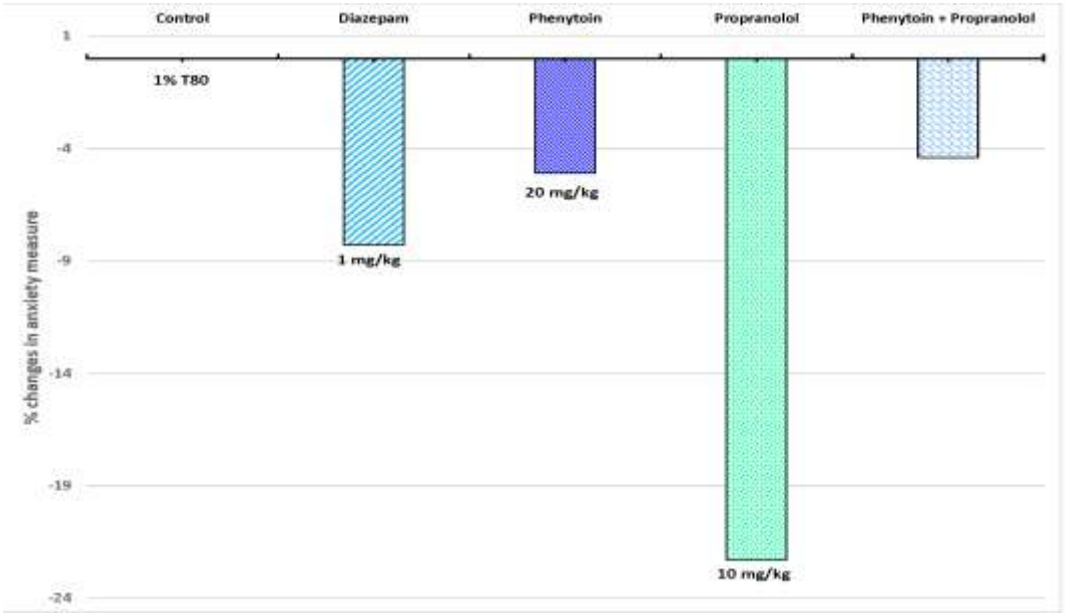


**Figure 1:** Percentage changes in the anxiety measure

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In **Table 2**, the total lines crossed were significantly decreased after diazepam, phenytoin, propranolol or their combined administration compared to the control treated group at p=0.002, 0.001, 0.002, and 0.001, respectively. Although the combined treatment showed a decrease in the total lines crossed statistically was insignificant compared to the propranolol-treated group (p=0.418) and compared to the phenytoin (p= 0.211) treated group. There was no significant change in the total number of arm entries after different treatments of the experiment (**Table 3**).

In **Figure 2**, administration of phenytoin and propranolol each alone produced a decrease in the locomotor activity of the mouse, the combined treatment produced a decrease in locomotor activity less than the additive effect. Administration of phenytoin with propranolol partially antagonizes each other with a different mechanism they produced.

**Table 2:** Effect of phenytoin on spontaneous motor activity of propranolol in a plus maze test

| Treatment groups | Total lines crossed | P Compared to control | P Compared to phenytoin | P Compared to propranolol |
|---|---|---|---|---|
| Control (01.0% T80) | 29.50±8.609 | | | |
| Diazepam (01.0 mg/kg) | 09.33±2.445 | 0.002 | | |
| Phenytoin (20 mg/kg) | 02.83±0.654 | 0.000 | | |
| Propranolol (10 mg/kg) | 08.33±2.076 | 0.002 | | |
| Propranolol + Phenytoin | 06.00±2.098 | 0.001 | 0.211 | 0.418 |

**Table 3:** Effect of phenytoin and propranolol on the total number of entries in plus maze test

| Treatment group | Total number of entries | P Compared to control | P Compared to phenytoin | P compared to propranolol |
|---|---|---|---|---|
| Control (01.0% T80) | 4.00±1.317 | | | |
| Diazepam (01.0 mg/kg) | 9.33±2.445 | 0.05 | | |
| Phenytoin (20 mg/kg) | 2.83±0.654 | 0.657 | | |
| Propranolol (10 mg/kg) | 8.33±2.076 | 0.108 | | |
| Propranolol + Phenytoin | 6.00±2.098 | 0.448 | 0.234 | 0.377 |

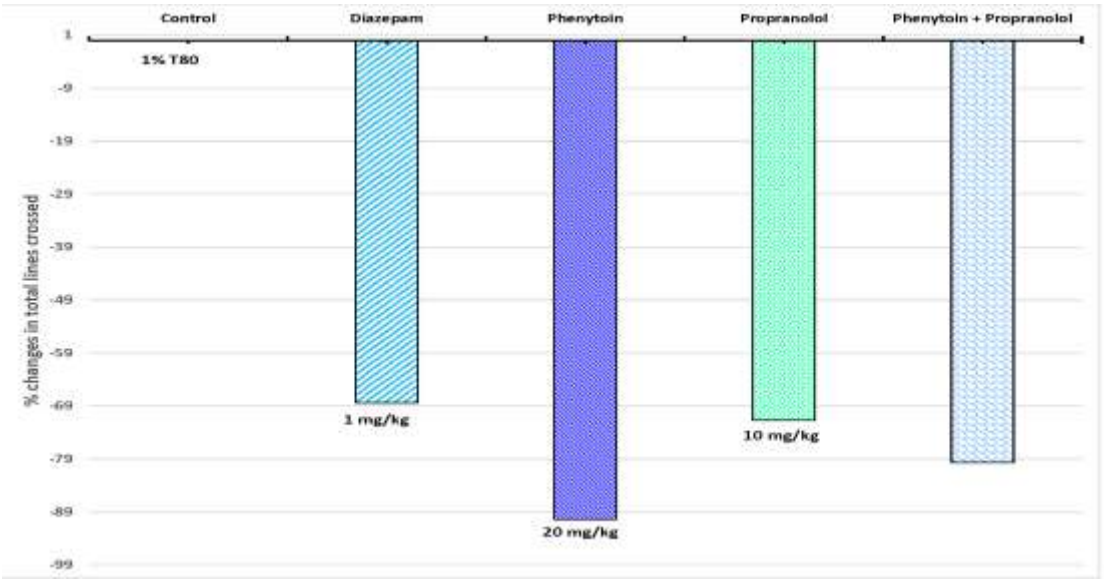


**Figure 2:** Percentage changes in the total lines crossed

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## Discussion

In this study, propranolol alone produces an anxiolytic-like effect which is abolished when administered with phenytoin. The prevalence of anxiety mental condition has risen in recent years [31]. Benzodiazepines such as diazepam are well-known drugs that help people feel calmer through their sedating, hypnotic, anti-anxiety and muscle-relaxant effects. These effects occur, at least in part, by enhancing the neurotransmitter, gamma-aminobutyric acid [32]. Beta-blockers, such as propranolol, can be helpful in the treatment of physical symptoms of anxiety, especially social anxiety. Physicians prescribe them to control rapid heartbeat, shaking, trembling and blushing in anxious situations for several hours [33]. Propranolol acts as a non-selective beta-blocker, thereby inhibiting the effects of epinephrine and norepinephrine [6, 34]. Propranolol is also speculated to inhibit the activity of the norepinephrine transporter [35]. They are hormones responsible for modulating the "fight or flight" response; this response occurs in a stressful situation and results in an increase in heart rate and force of myocardial contraction, along with the constriction of blood vessels in many parts of the body [36]. Beta-blockers seem to mitigate strain on the heart by blocking epinephrine from binding to beta-receptors and reducing the intensity of oxidative stress [37]. Propranolol acts peripherally to block the somatic components of the anxiety state; these symptoms include tachycardia - palpitations, tremor and sweating [38]. There is evidence to suggest that propranolol may act upon serotonin (5-HT) receptor sites such as 5-$HT_{1B}$, 5-$HT_{1A}$, and 5-$HT_2$. Thus, it may be necessary to consider that its ability to modulate serotonergic transmission may contribute to its anxiolytic effect [39]. Propranolol hydroxylation is mainly catalysed by CYP2D6 in human liver microsomes whereas the activity of CYP3A4 was relatively low [40, 41]. Propranolol is also metabolized by CYP1A2 [41]. In rat liver microsomes, propranolol has been found to be a selective inhibitor of CYP2D6 isoenzyme-dependent reactions and a nonspecific inhibitor of other cytochrome P450 isoenzymes. It is assumed that the non-competitive inhibition is due to the covalent binding of reactive metabolites derived from propranolol to hepatic microsomal proteins [41, 42]. Inhibition of CYP2D by propranolol suggests caution when prescribing propranolol with other CYP2D6 substrates. Therefore, co-medication with propranolol increased drug concentrations of other CYP2D6 substrates and increased adverse events [41] or caused serious complications in diseased patients due to drug-drug interactions [43].

Phenytoin is a hydantoin derivative and non-sedative antiepileptic agent with anticonvulsant activity [44, 45]. It is often described as a non-specific sodium channel blocker and targets almost all voltage-gated sodium channel subtypes [46]. More specifically, phenytoin prevents seizures by inhibiting the positive feedback loop that results in the neuronal propagation of high-frequency action potentials [44]. The reduction of potentiation prevents cortical seizure foci from spreading to adjacent areas, stabilizing the threshold against hyperexcitability [47]. It is a potent enzyme inducer, induces the cytochrome P450 isoenzyme [48] subfamilies CYP3A4 and CYP2D6 [49, 50] which are responsible in the metabolism of propranolol, thereby, decreasing their serum concentration [51, 52]. Blood levels of propranolol may be decreased by co-administration with inducers such as rifampin, ethanol, phenytoin and phenobarbital [53]. From the above explanation, phenytoin is a potent enzyme inducer, which induces the cytochrome P450 isoenzyme, while propranolol inhibits them. This explains that phenytoin and propranolol co-administration may antagonize each other as observed in this study. It seems that phenytoin as an enzyme inducer is more powerful compared to propranolol's effect on cytochrome P450.

All treatments produced a significant decrease in spontaneous motor activity (total lines decreased) compared to the control. This effect was not shown by the total number of entries. The combined treatment of propranolol and phenytoin showed no significant difference compared to phenytoin alone or propranolol alone. It seems both partially antagonize each other. Using a plus-maze model of exploratory behaviour, scoring the total number of lines crossed was more sensitive to the changes in locomotor activity compared to scoring the total number of entries in this model. Beta-adrenoceptor antagonists are liable to produce behavioural side effects

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such as drowsiness, fatigue, lethargy, sleep disorders, nightmares, depressive moods and hallucinations. These undesirable actions indicate that beta-blockers affect not only peripheral autonomic activity but also some central nervous mechanisms. In experimental animals, beta-blockers have been found to reduce spontaneous motor activity [54]. It was proposed that the propranolol sedative effect in the brain is generally linked to the GABA neural mechanism [55]. Another finding showed that propranolol decreases exploratory behaviour or a sedation effect. This may be attributable to its central cell membrane stabilizing effect [56]. Antiepileptic drugs are CNS depressants, they stabilize cell membranes in the cerebral cortex [57]. Phenytoin's adverse effects potentially include sedation and locomotor dysfunction [47]. In addition, phenytoin has mild blocking action at the neuromuscular junction [58] and this may decrease the locomotor activity. It is found that phenytoin decreased the spontaneous motor activity in a dose-dependent fashion [59]. The decrease in locomotor activity could be translated as sickness behaviour, a coordinated set of adaptive behavioural changes triggered by activation of the peripheral innate immune system and the production of pro-inflammatory cytokines [60, 61]. Cytokines are responsible for this symptomatology observed in response to inflammation (anorexia and decreased motor activity) [62]. Phenytoin treatment causes a broad spectrum of side effects. It is reported that TNF-α induces IL-1β production [63] and prostaglandin $E_2$ formation in gingival fibroblasts that were up-regulated by phenytoin [64]. It is mentioned that phenytoin is reported to elevate the levels of IL-1*β*, IL-2, IL-5, IL-6 and TNF-*α* [65, 66]. Propranolol has anti-inflammatory properties due to its suppressive effect on inflammatory cytokines (IL-13 and TNF-α). Thus, propranolol and its long-term usage may be useful in treating inflammatory-based diseases [67].

*Conclusion:* In mice, propranolol has an anti-anxiety effect and phenytoin antagonizes the propranolol anxiolytic effect when administered together. Propranolol, phenytoin and their combination decrease the spontaneous motor activity. Thus, propranolol and phenytoin partially antagonize each other in the spontaneous motor activity. However, careful consideration of treatment with cytochrome P450 substrate may be required in patients taking propranolol.

**Author contributions:** SMA conceived and designed the study. MTE, OIA & SFA have collected and contributed to data analysis. SMA & MTE contributed to drafting and revising the manuscript. All authors contributed significantly. All authors have approved the final version of the manuscript and agreed to be accountable for its contents.
**Conflict of interest:** The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
**Ethical issues:** Including plagiarism, informed consent, data fabrication or falsification and double publication or submission were completely observed by the authors.
**Data availability statement:** The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
**Author declarations:** The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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