

# Effect Of 30-Day Administration Of Cellgevity® Supplement On Selected Rat Liver Cytochrome P450 Enzyme Activity And Supplement Interaction With Carbamazepine

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## Research article

**Keywords:** Carbamazepine, Cellgevity®, Cytochrome P450, Interaction, Pharmacokinetics, Supplement

**Posted Date:** December 31st, 2019

**DOI:** <https://doi.org/10.21203/rs.2.19774/v1>

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# Abstract

## Background

There is considerable evidence that many patients concurrently take dietary supplements with conventional drugs, with a risk of potential drug-supplement interaction. The aim of this study was to determine the effect of Cellgevity® supplement on selected rat liver cytochrome P450 (CYP) enzymes and on the pharmacokinetics of carbamazepine.

## Methods

Sprague-Dawley (SD) rats were put into 5 groups and modulation of CYP enzyme activity by Cellgevity® was determined by comparing the enzyme activity of Cellgevity-treated groups with the negative control group after 30 days of treatment. For the effect of Cellgevity® on the pharmacokinetics of carbamazepine, 12 SD rats were put into 2 groups; one group received an oral administration of carbamazepine plus Cellgevity®, and the other carbamazepine plus normal saline. Blood samples were collected at specific time points and analyzed for levels of carbamazepine.

## Results

Activities of CYP1A1/2, CYP2C9 and CYP2D6 were significantly increased by Cellgevity®. The pharmacokinetic parameters for rats administered carbamazepine with Cellgevity® vis-a-vis carbamazepine with normal saline were changed as follows:  $C_{max}$ ; 20  $\mu\text{mol/L}$  vs 11  $\mu\text{mol/L}$ ,  $AUC_{0\rightarrow 24}$ ; 347  $\mu\text{mol.h/L}$  vs 170  $\mu\text{mol.h/L}$ ,  $K_e$ ; 0.28  $\text{h}^{-1}$  vs 0.41  $\text{h}^{-1}$ , and  $t_{1/2}$ ; 2.3 h vs 1.7 h, respectively.

## Conclusions

Cellgevity® increased the activity of rat CYP1A1/2, CYP2C9 and CYP2D6, and also altered the pharmacokinetics of carbamazepine in rats.

## Background

Dietary supplements may be vitamins, minerals or herbal products that improve the well-being of individuals (1). This clearly denotes the use of these supplements as an addition to the dietary requirement that may not be met by daily meals. However, under no circumstance should dietary supplements be used as a replacement for daily meals. The United States Food and Drugs Administration also prohibits the indication of dietary supplements as treatment for diseases or any indication that connotes primary drug therapy (1).

With the increase in the incidence of non-communicable diseases (NCDs) such as diabetes, cardiomyopathies, cancers and epilepsy, which are often associated with oxidative stress, people usually resort to the use of dietary supplements (with antioxidant potential) to prevent these diseases (2). Interestingly, some individuals who use dietary supplements have the notion that these agents may

enhance the effects of conventional drugs (3), possibly because some synergy between dietary supplements and conventional drugs have been reported (4). However, this notion may not always be true.

Available on the market currently are a number of dietary supplements known to replenish levels of reduced glutathione, a free radical scavenger in the cell. One such supplement, Cellgevity®, contains a glutathione precursor molecule, riboceine (D-ribose-L-cysteine). Riboceine is known to deliver cysteine into cells and enhance reduced glutathione level in the body (5, 6). The other constituents of Cellgevity® are broccoli seed extract, turmeric root extract, resveratrol, grape seed extract, quercetin, curcumin, milk thistle, vitamin C, selenomethionine, cordyceps, black pepper and aloe extract. Some of these constituents are known as inducers and/or inhibitors of CYP enzymes (7, 8, 16). Max International, the marketer and distributor of Cellgevity®, has branches in 14 countries (United States, Nigeria, Cote d'Ivoire, New Zealand, Singapore, Costa Rica, Columbia, Philippines, El Salvador, Malaysia, Guatemala, Ghana and Hong Kong). Cellgevity® has gained popularity in these countries probably due to the knowledge that the supplement has a high antioxidant potential (9).

Reports suggest that there could be clinically important modulation of cytochrome P450 (CYP) enzymes by supplements and/or herbal products. This could result in adverse or sub-therapeutic effects of concurrently administered conventional drugs. For example, St. John's wort was reported to decrease the serum concentration of theophylline (a bronchodilator) as a result of CYP450 enzyme induction (10). This interaction between St John's wort and theophylline could lead to sub-therapeutic effect of normal doses of theophylline when there is co-administration. For this reason, patients are often advised not to take theophylline concomitantly with St. John's wort. In a previous study, we reported that Cellgevity® at 4 and 8 mg/kg significantly inhibited rat liver CYP2C9, CYP2B1/2B2 and CYP3A4 over a 7-day treatment period (11). Although there is evidence that food supplements could be effective at low dose in preventing some NCDs (COSMOS; NCT02422745), food supplements should also be tested at animal equivalent of the human dose (that is to mimic the human dose) when investigating their effectiveness in animal models. Therefore, since xenobiotics are known to modulate CYP enzymes depending on factors such as dose and treatment duration (12), the current study is follow-up to one we previously reported (11), but with Cellgevity® doses calculated after scaling from humans, and Cellgevity® administered over a longer period of time (30 days).

With reports suggesting concurrent administration of Cellgevity®, known to inhibit rat liver CYP3A4 (11), and carbamazepine also known to be extensively metabolized by CYP3A4, there is the potential for interaction between these two agents. Carbamazepine is one of the most commonly prescribed drugs in the management of epilepsy. Due to the chronic nature of this disease, and the fact that patients have to take carbamazepine for a long time (lifetime in most cases), there is the potential for clinically significant interactions to occur between carbamazepine and co-administered agents like dietary supplements, herbal products and food (13). Therefore, the current study also sought to determine the effect of Cellgevity® on the pharmacokinetics of carbamazepine.

# Methods

## Animals care and safety

This research was approved by the College of Health Sciences Ethical and Protocol Review Committee (Protocol ID: CHS-Et/M.9 – P1.16/2017-2018) of the University of Ghana. All animal procedures and techniques used in this study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Male Sprague-Dawley (SD) rats, weighing 150-200 g and 6-8 weeks old, were obtained from Center for Plant Medicine Research, Mampong, Eastern Region, Ghana. The animals were housed in stainless steel cages. Each rat occupied a minimum space of 2 cubic feet (61 cm x 31 cm x 31 cm) with softwood shavings as bedding for their comfort. They were fed with normal pellet diet (AGRIMAT, Kumasi, Ghana), given water *ad libitum*, and maintained under standard laboratory conditions (temperature ~25°C, relative humidity 60-70%, and 12 h light-dark cycle). The animals' feeding and water troughs were cleaned regularly to prevent contamination. Animals were acclimatized under the above conditions for 7 days before the experiment was commenced.

## Hepatic enzyme induction/inhibition studies

### Animal grouping and treatment administration

In determining the influence of Cellgevity® on CYP enzymes, male SD rats were put into five groups (6 rats in each group). Group 1 was administered distilled water, the vehicle used in dissolving Cellgevity® purchased from Max International (Ghana), and that served as the negative control (N-C) group. Groups 2, 3 and 4 received daily a low dose (L-D) of 38.63 mg/kg, a medium dose (M-D) of 77.25 mg/kg and a high dose (H-D) of 154.50 mg/kg of Cellgevity®, respectively. The doses of Cellgevity® administered to SD rats were animal equivalent of the human dose, calculated as described by Nair AB and Jacob SA (14), the human dose being 12.46 mg/kg *per os*. The SD rats in Group 5 received an oral dose of phenobarbital (Kinapharma, Ghana) 15 mg/kg daily, and that served as positive control (P-C). All administrations were for 30 days. After the 30-day period, animals were then sacrificed by cervical dislocation. Livers were excised and washed in ice-cold saline solution and weighed. Livers were then stored at -80°C until use.

### ***Microsomal Preparation***

Livers were thawed and homogenized in potassium phosphate buffer (pH 7.4) using a mortar and pestle on ice. Homogenized samples were first centrifuged at 4,000 rpm for 20 min. The supernatant was taken-up and re-centrifuged (Beckman Avanti J-25, USA) at 25,000 rpm for 2 h. The pellets, which constituted the microsomes were collected and stored at -80°C until use.

### CYP2C9 (Diclofenac Hydroxylation) and CYP2D6 (Dextromethorphan O-demethylation) Assays

The assay was performed as previously described (15), with some modification. A volume of 350  $\mu\text{L}$  of 0.1M potassium phosphate buffer (pH 7.4), 50  $\mu\text{L}$  of 1 mM substrate (diclofenac for CYP2C9 assay and dextromethorphan for CYP2D6 – both substrates purchased from Sigma-Aldrich, USA) and 50  $\mu\text{L}$  of 2.5 mg/mL microsome (obtained from rat livers from respective groups) were mixed separately in Eppendorf tubes. The mixtures were pre-incubated at 37°C for 5 min. A volume of 50  $\mu\text{L}$  of 1 mM nicotinamide adenine dinucleotide phosphate (NADPH) [Sigma-Aldrich, USA] was added, mixed and incubated at 37°C for 45 min. A 100  $\mu\text{L}$  stopping solution ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) was added and the mixture centrifuged at 4000 rpm for 5 min. The supernatants were aliquoted into High-Performance Liquid Chromatography (HPLC) [Shimadzu, Japan] vials.

Samples were analyzed using HPLC. The chromatographic system consisted of a binary solvent delivery system (LC - 20AB), a degasser (DGU-20A3), an auto-sampler (SIL - 20AHT), a column temperature controller (CTO - 10AS VP) and a photodiode array detector (SPD - M20A) for CYP2C9 metabolites and fluorescence detector (RF - 10A<sub>XL</sub>) for CYP2D6 metabolites. The following chromatographic conditions were used for the analysis of CYP2C9; column, C18 (Shimadzu, Japan), diameter 5  $\mu\text{m}$ , length x width 150 mm  $\times$  4.6 mm; flow rate, 1 mL/min; column temperature, 40°C; injection volume, 20  $\mu\text{L}$ ; mobile phase, 20 mM potassium phosphate buffer (pH 7.4)/methanol/acetonitrile (60:22.5:17.5, v/v/v). The same chromatographic conditions were used for the analysis of the CYP2D6, with modification to the mobile phase (acetonitrile/distilled water/triethylamine; 24:75:1, v/v/v).

*CYP1A1/1A2 - Ethoxyresorufin O-deethylase (EROD), CYP2B1/2B2 - Pentoxyresorufin O-depenty/lase (PROD) and CYP3A4 - Benzyloxyresorufin O-debenzylase (BROD) Assays*

The assays were performed as previously described (16, 17), with some modification. In brief, microsomes (CYP enzymes) were tested in a total volume of 100  $\mu\text{L}$ . Aliquots of 70  $\mu\text{L}$  potassium phosphate buffer (pH 7.4) were placed into a 96-well black plate. This was followed by addition of 10  $\mu\text{L}$  of 50  $\mu\text{M}$  substrate concentration (resorufin ethyl ether for CYP1A1/2, pentoresorufin for CYP2B1/2 and resorufin benzyl ether for CYP3A4; all substrates purchased from Sigma-Aldrich, USA). The final substrate concentration in 100  $\mu\text{L}$  total reaction volume was 5  $\mu\text{M}$  with 0.25% (v/v) dimethyl sulfoxide (DMSO). It is noteworthy that CYP activities were not expected to be affected at the DMSO concentration used in this experiment (18). Aliquots of 10  $\mu\text{L}$  enzyme (microsome from each rat liver from respective Groups) corresponding to 1 mg/mL protein concentration and the vehicle was added in triplicates. The mixtures were pre-incubated at 37°C for 5 min. A volume of 10  $\mu\text{L}$  of NADPH was then added to each well and the setup was incubated for 10 min for CYP1A1/2, 20 min for CYP2B1/2 and 30 min for CYP3A4 assays, respectively. Aliquots of 40  $\mu\text{L}$  of stopping solution (20% 0.5 M Tris: 80% acetonitrile) were added to each well and shaken gently. Fluorescence of wells was read at wavelengths of 530 nm excitation and 586 nm emission. Triplicate experiments were performed. The average absorbance of the blank was subtracted from the average absorbance of each sample.

**Effect of Cellgevity® on the pharmacokinetics of carbamazepine**

### Animal grouping and treatment administration

Twelve male SD rats were obtained for this aspect of the study. The animals were put into 2 groups (Group 1 and Group 2) of 6. Group 1 was administered carbamazepine plus saline and Group 2, Cellgevity® plus carbamazepine. A dose of 77.25 mg/kg/day of Cellgevity® plus 80 mg/kg of carbamazepine, both scaled from humans (14), were administered orally to rats in Group 2. Rats in Group 1 received 80 mg/kg/day of carbamazepine plus normal saline (the same volume was calculated per rat for the Cellgevity® dose).

### Blood sample collection

After administration of agents, every 24 h for 14 consecutive days to Groups 1 and 2, tail vein blood samples were taken following the dose administered on the 14<sup>th</sup> day. Samples were drawn after 0.5, 1, 4, 12 and 24 h. Blood was collected into microtainer gel tubes and centrifuged at 2000 rpm for 5 min to separate serum, and this was stored at -20°C until analysis was done.

### Assay for carbamazepine in serum

Due to low sample volumes, serum samples of rats from the same group (6 animals) at each time point were pooled together, such that, for instance, serum samples of Group 1 rats at time 4 h, were pooled together to obtain a single sample. Usually, challenges with low sample volume can be circumvented by the approach of sample-pooling (19). Analysis of carbamazepine in serum was done by Fluorescence Polarization Immunoassay (FPIA) [Cobas Integra® 400 Plus, Roche, Philippines].

### Statistical analysis

CYP activity of treatment groups was expressed as a percentage relative to the negative control group. All values were expressed as mean  $\pm$  standard deviation. Differences between groups were tested for significance using a One-Way Analysis of Variance (ANOVA). This was followed by post-hoc analysis using Bonferroni's Multiple Comparison Tests.  $P$ -values  $< 0.05$  were considered statistically significant.

Non-compartmental pharmacokinetic analysis was used to determine the various pharmacokinetic parameters of carbamazepine. The maximum serum drug concentration ( $C_{max}$ ) and its corresponding time ( $T_{max}$ ) were determined by visual inspection of the concentration-time curve. The linear trapezoidal rule was applied in extrapolating area under the concentration-time curves (AUCs) for the two groups. The elimination rate constant ( $K_e$ ) was determined from the slope of the concentration-time curve, and this was then used to calculate the elimination half-life ( $t_{1/2}$ ).

## **Results**

### **Hepatic enzyme induction/inhibition studies**

#### CYP2C9 activity after 30-day treatment

CYP2C9 enzyme activity in the treatment groups was estimated relative to the negative control (N-C) group. CYP2C9 enzyme activity was found to be elevated in the phenobarbital- and Cellgevity®-treated groups in comparison with the negative control group. The phenobarbital- and Cellgevity®-treated groups were found to differ significantly from the negative control. This increase in rat CYP2C9 enzyme activity by Cellgevity® was found to be dose-dependent. A representation of the effect of Cellgevity® on rat CYP2C9 enzyme is shown in Figure 1.

#### CYP2D6 activity after 30-day treatment

CYP2D6 enzyme activity in the treatment groups was estimated relative to the N-C group. CYP2D6 enzyme activity was found to be elevated 2.5-fold in the phenobarbital-treated and 2-fold in the Cellgevity®-treated groups in comparison with the negative control (N-C). The positive control (P-C) and H-D Cellgevity®-treated groups differed statistically ( $p < 0.01$ ) from N-C group. The L-D and M-D groups also differed statistically ( $p < 0.05$ ) in comparison to the N-C group. There was no significant difference between the mean CYP2D6 activities of the L-D, M-D and H-D Cellgevity®-treated rats. CYP2D6 enzyme activity after the 30-day treatment is shown in Figure 2.

#### CYP1A1/2 activity after 30-day treatment

CYP1A1/2 enzyme activity in the treatment groups was estimated relative to the N-C group. CYP1A1/2 enzyme activity was found to be elevated in the phenobarbital- and Cellgevity®-treated groups in comparison with the N-C group. The Cellgevity®-treated L-D and M-D groups showed elevated CYP activity compared to N-C group, but these differences were not statistically significant. However, a significant difference was found between the H-D group and the N-C group. There was somewhat a dose-dependent increase in the effect of Cellgevity® on rat CYP1A1/2 enzyme activity. Levels of CYP1A1/2 enzyme activity after 30-day treatment are shown in Figure 3.

#### CYP2B1/2 activity after 30-day treatment

CYP2B1/2 enzyme activity in the treatment groups was estimated relative to the N-C group. CYP2B1/2 enzyme activity was found to be elevated in the phenobarbital- and Cellgevity®-treated groups in comparison with the N-C group. The Cellgevity®-treated groups showed elevated levels compared to N-C group, but the differences were not statistically significant. There was also no dose-dependent effect of Cellgevity® on rat CYP2B1/2 enzyme activity. Levels of CYP2B1/2 enzyme activity after 30-day treatment are shown in Figure 4.

#### CYP3A4 activity after 30-day treatment

CYP3A4 enzyme activity in the treatment groups was estimated relative to the N-C group. CYP3A4 enzyme activity was found to be elevated in the phenobarbital- and Cellgevity®-treated groups in comparison with the N-C group. The Cellgevity®-treated groups showed elevated levels compared to the N-C group, but the differences were not statistically significant. There was also no dose-dependent effect

of Cellgevity® on rat CYP3A4 enzyme activity. Levels of CYP3A4 enzyme activity after 30-day treatment are shown in Figure 5.

### Overall Effect of Cellgevity® on Rat CYP Enzyme Activity

When Cellgevity®-treated groups were compared to the N-C group, the activities of CYP3A4 and CYP2B1/2 did not differ significantly when compared to the N-C group. However, CYP1A1/2, CYP2C9 and CYP2D6 activity in rats treated with Cellgevity® were found to be significantly increased compared to the N-C group. Additionally, the increase in CYP2C9 activity was found to be dose-dependent. The overall effect of Cellgevity® on selected CYP enzymes is shown in Table 1.

### **Effect of Cellgevity® on the pharmacokinetics of carbamazepine**

The concentration-time curves of carbamazepine in rats administered carbamazepine with Cellgevity® and carbamazepine with normal saline is shown in Figure 6. From the concentration-time curve, rats administered carbamazepine with Cellgevity® had a higher peak at 4 h compared to the rats administered carbamazepine with normal saline.

The peak concentration for rats administered carbamazepine with Cellgevity® was 2-fold greater compared to carbamazepine with saline. Total drug exposure at the last sample time point ( $AUC_{0\rightarrow 24}$ ) was also about 2-fold greater in rats administered carbamazepine with Cellgevity® compared to carbamazepine with saline. Pharmacokinetic parameters obtained from the concentration-time curves for the two groups are shown in Table 2.

## **Discussion**

This study was a follow-up on an earlier one that sought to investigate the potential of Cellgevity® to modulate CYP enzymes in rats. In the earlier study, low doses of Cellgevity® (4 mg/kg and 8 mg/kg) were used over a period of 7 days (11). In that study, Cellgevity® was found to inhibit rat liver CYP3A4, CYP2C9 and CYP1A2 after the 7-day treatment period (11). In the current study, animal equivalent doses of Cellgevity® per serving in humans (12.46 mg/kg) were used in SD rats. Since xenobiotics are known to modulate CYP enzymes depending on factors such as dose and treatment duration (12), this study, therefore, sought to investigate the effect of Cellgevity® on rat liver CYP enzymes using 3 doses of Cellgevity® calculated after scaling from humans, and administering Cellgevity® over a period of 30 days.

In the present study (after the 30-day treatment period) Cellgevity® was found to have increased the activity of CYP1A1/2, CYP2C9 and CYP2D6 significantly. These results are contrary to what was reported by N'guessan et al., (11), where Cellgevity® significantly inhibited rat CYP2B1/2B2, CYP3A4, and CYP2C9 after a 7-day treatment period. Horn, Reichert (12) showed that CYP activity is both dose and treatment duration dependent. On the other hand, Pichard-Garcia, Hyland (20) reported that higher concentrations of eletriptan induced CYP3A in the culture medium. However, lower doses of eletriptan did not cause CYP3A

induction. Organisms after exposure to xenobiotics or foreign chemicals often develop adaptive mechanisms where they increase metabolism in an attempt to get rid of the insulting agent.

Indeed, it may not be entirely prudent to extrapolate animal studies to humans, but these data give credence to the fact that dietary supplements could modulate CYP enzymes in humans. If this increase in enzyme activity observed for CYP1A1/2, CYP2C9 and CYP2D6 (dose-dependent in the case of CYP2C9) are clinically relevant, then emphasis should be made on maximum therapeutic daily doses of Cellgevity® in humans.

There are reports of potential interaction between dietary supplements/herbal products and conventional drugs. The commonest of these interactions appear to occur at the level of drug metabolism; especially with liver microsomal enzymes. The current study, therefore, also sought to determine the effect of Cellgevity® on the pharmacokinetics of carbamazepine in SD rats. The total carbamazepine exposure (AUC) for rats administered carbamazepine with normal saline was 347  $\mu\text{mol}\cdot\text{h}/\text{L}$ , as against 170  $\mu\text{mol}\cdot\text{h}/\text{L}$  for rats administered carbamazepine with Cellgevity®. With elimination rates being 0.28  $\text{h}^{-1}$  for carbamazepine with Cellgevity® and 0.41  $\text{h}^{-1}$  for carbamazepine with normal saline, this meant that there was a slower clearance of carbamazepine in rats administered carbamazepine with Cellgevity®. This ultimately led to a longer half-life (2.3 h) among rats administered carbamazepine with Cellgevity®. It can, therefore, be inferred from the current study that Cellgevity® had some level of interaction with carbamazepine, possibly through inhibition of CYP3A, the enzyme that is known to metabolize carbamazepine.

Anecdotal reports that suggested that epileptic patients were taking diosmin (a widely used flavonoid in the treatment of varicose veins and haemorrhoids) along with carbamazepine, raised concerns that led to a study to ascertain possible interaction between these two agents in an animal model (21). In that study, diosmin significantly enhanced  $C_{\text{max}}$ , AUC, and  $t_{1/2}$  of carbamazepine as compared to control rats. Diosmin also significantly decreased  $k_e$  and apparent oral clearance (CL/F) of carbamazepine as compared to control rats. This, therefore, corroborates findings from the current study, that there is a potential for herbal medicines, dietary supplements, and food to interact with conventional drugs *in vivo* (22), and that studies of this nature ought to be conducted to ascertain such interactions.

## Conclusion

In the current study, Cellgevity® was found to cause an appreciable increase in the activities of rat liver CYP1A1/2, CYP2C9 and CYP2D6 after a 30-day treatment period. Additionally, Cellgevity® was found to alter the pharmacokinetics of carbamazepine in Sprague-Dawley rats. Although this study was conducted in an animal model, this finding is noteworthy, as this may serve as a basis for future studies in humans.

## Abbreviations

ANOVA: One-Way Analysis of Variance

AUC: Area Under the Concentration-time Curve

$C_{\max}$ : Maximum Serum Drug Concentration

CYP: Cytochrome P450 Enzymes

DMSO: Dimethyl Sulfoxide

FPIA: Fluorescence Polarization Immunoassay

H-D: High Dose

HED: Human Equivalent Dose

HPLC: High-Performance Liquid Chromatography

$K_e$ : Elimination Rate Constant

L-D: Low Dose

M-D: Medium Dose

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

N-C: Negative Control

NCD: Non-Communicable Disease

P-C: Positive Control

rpm: Revolutions Per Minute

SD: Sprague-Dawley

$t_{1/2}$ : Elimination Half-life

$T_{\max}$ : Time to Reach Maximum Serum Drug Concentration

## **Declarations**

### **Acknowledgement**

We would like to acknowledge Jessica Twumasi-Ankrah and all laboratory technicians at the School of Pharmacy and Noguchi Memorial Institute for Medical Research, University of Ghana, for their help and technical support.

### **Funding**

This project was made possible with financial support from Building a New Generation of Academics in Africa (BANGA-Africa) Project (Grant Number: UG-BA/PD-006/2017-2018), University of Ghana, which is funded by the Carnegie Corporation of New York. The funding body, BANGA-Africa Project, played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

### **Authors' contributions**

BBN and SKA conceived the study and designed the methodology. MA, SYA, AA, EAD performed the experimental work under the supervision of SKA and BBN. SKA, BBN, KFMO, IJAG, RAO, MA, SYA, AA and EAD conducted the analysis of data. SKA, BBN, KFMO, IJAG and RAO were involved in the writing of the manuscript. All authors read and approved the final manuscript.

### **Availability of data and materials**

The data used to support the findings of this study are available from the corresponding author upon request.

### **Competing interests**

The authors declare that they have no competing interests.

### **Consent for publication**

Not applicable.

### **Ethics approval and consent to participate**

This research was approved by the College of Health Sciences Ethical and Protocol Review Committee (Protocol ID: CHS-Et/M.9 – P1.16/2017-2018) of the University of Ghana.

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## Tables

**Table 1:** Summary of the effect of Cellgevity® on selected rat CYP enzyme activity

CYP ISOFORM	ASSAY	EFFECT OF CELLGEVITY® ON CYP ACTIVITY
CYP3A4	BROD	No significant increase in enzyme activity
CYP2B1/2	PROD	No significant increase in enzyme activity
CYP1A1/2	MROD	Significant increase in enzyme activity (H-D: p < 0.001)
CYP2C9	Diclofenac Hydroxylation	Significant increase in enzyme activity (L-D: p < 0.05; M-D and H-D: p < 0.001)
CYP2D6	Dextromethorphan Demethylation	O- Significant increase in enzyme activity (L-D and M-D: p < 0.05; H-D: p < 0.01)

*L-D = Low dose (38.63 mg/kg) of Cellgevity®; M-D = Medium dose (77.25 mg/kg) of Cellgevity®; H-D = High dose (154.50 mg/kg) of Cellgevity®*

**Table 2:** Pharmacokinetic parameters of carbamazepine for the two groups of rats

PARAMETERS (UNIT)	CARBAMAZEPINE + CELLGEVITY®	CARBAMAZEPINE + NORMAL SALINE
$C_{max}$ ( $\mu\text{mol/L}$ )	20	11
$T_{max}$ (h)	4	4
$k_e$ ( $\text{h}^{-1}$ )	0.28	0.41
$t_{1/2}$ (h)	2.3	1.7
$AUC_{0 \rightarrow 24}$ ( $\mu\text{mol.h/L}$ )	347	170
$AUC_{0 \rightarrow \infty}$ ( $\mu\text{mol.h/L}$ )	375	178

## Figures

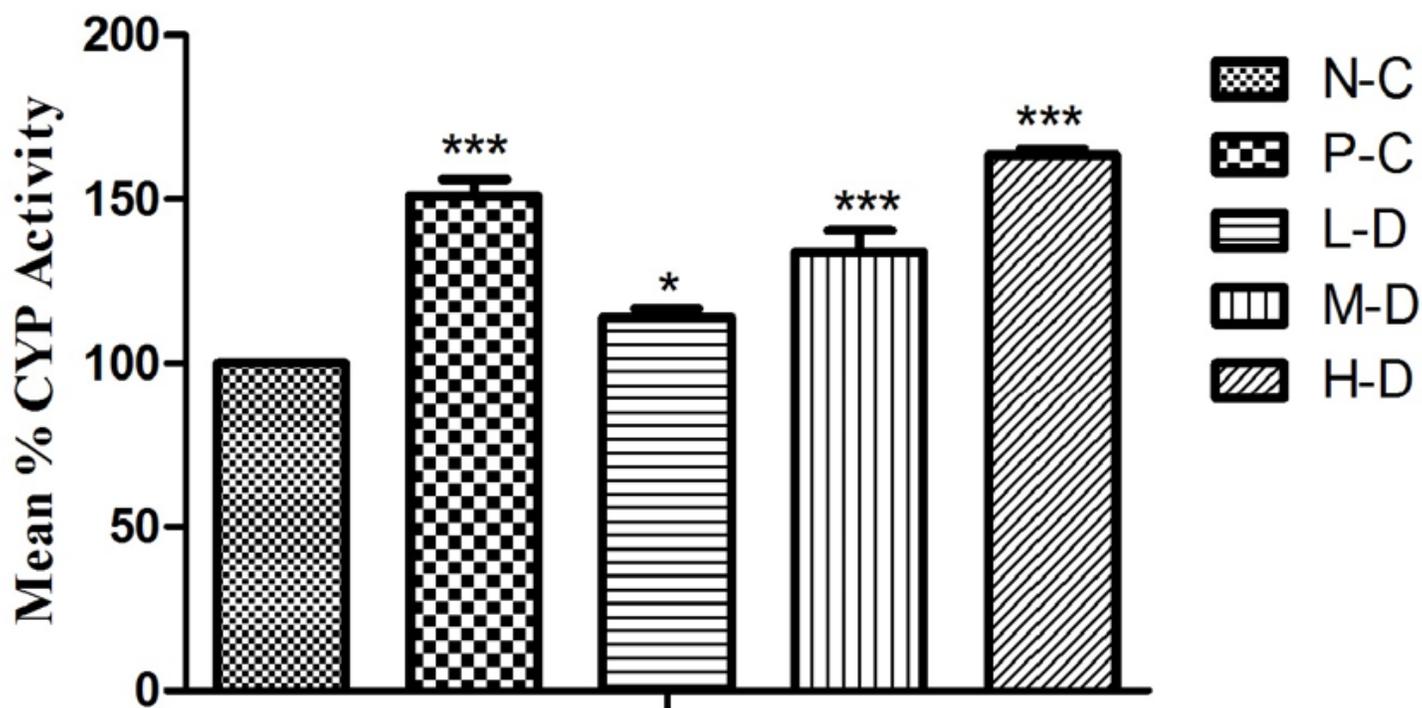


Figure 1

Rat liver CYP2C9 activity for various treatment groups after 30-day administration: N-C = Negative control; P-C = Positive control (Phenobarbital); L-D = Low dose (38.63 mg/kg) of Cellgevity®; M-D =

Medium dose (77.25 mg/kg) of Cellgevity®; H-D = High dose (154.50 mg/kg) of Cellgevity®. Data represents mean  $\pm$  standard deviations. \* and \*\*\* represent values significantly different from the negative control as indicated by  $p < 0.05$  and  $p < 0.001$ , respectively

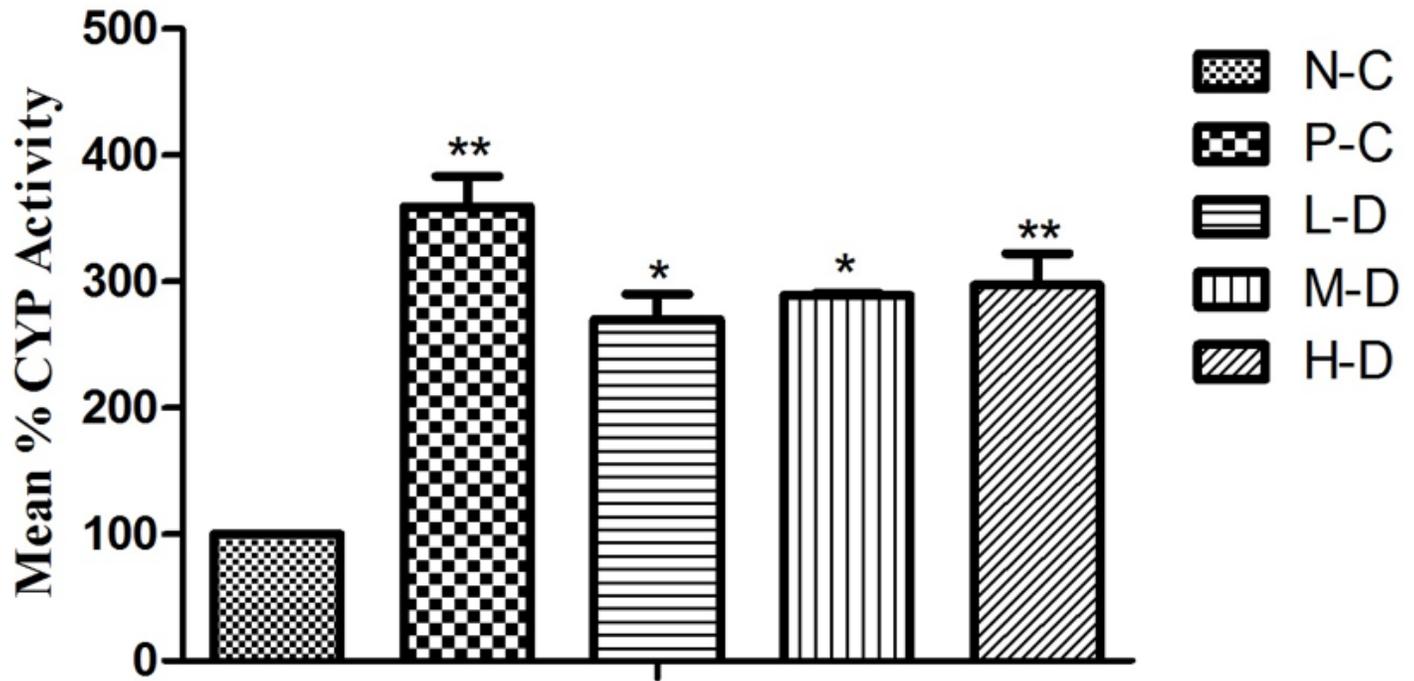


Figure 2

Rat liver CYP2D6 activity for various treatment groups after 30 day administration: N-C = Negative control; P-C = Positive control (Phenobarbital); L-D = Low dose (38.63 mg/kg) of Cellgevity®; M-D = Medium dose (77.25 mg/kg) of Cellgevity®; H-D = High dose (154.50 mg/kg) of Cellgevity®. Data represents mean  $\pm$  standard deviations. \* and \*\* represent values significantly different from the negative control as indicated by  $p < 0.05$  and  $p < 0.01$ , respectively

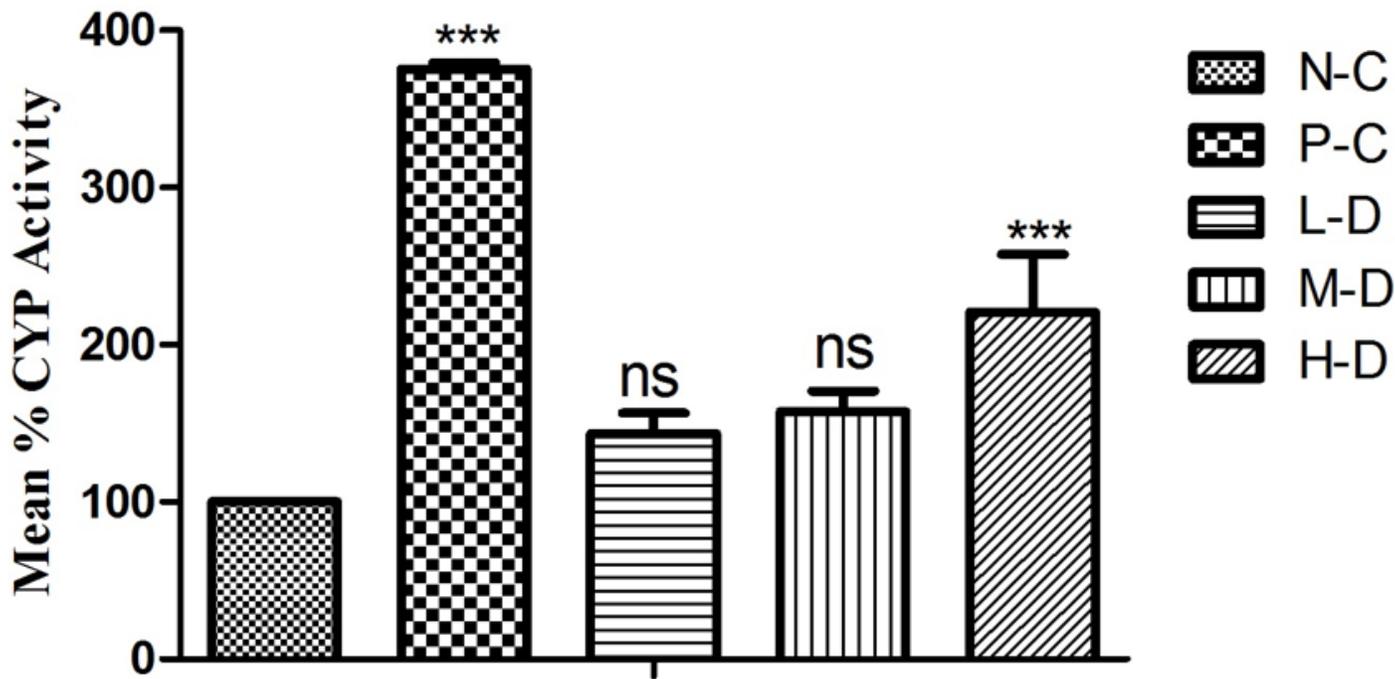
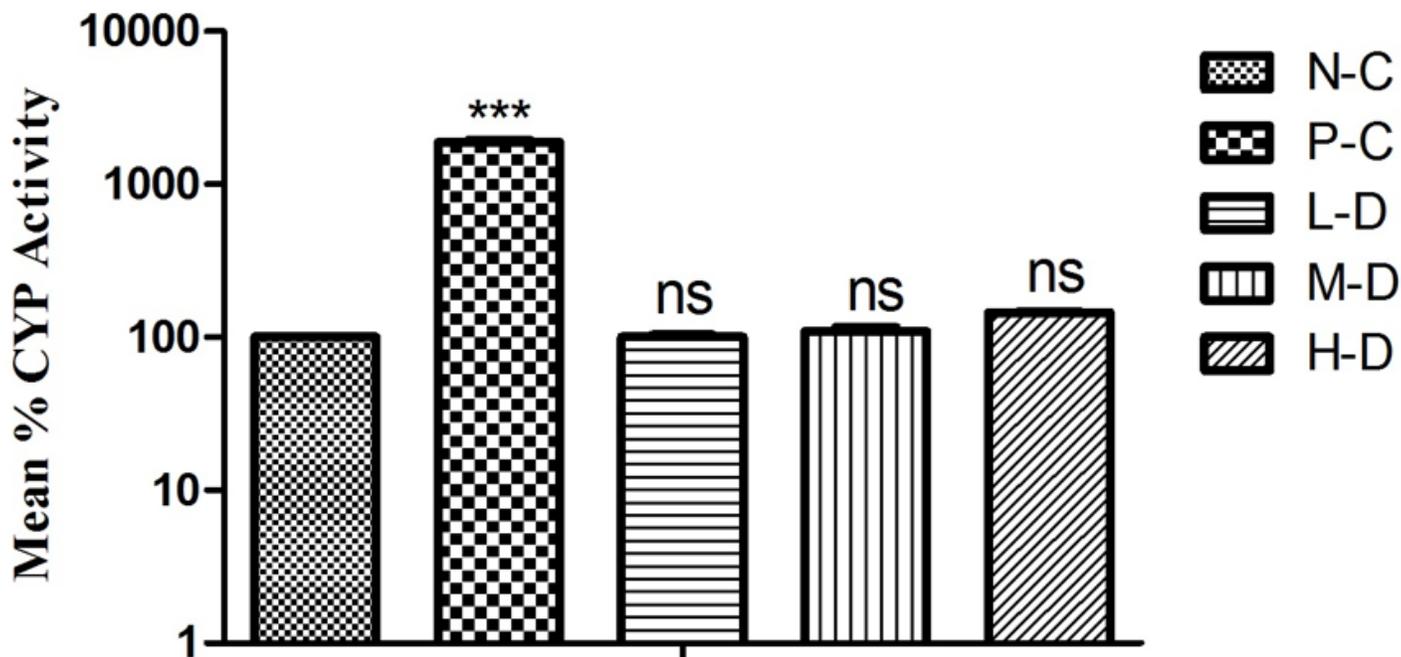


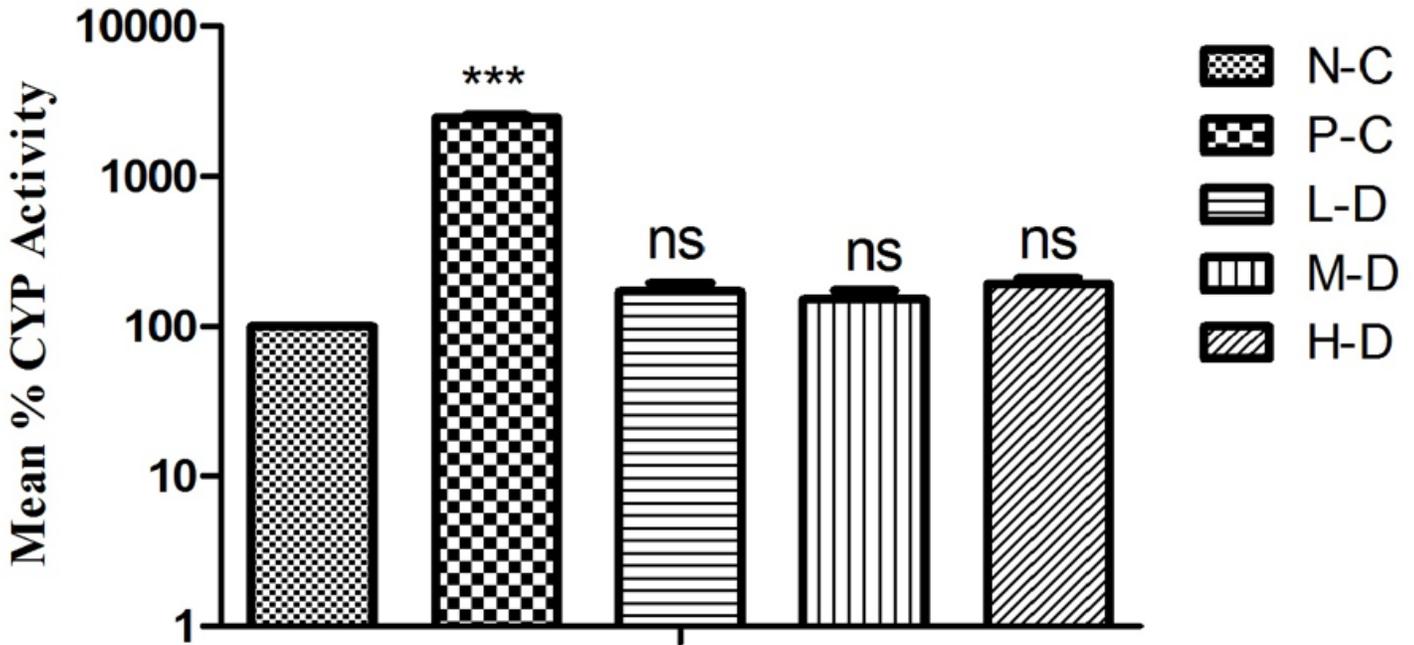
Figure 3

: Rat liver CYP1A1/2 activity for various treatment groups after 30 day administration: N-C = Negative control; P-C = Positive control (Phenobarbital); L-D = Low dose (38.63 mg/kg) of Cellgevity®; M-D = Medium dose (77.25 mg/kg) of Cellgevity®; H-D = High dose (154.50 mg/kg) of Cellgevity®. Data represent mean  $\pm$  standard deviations. \*\*\* represents values significantly different from the negative control as indicated by  $p < 0.001$ , and ns means not significantly different



**Figure 4**

Rat liver CYP2B1/2 activity for various treatment groups after 30 day administration: N-C = Negative control; P-C = Positive control (Phenobarbital); L-D = Low dose (38.63 mg/kg) of Cellgevity®; M-D = Medium dose (77.25 mg/kg) of Cellgevity®; H-D = High dose (154.50 mg/kg) of Cellgevity®. Data represent mean  $\pm$  standard deviations. \*\*\* represents values significantly different from the negative control group as indicated by  $p < 0.001$



**Figure 5**

Rat liver CYP3A4 activity for various treatment groups after 30 day administration: N-C = Negative control; P-C = Positive control (Phenobarbital); L-D = Low dose (38.63 mg/kg) of Cellgevity®; M-D = Medium dose (77.25 mg/kg) of Cellgevity®; H-D = High dose (154.50 mg/kg) of Cellgevity®. Data represents mean  $\pm$  standard deviations. \*\*\* represents values significantly different as indicated by  $p < 0.001$

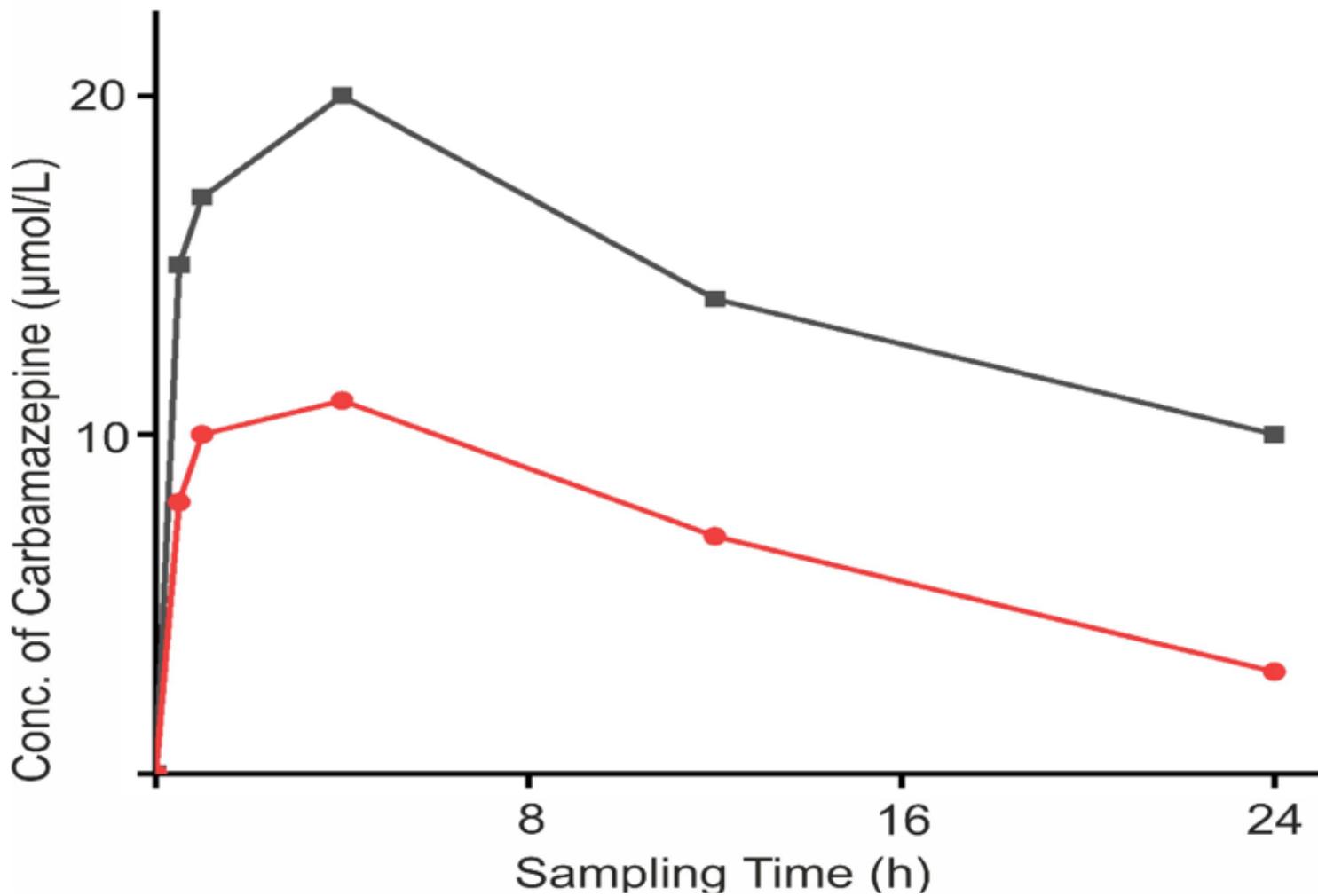


Figure 6

Concentration-time curves of carbamazepine with Cellgevity® (grey solid line; filled squares) and carbamazepine with normal saline (red solid line; filled circles)