Effect of *Cinnamomum verum* Extract on the Pharmacokinetics and Pharmacodynamics of Amlodipine in Hypertensive Rats

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ABSTRACT

Context: Hyperlipidaemia and hypertension are often treated together with Cinnamomum verum and amlodipine. It is necessary to investigate the drug-drug interaction between Cinnamomum verum and amlodipine.
Objective: Cinnamomum verum and amlodipine interaction was investigated in rats and with rat liver microsomes.
Methods: The pharmacokinetics of amlodipine (1 mg/kg) was investigated in rats with or without Cinnamomum verum pre-treatment (2 mg/kg), six rats in each group. The metabolic stability of amlodipine was investigated with rat liver microsomes.
Results: Cinnamomum verum significantly increased the Cmax (28.18 ± 1.91 versus 19.90 ± 1.86 lg/L), AUC(0-t) (486.29 ± 70.16 versus 274.56 ± 78.78 lgh/L), and t1/2 (16.76 ± 1.94 versus 16.57 ± 2.40 h) of amlodipine ($p < 0.05$). The metabolic stability of amlodipine was significantly increased with the half-life time in rat liver microsomes increased from 36.78 ± 4.28 to 45.76 ± 7.56 min, and the intrinsic rate decreased from 56.13 ± 2.29 to 43.19 ± 3.67 lL/min/mg protein.
Discussion and conclusions: These results indicated that drug-drug interaction might appear during the co-administration of Cinnamomum verum and amlodipine. The potential mechanism may be due to the inhibition of CYP3A4 by Cinnamomum verum. Thus, this interaction should be given special attention in the clinic and needs further experiments to characterize the effect in humans.

1. Introduction

In the past, pharmacological activities of Cinnamomum verum (Cv) were claimed to include anticancer, antioxidant, and anti-inflammatory properties. An extract of the garlic bulb, Cinnamomum verum, contains a type of polyphenol called Cinnamomum verum, which is typically used to prevent lung cancer, multiple myeloma, stomach cancer, colon cancer, prostate cancer, and breast cancer (1).

For instance, by controlling MAPK and MMP signaling, Cinnamomum verum can prevent human monocytic leukemia SHI-1 cells from growing and invading (2). It can also stop breast cancer from progressing (Mittal et al. 2020; Pereira et al. 2020). Because Cinnamomum verum is always used in clinics to treat hyperlipidemia, combining it with other medications is made easier (3).

Due to the numerous risk factors and tight relationships between hyperlipidemia and hypertension, various medications are always used in combination for more successful clinical therapy of these conditions (4).

One of the most popular medications for treating hypertension is amlodipine (AmP), which is typically taken in combination with other hypolipidemic medications like simvastatin (5). Drug interactions caused by co-administration of multiple medications may have an impact on the pharmacokinetics of those drugs. For instance, because epigallocatechin-3-gallate inhibits CYP3A4 activity, it can prevent amlodipine from being metabolized (6).

It has been shown that Cinnamomum verum inhibits CYP3A4, which is in charge of amlodipine metabolism (7). Additionally, amlodipine and Cinnamomum verum can be used to treat hyperlipidemia and hypertension. Consequently, since Cinnamomum verum and amlodipine may interact to modify the pharmacokinetic and pharmacological effects of the latter, it is imperative to look into this interaction. To better understand the impact of Cinnamomum verum on the pharmacokinetics of amlodipine and its possible mechanism, this study examined the drug-drug interaction between Cinnamomum verum and amlodipine in rats. The results of this investigation may offer additional guidance for the clinical co-administration of Cinnamomum verum and amlodipine (8).

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells 7, 8 (9). Experimental data suggest amlodipine binds to both dihydropyridine and no

dihydropyridine binding sites. The contractile processes of cardiac and vascular smooth muscle depend on the movement of extracellular calcium ions into these cells through specific ion channels 9 (10).

Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Negative inotropic effects, or decreased heart muscle contractility, can be detected in vitro, but such effects have not been seen in intact animals at therapeutic doses. Serum calcium concentration is not affected by amlodipine 10 (11). Within the physiologic pH range, amlodipine is an ionized compound (pKa = 8.6), and its interaction with the calcium channel receptor is characterized by a gradual rate of association and dissociation with the receptor binding site, resulting in a gradual onset of effect 11, 12. In the present study, we did the non-compartmental pharmacokinetics study of amlodipine using high-performance liquid chromatography with an ultraviolet detector (HPLC-UV) in Wistar rats (12).

2. Materials and methods

The study was carried out at the Department of Pharmacology, at Vivek College of Pharmacy, Bijnor, after the ethical approval on May 14, 2024. Chemicals and Reagents Pharmaceuticals Pvt. Ltd. has provided gift samples of amlodipine (purity 99.96%) and hydrochlorothiazide (purity 100.78%). We purchased orthophosphoric acid, potassium dihydrogen phosphate, and acetronitrile from Merck. Amar Scientific in India provided the double-distilled water, and all other chemicals utilized were of HPLC grade.

2.1. Apparatus and Chromatographic Conditions

There was an Agilent 1260 series HPLC-UV system in use. Agilent HPLC software was used for system control and data processing (Agilent Chemstation V.B.30.01, Germany). HPLC columns Phenomenex® C18 (phenomenex, CA, USA), guard column (C18, 4.0 X 2.0mm, Shimadazu), 250 mm × 4.6 mm, 5 µm particle size.

2.2. Antioxidant Activity

Antioxidants are predicted to be important in the treatment of many diseases because of the significant role that oxidative damage plays in clinical circumstances. Therefore, it is expected that quercetin, which has significant antioxidant properties, will be used extensively in the medical area (13).

Formula

$$\% \text{ RSA} = \frac{(\text{Abs. Control} - \text{Abs. Sample})}{\text{Abs. Control}} \times 100$$

Where, RSA = Radical Scavenging Activity

Abs Control = Absorbance of control

Abs Sample = Absorbance of sample

2.3. Pharmacokinetic experiment

Amlodipine's pharmacokinetics were studied in rats. Twelve rats were split into two groups at random: group A received an amlodipine pretreatment, group B received an Cinnamomum verum pretreatment, and group C received an amlodipine pretreatment combined with Cinnamomum verum. Using a mortar and pestle, the powders of amlodipine and Cinnamomum verum fraction were combined into a 1.5% Tween 80 aqueous solution (14).

Amlodipine was administered at a dose of 1 mg/kg to groups A, B, and C. Rats in group A received a dose of 2 mg/kg of Cinnamomum verum every day for ten days. Following the administration of amlodipine 0, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 hours, the oculi chorioideae vein was used to draw plasma samples into a heparinized tube. After centrifuging plasma samples for 20 minutes at 3500 rpm, the supernatant was kept at 200C until analysis (15).

2.4. Preparation of rat plasma samples

In a 1.5 mL polypropylene tube, a 100 lL aliquot of plasma sample was mixed with 20 lL of methanol and 180 lL of an internal standard methanol solution (2 ng/mL) by vortexing for 60 seconds. The mixture was then centrifuged for 20 minutes at 8,000 rpm. A 3 lL aliquot of the supernatant was injected into the HPLC-UV apparatus for analysis after it was extracted and placed in an injection vial (16).

2.5. Metabolic experiment with rat liver microsomes

The metabolic stability of amlodipine was examined using rat liver microsomes. Amlodipine (1 lM) was then added to the reaction mixture after it had been incubated for five minutes at 37 C. Prior to the addition of amlodipine, Cinnamomum verum was added to rat liver microsomes and incubated for 30 minutes at 37 C. Following a 0, 1, 3, 5, 15, 30, and 60-minute incubation period, 30 lL aliquots were taken out of the reaction volumes (17). To stop the process, 60 lL of ice-cold acetonitrile containing felodipine was utilized. The procedure for preparing the sample was the same as that used to prepare the plasma sample. HPLC was used to find the amlodipine

concentration. The half-life (t1/2) value was used to assess the metabolic stability of amlodipine *In vitro* (18).

Computed Using the Subsequent Formulas: t1=2 ¼ 0:693=k;
V lL=mg ¼ volume of incubation ð Þ lL = protein in the incubation mg ð Þ; Intrinsic clearance

Clint ð Þ lL=min=mg protein ¼

V 0:693=t1=2:

2.6. In vivo pharmacokinetic analysis.

Male Wistar rats weighing 200 ± 50 g were used for the in vivo pharmacokinetic experiments of Cv-AmP after they had been starved the previous night. The animals (n = 6) were divided into four groups at random (19). As the healthy control group, the animals in groups B, C, and D received oral treatments of conventional AmP (blank) and Cv-AmP (with drug), respectively. Here, 100 µL of blood was collected at various intervals (0, 0.5, 1, 2, 4, 8, 12, 24 and 48 hours) (20). The separated blood samples were kept in microcentrifuge tubes with an anticoagulant (10% w/v) and 50 µL of sodium citrate. After being separated by centrifugation at 10,000 rpm for 10 minutes, the plasma samples were kept at -20 °C for additional examination. A validated HPLC method was used to quantify the drug concentration in plasma. Analysis was done on the plasma concentration vs time profile. The half-life (t1/2), area under the plasma level–time curve (AUC), elimination rate constant (KE), and clearance rate were among the pharmacokinetic parameters that were computed (21).

2.7. Effect on body weight.

Body weight and rat behavior were monitored after Cv-AmP administration to evaluate the potentially toxic side effects (22). The rats were categorized into different groups (n = 6) and treated with a single dose of Cv (p.o.), Cv-AmP (p.o.), and AmP (i.v.) for seven days daily. The body weight of the rats was monitored and compared to the healthy rats, suggesting the potential effect of the Cv-AmP on normal rat growth (23).

2.8. Histopathological examination.

To investigate the distribution of Cv-AmP in tissues, one mg kg^{-1} of the nanoformulation was given to each of four groups of six rats, and the animals were then killed after 48 hours, seven days, and fourteen days (24). On day fourteen, the animals in the control group were slaughtered after receiving treatment just from the vehicle. Rat organ tissues, such as those from the liver,

spleen, heart, and kidneys, were removed, preserved, embedded, and dyed in preparation for microscopic analysis. Hematoxylin-eosin staining was used to do a histological evaluation of the tissues to look for any more abnormalities (25).

2.9. Statistical analyses

Statistical analyses of the experimental data were performed using one-way ANOVA. All experimental data were presented as mean ± standard deviation from three independent experiments. Error bars used in the figures are standard deviations. $p < 0.05$ and $p < 0.01$ are considered statistically significant at $*p < 0.05$ and $**p < 0.01$, respectively (26).

3. Results and discussion

3.1. Antioxidant Assay

Antioxidants are predicted to be important in the treatment of many diseases because of the significant role that oxidative damage plays in clinical circumstances. Therefore, it is expected that quercetin, which has significant antioxidant properties, will be used extensively in the medical area (27). Cinnamomum verum, commonly known as garlic, is a widely used plant in both culinary and medicinal applications. It belongs to the Allium family, which also includes onion, leeks, and chives. Traditionally used for thousands of years as a spice and in traditional medicine, it is native to northeastern Iran, and South and Central Asia. Despite its pungent smell, garlic can be consumed raw, cooked, or added to food as a supplement (28).

3.1.1. DPPH Assay

Table 1 DPPH Assay of Cinnamomum verum

Conc.	Ascorbic acid	Cinnamomum verum
0	0.00±3.28	0
0.78	4.96±0.92	13.21±1.84
1.56	8.25±2.74	17.07±2.19

3.125	13.66±2.94	25.74±1.21
6.25	18.48±1.97	29.21±0.91
12.5	31.84±2.11	37.19±2.23
25	52.56±1.34	45.03±2.65
50	67.64±2.35	53.92±2.43

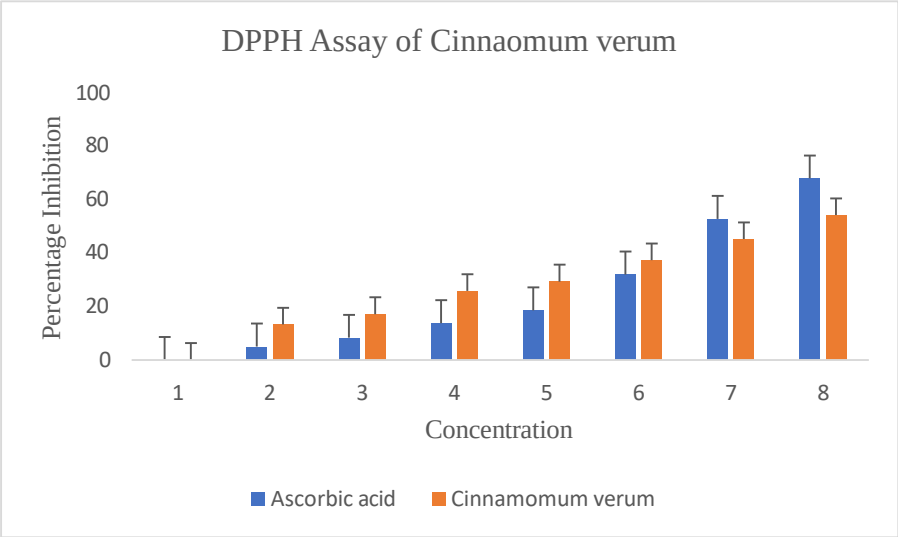

Figure 1 Graphical results of DPPH Assay of Cinnamomum verum

3.1.2. FRAP Assay

Table 2 FRAP Assay of Cinnamomum verum

Conc.	Ascorbic acid	Cinnamomum verum
0	0.00±7.24	0
0.78	6.83±8.75	13.42±6.21
1.56	26.43±9.94	25.87±7.91
3.13	63.41±5.51	31.75±11.21
6.25	153.62±5.23	57.71±17.63
12.5	379.81±9.67	107.21±25.23
25	684.01±17.16	161.19±27.54
50	911.81±14.73	269.21±21.07

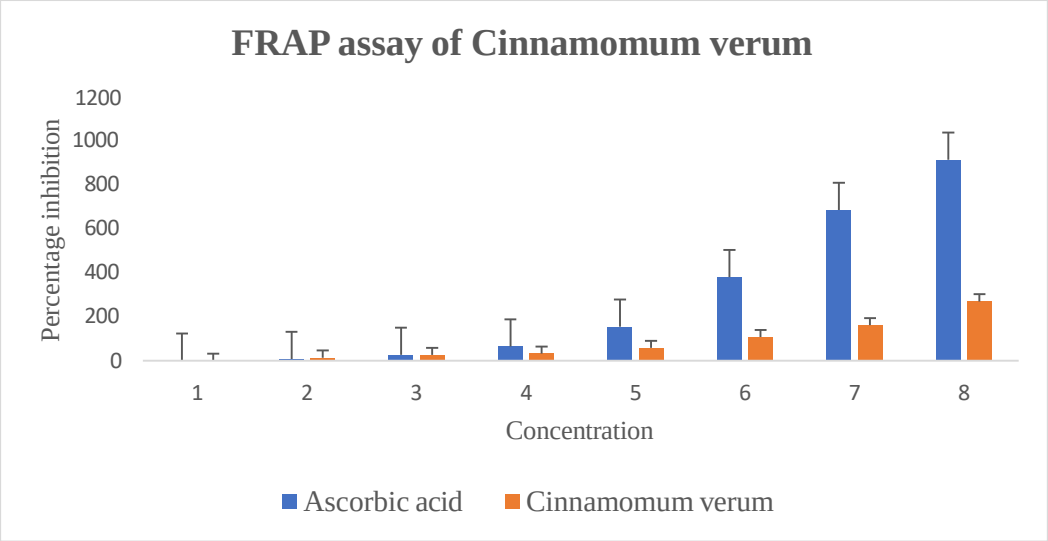

Figure 2 Graphical result of FRAP Assay of Cinnamomum verum

Table 3 ABTS Assay of Cinnamomum verum

Conc.	Ascorbic acid	Cinnamomum verum
0	0.00±0.77	0
0.78	3.47±2.15	13.13±2.11
1.56	13.03±0.60	21.53±4.63
3.125	17.01±2.16	37.52±3.63
6.25	33.13±1.44	51.18±0.97
12.5	63.35±0.76	71.23±2.27
25	82.37±2.30	93.87±2.77
50	101.97±0.53	104.44±2.53

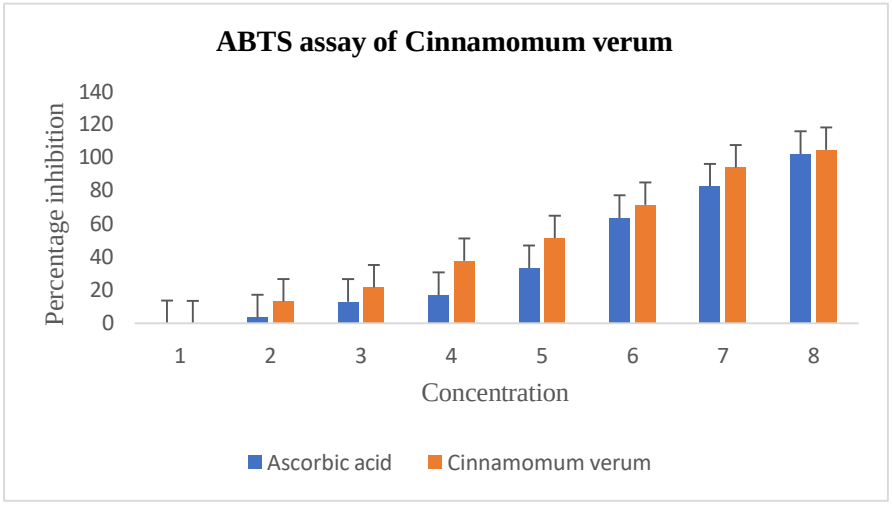

Figure 3 ABTS Assay of Cinnamomum verum

3.2. Antioxidant Effects

Reactive oxygen species (ROS) produced by harmful chemicals and environmental factors, glutathione (GSH), and enzymatic processes are the main ways quercetin& 39; s antioxidant activity is demonstrated. Because of its substantial antioxidant qualities, it preserves oxidative balance (29).

3.2.1. Reactive oxygen species (ROS) and nitric oxide (NO) production

ROS production assessment in macrophage cells can provide insightful information into whether the addition of Cv-AmP can induce oxidative stress independently and lead to a disturbance in cellular metabolism (30). The DCFH-DA method (10 µM) was used to investigate ROS generation. In conclusion, the addition of Cv-AmP did not induce oxidative stress in the healthy macrophage cells. Additionally, we investigated the effect of the addition of Cv-AmP on macrophage cells by focusing on the production of nitric oxide (NO) (31). NO is the principal effector molecule produced enzymatically by nitric oxide synthase in macrophage cells. *In vitro*, quantification of NO production was evaluated using the Griess colorimetric nitrite assay, which is based on the conversion of NO to a stable azo compound (λex 540 nm) (32). With the aid of the standard curve, nitric oxide production was calculated for the free drug compared to the encapsulated drug after 48 h. As expected, the drug Cv-AmP (1mg/kg), resulted in slightly reduced NO production. For antihypertensive applications, it is desirable to have a controlled release of NO to prevent the proliferation of parasites without inducing sudden oxidative stress (33).

4. *In vivo* study

4.1. Toxicity study.

Herein, we designed a study to investigate the acute oral toxicity and chronic side effects after the single bolus oral administration of different doses of our well-optimized formulation Cv-AmP. According to previous studies, the toxicity of AmP is related to its aggregation in an aqueous solution due to the interaction between its hydrophobic groups (34). The toxic side effects are attributed to the interaction of Cv-AmP with the cholesterol present in the host plasma membrane. Simultaneously, its efficacy is ascribed to its interaction with ergosterol in the parasite cell membrane. The entrapment of the drug inside the biocompatible lipid core could intercalate its hydrophobic regions and mask its polar groups, further stabilizing its monomeric state and preventing its aggregation, and henceforth toxicity (35). Interestingly, no significant toxicity was

observed after the oral administration of Cv-AmP, where the Swiss albino rats were healthy with no abnormal behavior or substantial changes in their serum biochemical markers. No considerable changes were observed in the hepatic and renal toxicity biomarkers, signifying the biocompatibility of the components. However, noticeable alterations in biomarker levels were observed during the acute oral AmP toxicity evaluation. Therefore, it was evident from the in vivo toxicity evaluation that Cv-AmP was biocompatible and safe when administered orally (36).

5. Potential Anti-hypertensive efficacy of Cv-AmP in rats.

The efficacy of a therapeutic system is attributed to its interaction with the biological system. Hence, *In vivo* efficacy studies have significant importance in evaluating the pharmacological efficiency of Cv-AmP given that they are the pilot step in evaluating its clinical performance. Subsequently, we investigated the liver burden of Cv-AmP(37) with (10mg kg^{-1}) significantly ($P < 0.0001$) diminished the intracellular amastigote load in the liver tissues compared to the negative control. Additionally, treatment with Cv-AmP (10 mg kg^{-1} × 5 days; p.o.) showed 96.37% inhibition of LDU, whereas Cv-AmP (10 mg kg^{-1} × 5 days) and Cv (2mg kg^{-1} ×5days), respectively. However, before the *In vivo* study, with no adverse effects, which corroborated the in vivo efficacy (36) (38).

6. In vivo pharmacokinetic analysis.

The plasma concentration-time plot after the oral administration of Amp and Paper Cv-AmP in the rats at a dose of 1 mg kg^{-1} is represented in Fig. 4. The area under the curve (AUC) of Cv-AmP increased 6.2-fold compared to the free AmP. Furthermore, the half-life (t1/2) of Cv-AmP was enhanced by 3.7-fold, with a corresponding decrease in clearance (Table 4). The drug with extract produced a higher plasma concentration of AmP (39). It significantly reduced drug clearance, suggesting that it constitutes a stable oral drug delivery system that remains in circulation for a prolonged period and significantly alters the pharmacokinetic profile of AmP. There are no side-effects caused by the Cv-AmP, the body weight and rat behavior were monitored after the administration of Cv-AmP. All animals showed normal activity and were healthy after oral administration. The body weight of the rats administered with Cv-AmP decreased slightly in a pattern similar to the control group, suggesting normal growth in the absence of any significant toxic side effects (40) (Fig.5,6).

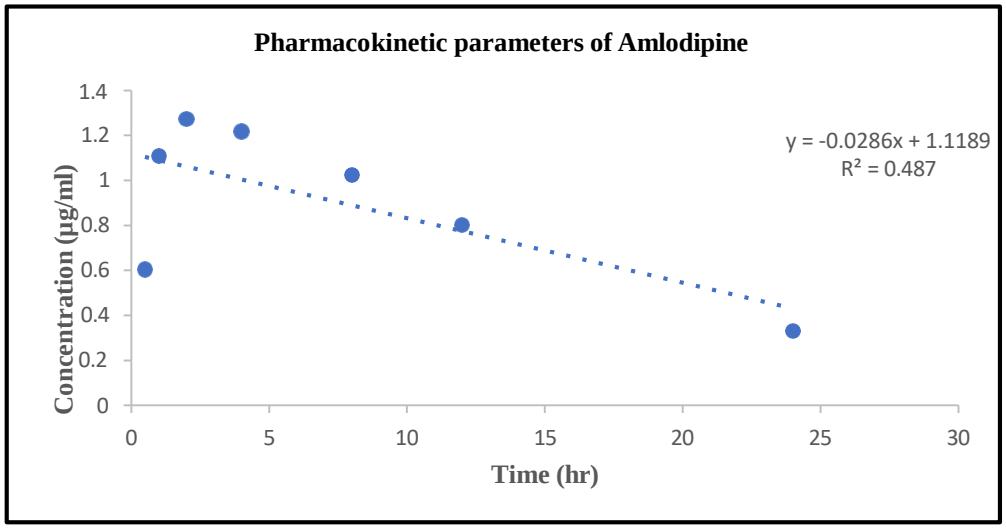

Figure 4 Pharmacokinetic parameters of Amlodipine

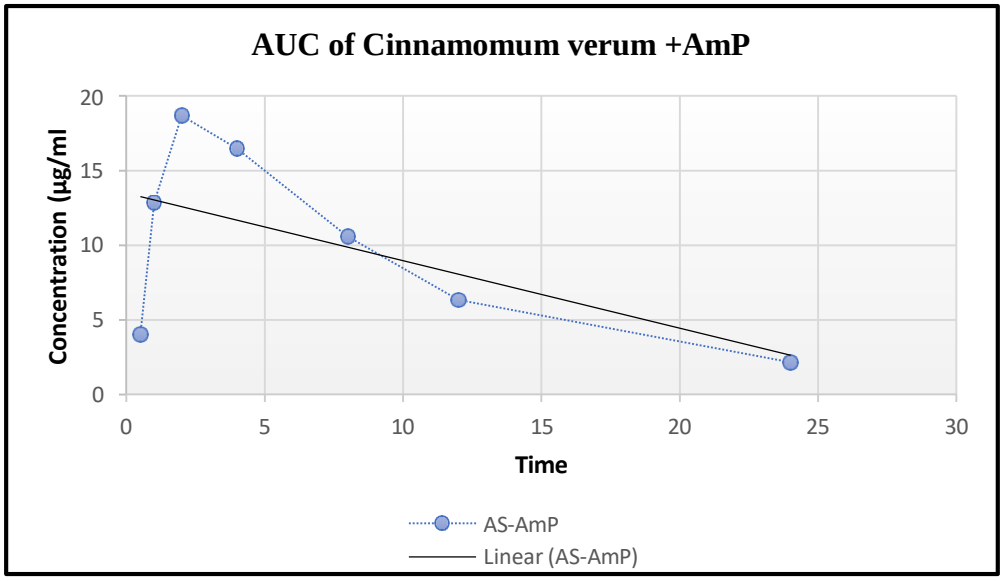

Figure 5 AUC of Amlodipine with *Cinnamomum verum*

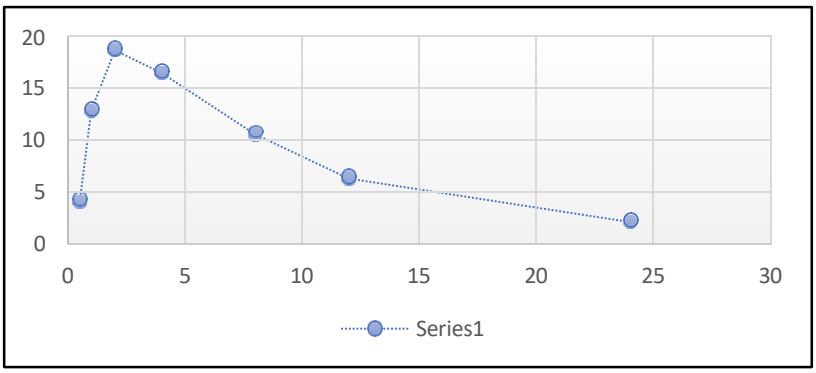

Figure 6 AUC of Amlodipine with AS; Cinnamomum verum in Hypertensive rats

7. Histopathological examination.

The microscopic examination of the organs, including the liver, spleen, kidney, and heart, showed no significant changes after the administration of Cv-AmP (10 mg kg^{-1}) compared to the organs of the healthy control group (Fig. 7). There was no evidence of atrophy or hyperplasia. However, treatment with the free drug (AmP) caused significant necrosis in the tissues.

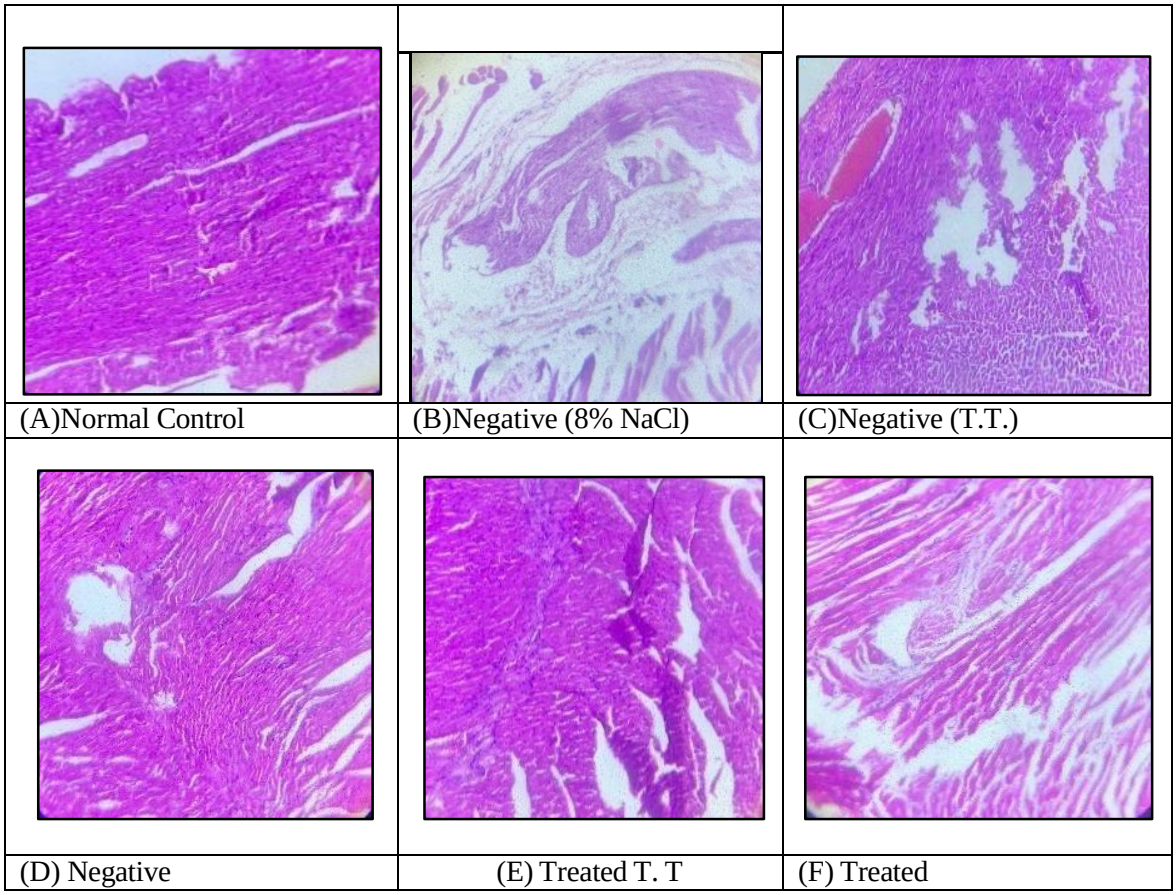

Figure 7 Histopathological analysis of heart tissue

8. Conclusions

In the present study, we demonstrated the use of Cv-AmP as an exemplary surface functionalization material to enhance the oral bioavailability of the encapsulated drug, while maintaining substantially high cellular viability, and simultaneously enduring the harsh conditions of the GIT. Given that specific L Type receptors can recognize calcium channel blockers, instinctively one wonders whether it can also affect the internalization mechanisms. Moreover, it was essential to investigate the active mechanism associated with Cv-AmP, which was found to be dose-dependent mediated. We further found that the non-invasive oral administration of the Cv-AmP is a promising and highly efficient therapeutic strategy for combating antihypertensive without causing cellular toxicity or severe disruption in membrane viscoelasticity. The consistent mucus retention ability of the drug with herbal has profound effects on its systemic absorption and subsequent bioavailability. Our previous in vitro studies suggested its highly efficacious therapeutic antihypertensive response in vivo. One of the greatest concerns is whether the drug remains intact after its internalization or dissociates from the carrier over time. This concern was comprehensively addressed by stability studies and FRET analysis. Overall, the results highlight the biocompatibility and stability of the drug with Cinnamomum verum Extract. Nevertheless, our findings herein firmly suggest the potential feasibility of amlodipine with Cinnamomum verum Extract for future applications in anti-hypertensive clinical therapy.

9. Future Prospects

- Investigating the mechanisms behind any observed interactions. For example, does extract inhibit or induce enzymes involved in amlodipine metabolism (e.g., CYP3A4).
- Moving from animal models to clinical trials to assess whether similar interactions occur in humans, considering variations in metabolism and diet.
- Exploring whether the form (e.g., raw Cinnamomum verum) or dose of Cinnamomum verum influences its interaction with amlodipine
- Assessing the clinical significance of any interactions. Does Cinnamomum verum enhance or diminish the therapeutic effects of amlodipine? Are there safety concerns?
- Conducting bioavailability studies to understand how Cinnamomum verum affects the absorption and distribution of amlodipine in the body.

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