Pharmacokinetics and Pharmacodynamicinteraction of amlodipine with Allium sativum in Hypertensive Rat Models

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ABSTRACT:

Amlodipine and Allium Sativum are commonly used in conjunction to treat hypertension and hyperlipidemia. Investigating the pharmacological connection between amlodipine and Allium sativum is essential. Rat liver microsomes and the interaction between Allium Sativum and amlodipine were investigated in rodents. Six rats per group were used to examine the biological effects of amlodipine (1 mg/kg) in rats, irrespective of whether they had previously received Allium Sativum therapy (2 mg/kg). Rat liver microsomes were used to study amlodipine's metabolic equilibrium. The Cmax (26.19 ± 2.21 vs 17.80 ± 1.56 lg/L), AUC(0-t) (507.27 ± 60.23 versus 238.68 ± 45.59 lg/L), and t1/2 (14.69 ± 1.64 versus 11.43 ± 1.20 h) of amlodipine were all considerably increased by Allium Sativum ($p < 0.05$).

The half-life of amlodipine in rat liver microsomes rose from 34.23 ± 3.33 to 44.15 ± 4.12 minutes, indicating an improvement in metabolic stability. From 40.49 ± 3.26 to 31.39 ± 2.78 lL/min/mg protein, the intrinsic rate decreased. These results indicated that the combination of amlodipine and allium sativum may result in drug-drug interactions.

One possible mechanism is that Allium Sativum inhibits CYP3A4. Therefore, this interaction necessitates extra research to describe the effect in humans as well as special attention in theclinic.

1. INTRODUCTION

It was previously asserted that *Allium sativum's* (AS) pharmacological actions included anti-inflammatory, antioxidant, and anticancer effects. A particular kind of polyphenol found in an extract of the garlic bulb, Allium sativum, is commonly used to prevent breast, prostate, colon, stomach, lung, and multiple myeloma cancers [1]. For example, Allium Sativum can stop the growth and invasion of human monocytic leukemia SHI-1 cells by regulating MAPK and MMP signaling [2]. Additionally, it can prevent the spread of breast cancer (Mittal et al. 2020; Pereira et al. 2020). Combining Allium Sativum with other drugs is simpler because it is always used in clinics to treat hyperlipidemia [3].

For more effective clinical treatment of hyperlipidemia and hypertension, several drugs are always used in combination because of the many risk factors and close connections between these disorders[4]. Amlodipine (AmP), one of the most widely used drugs for treating hypertension, is usually given with other hypolipidemic drugs such as simvastatin [5]. The pharmacokinetics of certain pharmaceuticals may be affected by drug interactions brought on by taking several prescriptions at once. For example, epigallocatechin-3-gallate can stop the metabolism of amlodipine because it inhibits CYP3A4 activity [6].

Allium sativum has been demonstrated to inhibit CYP3A4, the enzyme responsible for the metabolism of amlodipine [7]. Allium sativum with amlodipine can also be used to treat hypertension and hyperlipidemia. Therefore, it is essential to investigate the potential interaction between amlodipine and Allium Sativum, as this could alter the latter's pharmacokinetic and pharmacological effects. This study examined the drug-drug interaction between Allium Sativum and amlodipine in rats to elucidate the effect of Allium Sativum on amlodipine pharmacokinetics and likely mechanisms. The results of this investigation might offer more details regarding the medical administration of amlodipine and allium sativum together [8].

A dihydropyridine Ca+2 antagonist, also known as a calcium ion antagonist or slow-channel blocker, amlodipine prevents calcium molecules from entering cardiac muscle cells and artery smooth muscle cells [9]. According to experimental findings, amlodipine has no dihydropyridine binding sites but binds to both forms of dihydropyridine. External Ca+2 must enter these cells through certain ion channels in order for the smooth muscles of the heart and arteries to contract [10].

Amlodipine primarily affects smooth muscle cells in the vascular system rather than heart muscle cells by specifically blocking Ca+2 influx across cell membranes. Although effects have not been shown in intact animals at therapeutic dosages, adverse inotropic effects, or decreased heart muscle contractility, can be shown in vitro. Serum calcium levels are unaffected by amlodipine [11]. As an ionized molecule in the physiological pH range (pKa = 8.6), amlodipine interacts with the Ca+2 receptor in a slow pace of association and detachment from the receptor attachment site, which causes the effect to start gradually. Amlodipine's non-compartmental pharmacokinetics in Wistar rats were investigated using HPLC-UV [12].

2. Material and Methods

The study was done at the Department of Pharmacology, Vivek College of Pharmacy, Bijnor, following ethical permission on May 14, 2024 (Proposal No. VCTE/IAEC/PH/24/001).Ingredients and Reagents Pharmaceuticals Pvt. Ltd. has offered gift samples of amlodipine (99.96%) and hydrochlorothiazide (100.78%). We bought orthophosphoric acid, potassium dihydrogen phosphate, and acetonitrile from Merck. Amar Scientific in India provided the double-distilled water, and all other chemicals used was HPLC quality.

1.2. 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 thesignificant role that oxidative damage plays in clinical circumstances. Therefore, it isexpected that quercetin, which has significant antioxidant properties, will be usedextensively in the medical area[13].

Formula

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

Where, RSA = Radical Scavenging Activity

2.3. Pharmacokinetic experiment

Amlodipine's pharmacokinetics were studied in rats. 12 rats were split into 2 groups at random: A group received an amlodipine pretreatment, group B received an allium sativum pretreatment, and group C received an amlodipine pretreatment combined with allium sativum. Using a mortar and pestle, the powders of amlodipine and Allium Sativum fraction were combined into a 1.5% Tween 80 aq. solution[14].

Amlodipine was given at a dosage of 1 mg/kg to groups A, B, and C. Rats in group A were given 2 mg/kg of allium sativum every single 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 mixed with 20 lL of methanol and 180 lL of an internal standard methanol solution (2 ng/mL) by vortexing for 60 seconds. The solution was then spun around for 20 minutes at 8,000 rpm. Following extraction and placement in an injection vial, a 3 lL aliquot of the residue was inserted into the HPLC-UV equipment for analysis[16].

2.5. Metabolic experiment with rat liver microsomes

The physiological equilibrium of amlodipine was investigated using liver from rats' microsomes. Amlodipine (1 lM) was then added to the reaction mixture after it had been incubated for five minutes at 37 C. Before the addition of amlodipine, Allium Sativum was introduced to Rat microsomes from the liver and kept 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. Procedure for preparing the trial 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 steadiness 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 research of AS-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 AS-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]. Blood samples were stored in tubes filled with microcentrifuge with 10% w/v anticoagulant and 50 µL sodium citrate. After separating by spinning at 10,000 rpm for a period of ten minutes, the resulting plasma samples were stored at -20 °C for further analysis. A validated HPLC method was used to determine the drug concentration in plasma. Plasma concentration vs. time profiles were analyzed. The pharmacokinetic parameters that were calculated included the half-life (t1/2), area under the plasma level-time curve (AUC), excretion rate constant (KE), and clearance rate[21].

2.7. Effect on body weight.

In order to evaluate the possible harmful side effects of AS-AmP therapy, rats' body weight and activity levels were examined. [22]. For seven days, each of the six groups of rats received a single dosage of AS (p.o.), AS-AmP (p.o.), and AmP (i.v.). The body weight of the rodents was evaluated in comparison to that of healthy rats, indicating that the AS-AmP might have an effect on typical rat growth[23].

2.8. Histopathological examination.

Distribution of AS-AmP in tissues: One milligram kg−1 of the nano-formulation was administered to IV groups of VI rats. After receiving therapy for 48 hours, seven days, and fourteen days, the animals were put to death [24]. After solely receiving therapy from the vehicle, the animals in the untreated group were killed on day fourteen. In order to prepare for a thorough investigation, rat organ tissues—including the kidneys, liver, heart, and graveyard of red blood cells—were removed, preserved, embedded, and colored. The tissues were subjected to a histological examination using hematoxylin-eosin staining in order to search for any more anomalies [25].

2.9. Statistical analyses

The results of the experiment were statistically analyzed using one-way ANOVA. All research results from three different studies were reported as mean ± SD. Error bars in the figures represent standard deviations. *p < 0.05 and **p < 0.01 indicate statistical significance, respectively[26].

3. RESULTS AND DISCUSSION

3.1. Antioxidant Assay

Antioxidants are expected to have a vital role in the treatment of many diseases due to the considerable impact that oxidative damage plays in clinical settings. As a result, it is envisaged that quercetin, which has considerable antioxidant qualities, would be widely used in the medical field [27]. Allium sativum,

also known as garlic, is a popular plant in both culinary and medical settings. It is from the Allium family, which additionally includes onion, leek, and chives. It is native to northeast Iran, as well as South and Central Asia, and used as a spice&medicine for thousands of ages. Despite its pungent odour, garlic can be eaten raw, cooked, or added to meals as a supplement[28].DPPH Assay (**Table 1 & fig. 1**), FRAP Assay (**Table 2 & Fig. 2**), ABTS Assay (**Table 3 & Fig. 3**)

Table 1: DPPH Assay of Allium sativum

Conc.	Ascorbic acid	Allium Sativum
0	0.00±3.29	0
0.78	5.97±0.94	14.23±1.85
1.56	9.24±2.75	19.09±2.21
3.125	15.68±2.92	27.77±1.23
6.25	20.47±1.98	31.22±0.90
12.5	33.87±2.13	39.23±2.2
25	54.59±1.38	47.05±2.69
50	69.69±2.39	55.95±2.45

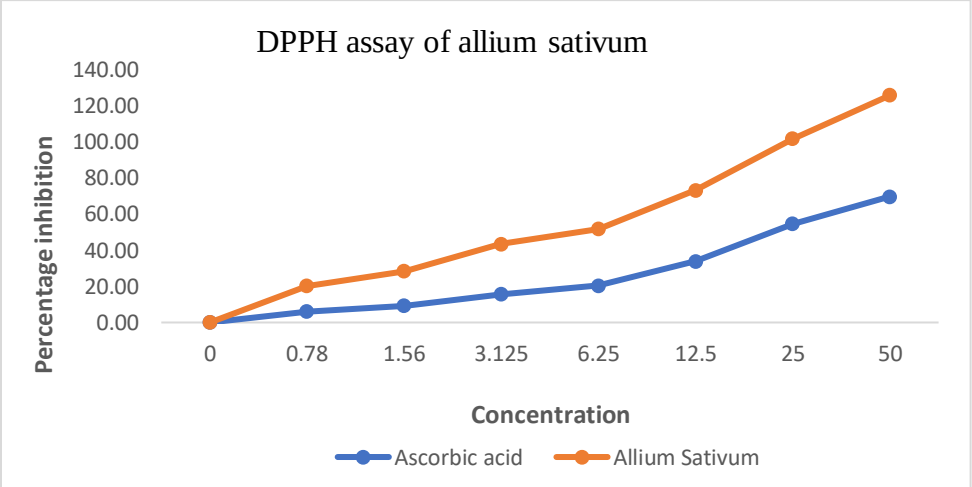

Figure 1: Graphical results of DPPH Assay of Allium sativum

Table 2: FRAP Assay of Allium sativum

Conc.	Ascorbic acid	Allium Sativum
0	0.00±7.27	0
0.78	5.85±8.78	15.45±6.24
1.56	28.45±9.98	27.89±7.92
3.13	64.43±5.52	33.79±11.2
6.25	155.64±5.26	59.72±17.65
12.50	382.84±9.69	109.2±25.23
25.00	687.02±17.19	163.23±27.56
50.00	913.8±14.75	273.2±21.08

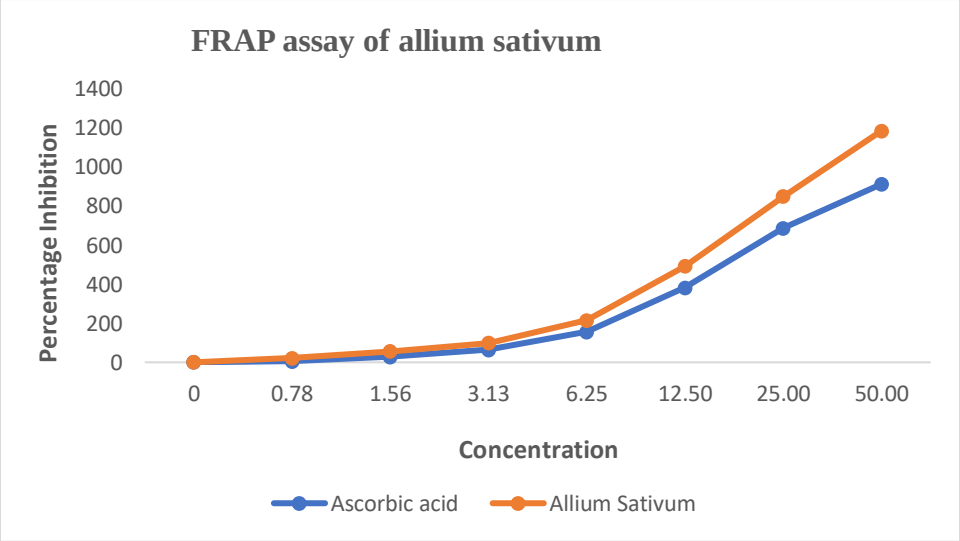

Figure 2: Graphical result of FRAP Assay of Allium sativum

Table 3: ABTS Assay of Allium Sativum

Conc.	Ascorbic acid	Allium Sativum
0	0.00±0.77	0
0.78	5.49±2.17	15.12±2.13
1.56	11.05±0.60	23.56±4.65
3.125	18.00±2.26	39.54±3.65
6.25	35.14±1.46	54.12±0.98
12.5	65.38±0.79	73.21±2.29
25	84.38±2.32	95.89±2.79
50	100.99±0.51	103.45±2.54

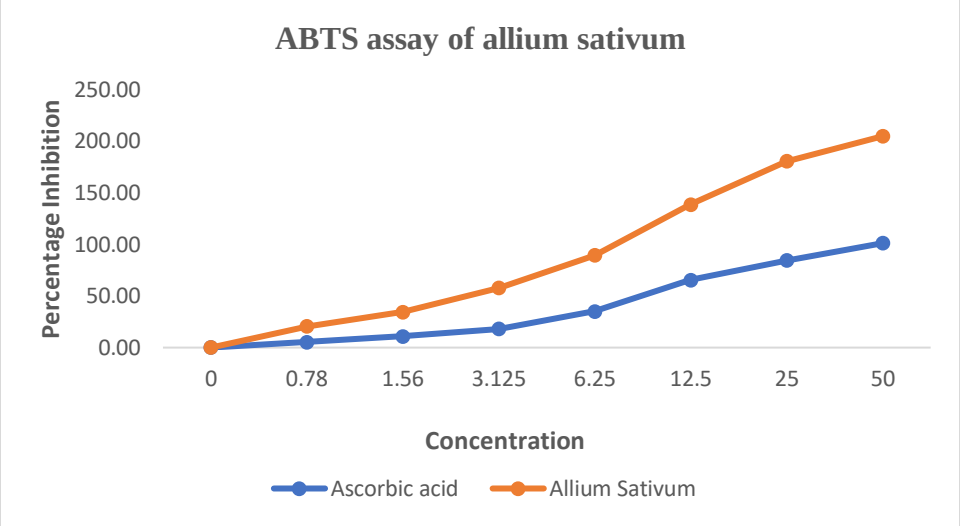

Figure 3: ABTS Assay of Allium Sativum

1.3. Antioxidant Effects

The major mechanisms by which quercetin's antioxidant activity is established are reactive oxygen species (ROS) created by hazardous chemicals and environmental variables, glutathione (GSH),

and reactions involving enzymes. Because of its significant antioxidant capabilities, it preserves oxidative balance[29].

Reactive oxygen species (ROS) and nitric oxide (NO) production:

Assessing the generation of reactive oxygen species (ROS) in macrophage cells may shed light on whether AS-AmP alone causes oxidative stress and modifies cellular metabolism[30]. In healthy macrophages, the injection of AS-AmP did not cause oxidative stress, according to the results of the DCFH-DA technique (10 µM) employed to measure ROS generation. In addition, we looked into how AS-AmP affected the production of nitric oxide (NO) in macrophages[31]. Nitric oxide (NO) is the primary effector molecule generated by nitric oxide synthase in macrophages. In vitro, NO levels were evaluated using the Griess colorimetric nitrite test. This assay transforms NO into a stable azo molecule, measurable at λex 540 nm[32]. The standard curve was used to calculate nitric oxide generation for the drug that was not bound against the encapsulated agent after 48 hours. As expected, the medication AS-AmP (1mg/kg) caused a modest reduction in NO generation. For antihypertensive uses, it is essential to have a regulated release of NO to limit parasite multiplication without causing abrupt oxidative stress [33].

1.4. In Vivo Study

We planned this research to look into short-term & long-term side effects of a single bolus given by the mouth of various doses of our properly optimized formulation AS-AmP. Previous research has linked AmP's toxicity to its agglomeration in an aqueous solution as a result of interactions between its aquaphobic groups[34]. The hazardous bad effects are caused by the relationship of AS-AmP with cholesterol in the host plasma cell membrane. Simultaneously, its effectiveness is linked to an association with ergosterol in infection cell sheaths. Entrapping the drug within the biocompatible lipid core could intercalate its aquaphobic portions and mask its polar groups, further stabilizing its monomeric form and preventing its aggregation, and hence toxicity[35]. Surprisingly, any type of major toxicity was not seen following the oral treatment of AS-AmP, as the Swiss albino rats were normal without aberrant behavior or notable alterations in their serum biochemical indicators. The indicators for the liver and renal toxicity showed no significant alterations, indicating that the components were biocompatiblethe in vivo toxicity analysis demonstrated that AS-AmP was biocompatible as well as secure when administered orally[36].

1.5.Potential Anti-Hypertensive Efficacy of AS-AmPIn Rat.

The efficacy of a treatment arrangement is determined by its relationship with the organic framework. As a result, *In Vivo*,usefulness studies are critical in determining the pharmacologic effectiveness of AS-AmP because they serve as a preliminary footstep in assessing its clinical performance. Subsequently, after therapy with test and reference therapies[37]. A medicinal system's efficacy is determined by how it interacts with the body's systems. As a result, *In Vivo*,effectivenesslessons are critical in determining the pharmacologic efficiency of AS-AmP because they are the first step in assessing its clinical efficacy. Following treatment with test and reference therapy medications.[36, 38].

1.6. In Vivo Pharmacokinetic Analysis.

Figure 4 shows the plasma concentration-time arc following the ingestion of Amp and Paper AS-AmP in rats at a dosage of 1 mg kg^{-1}. The area under the curve (AUC) of AS-AmP rose 6.2-fold when compared to free AmP. Furthermore, the t1/2 of AS-AmPremained increased by 3.72 times, resulting in a reduction in permission (**Table 4**). The medication containing extract produced a greater plasma concentration of AmP[39].It considerably slowedclearance the of drug, indicating i.e., steady oral drug delivery method that remains in circulation for an extended period and has a major impact on AmP's pharmacokinetic profile. The AS-AmP has no side effects, and the weight of animal and behaviour of the animal were evaluated following its delivery. Following oral delivery, all rodents resumed activity and appeared strong. The weight of rodents given AS-AmP fell somewhat in a pattern alike to the normal group, indicating regular growth in the absenteeism of any notable harmful side effects[40](**Fig.5,6**).

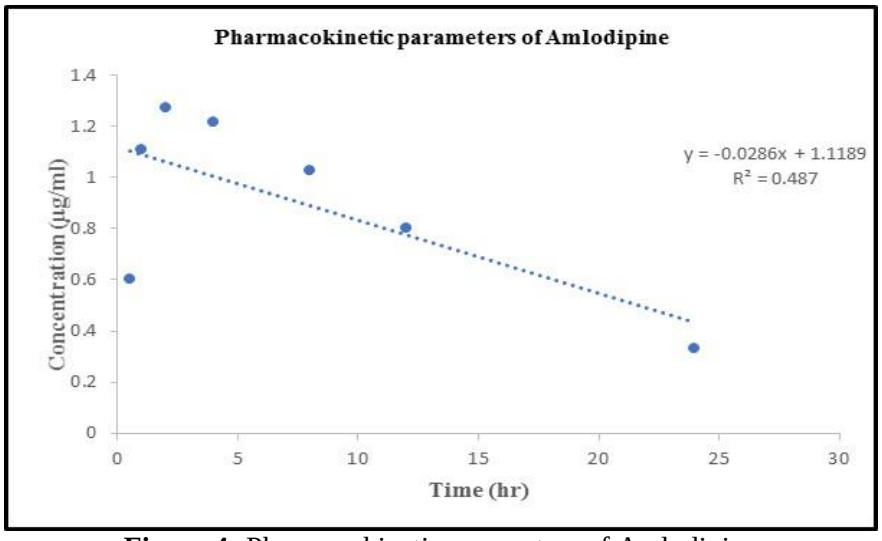

Figure 4: Pharmacokinetic parameters of Amlodipine

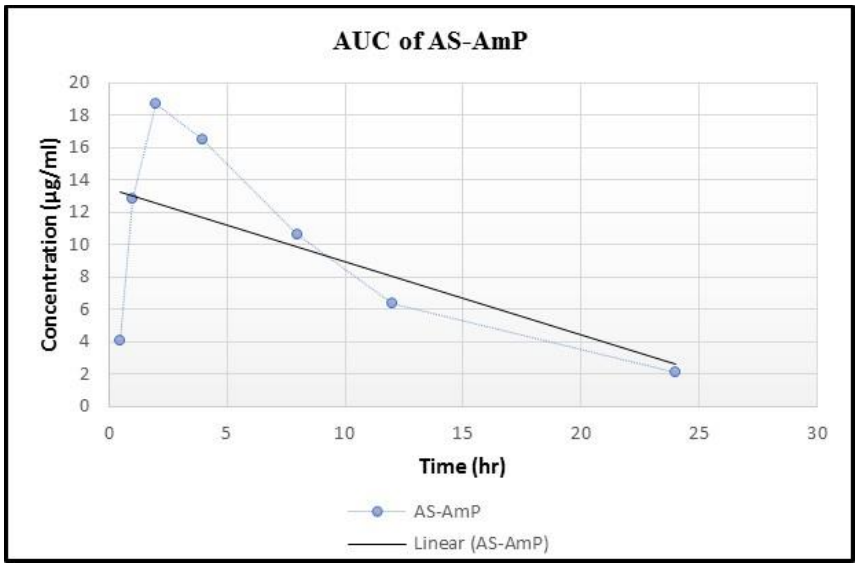

Figure 5: AUC of Amlodipine with AS; Allium Sativum

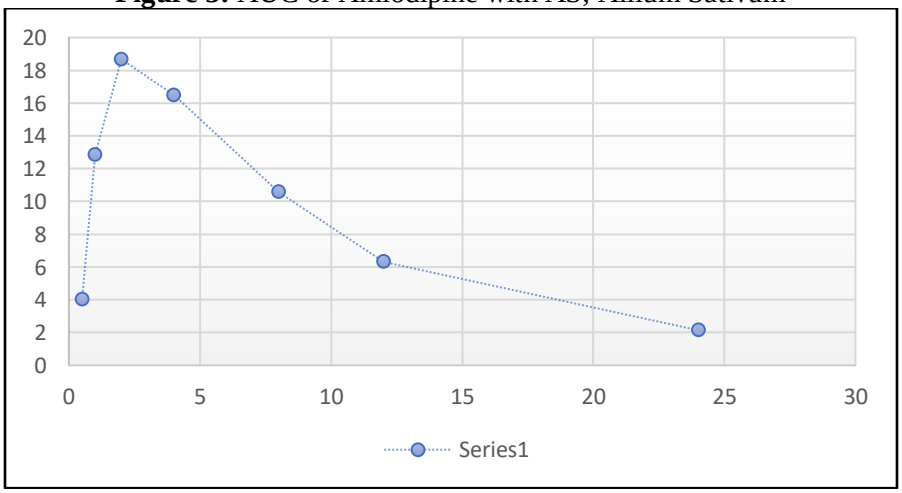

Figure 6: AUC of Amlodipine with AS; Allium Sativum in Hypertensive rats

Table 4 Pharmacokinetic parameters of Amlodipine and Amlodipine+ AS; Allium Sativum (mean ± SD, n = 6)

Comparative AUC Amlodipine Vs Amlodipine+ AS; Allium Sativum						
Time	Control Amlodipine Group			Amlodipine+ AS; Allium Sativum Group		
Hr	Plasma drug Concentration (Ug/ml)	Log (plasma drug Concentration (ug/ml)	AUC	Plasma drug Concentration (Ug/ml)	Log (plasma drug Concentration (ug/ml)	AUC
0.5	4.022	0.604442066	4.218	2.46	0.390935107	2.8525
1	12.85	1.108903128	15.76	8.95	0.951823035	14.535
2	18.67	1.271144318	35.15	20.12	1.303627976	32.44
4	16.48	1.216957207	54.12	12.32	1.090610708	42.32
8	10.58	1.024485668	33.8	8.84	0.946452265	26.42
12	6.32	0.800717078	50.76	4.37	0.640481437	38.1
24	2.14	0.330413773		1.98	0.29666519	

1.7. Histopathological Examination.

When AS-AmP (1 mg kg−1) was administered, microscopic analysis of the kidney, liver, spleen, and heart showed no appreciable changes in comparison to the organs in the control group (**Fig. 7**). There were no indications of hyperplasia or degeneration. Nevertheless, treatment with the free drug AmP caused a significant amount of tissue necrosis.

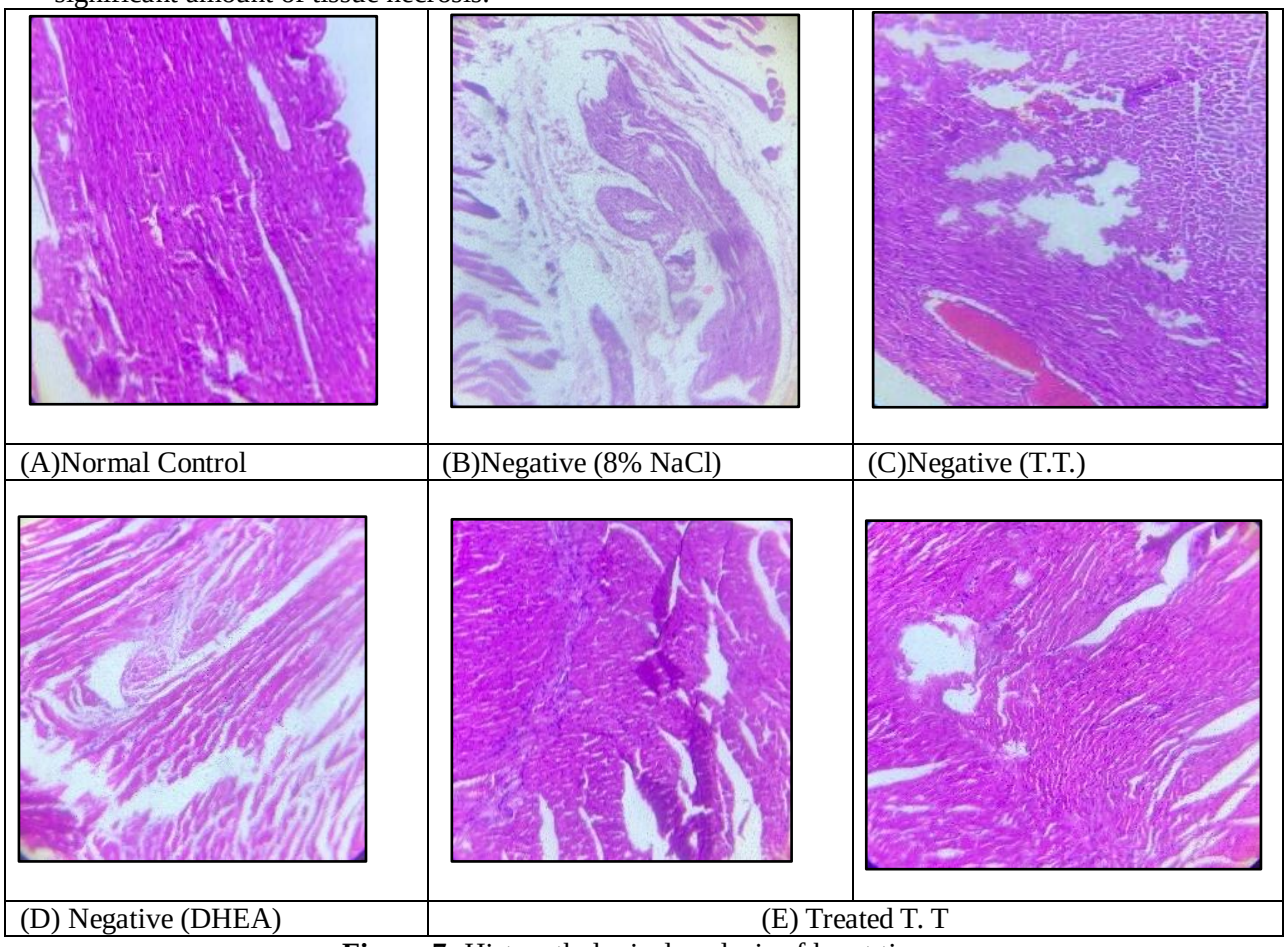

Figure 7: Histopathological analysis of heart tissue

4. CONCLUSION.

In this work, we showed how to use AS-AmP as an excellent surface functionalisation material for SLNs in order to maintain significantly enhanced viable cells and tolerate the harsh conditions of the gastrointestinal tract, all while improving the absorption of the medication encapsulated in the mouth. It is unknown how this affects internalisation mechanisms because certain L Type transmitters may be able to identify calcium channel blockers. Furthermore, it was necessary to investigate the active mechanism related to AS-AmP, which was revealed to be dose-dependently mediated. We also determined that simply eating the AS-AmP is a promising and exceedingly successful therapy strategy for hypertension that does not cause cellular toxicity or significant changes in membrane viscoelasticity. The drug's capacity to hold onto continuous mucus when taken with herbs has a significant effect on intestinal absorption and bioavailability. A very effective therapeutic antihypertensive effect was seen in vivo based on our prior in vitro investigations. One of the most pressing issues is whether the medication stays intact after internalization or gradually degrades from the carrier. Equilibrium study and FRET analysis thoroughly examined this issue. All things considered, the results show that the medication is secure and biocompatible when used with allium sativum extract.

Declaration of Competing Interest

The authors declare that they have no known competing financialinterests or personal relationships that could have appeared to influencethe work reported in this paper.

Ethical approval

All applicable procedures performed in studies involving animals were in accordance with the ethical standards of the institution ethical committee. In this paper authors do not contain any studies with human participants.

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