Trends in
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Protective Effects of Silymarin Against Amphotericin-B-associated Nephrotoxicity in Rats: *in* Vivo and *in* Silico Studies

Oluwafemi Ezekiel Kale[1,2*],Olaoluwa Temitope Talabi[3], Muinat Moronke Adeyanju[4] , Ambrose Oche Gorge[5], Temitope Funmi Kale[2], Adebola Dan Deru[6],Foluke Debora Adewunmi[6], Abidemi James Akindele[7], Olufunsho Awodele[7]

[1]Department of Pharmacology and Therapeutics, Olabisi Onabanjo University, Ago-Iwoye, Sagamu Campus, Ogun State, Nigeria.
[2]Department of Pharmacology, Benjamin Carson (Snr.) School of Medicine, Babcock University, Ilishan Remo, Ogun State, Nigeria.
[3]Department of Biochemistry, College of Medicine, PMB 12003, Idi-Araba Campus, University of Lagos, Nigeria
[4]Department of Biochemistry, Olabisi Onabanjo University, Ago-Iwoye, Sagamu Campus, Ogun State, Nigeria.
[5]Department of Biochemistry, University of Ilorin, *Ilorin*, Nigeria.
[6]Department of Biochemistry, Benjamin Carson (Snr.) School of Medicine, Babcock University, Ilishan Remo, Ogun State, Nigeria.
[7]Department of Pharmacology, Therapeutics and Toxicology, College of Medicine, PMB 12003, Idi-Araba Campus, University of Lagos, Nigeria

Keywords: Nephrotoxicity, Amphotericin, Silymarin; Wistar Rats; Molecular Docking.

Abstract: Amphotericin B (AmB) is a well-known antifungal drug but its use is limited by its nephrotoxicity. This study investigated the possible modulatory effect of Silymarin during AmB induced nephrotoxicity using both *in* vivo and *in* silico methods. Adult rats were divided into six groups of seven per group and assigned into the control, AmB (2.5 mg/kg, i.p.), silymarin(dose), AmB (2.5 mg/kg) plus silymarin (0.5, 1 and 2 mg/kg, p.o.) groups. Treatments lasted for seven days and all rats the were sacrificed 24 hours after the last administration. Changes in biochemical, antioxidant parameters, and kidney histopathology were assessed. Also, *in* silico analyses using the PyRx, and Auto DockVina exhaustive search docking function were also conducted. From the results, AmB administration produced significantly ($p < 0.05$) elevated serum creatinine, urea and uric acid levels by 187 %, 100.2 % and 58.4 % compared with the control group. Similarly, serum total cholesterol (TC) (80.2 %) and malondialdehyde (MDA, 145.5 %) levels were increased. Reduced glutathione (GSH) and high-density lipoprotein (HDL) levels were significantly ($p < 0.05$) lowered in AmB-administered rats. Silymarin treatments lowered creatinine, MDA, and increased GSH, HDL, catalase levels respectively. Silymarin significantly lowered ($p < 0.05$) TC levels and restored kidney damages in treated rats. Molecular docking results, indicated that silymarin had the highest binding energy of -5.2 kcal/mol and demonstrated potentials

*Corresponding author:
kale.oluwafemi@oouagoiwoye.edu.ng;
kalefemi@gmail.com
; +2347063368353
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at the active site of CYS18 and HIS19 hydrogen bonds residues or PHE47, TYR68, PRO31, MET18, LYS80, ILE82, and LEU33 hydrophobic interactions. Overall, these results showed that silymarin possesses the potential to offer chemo preventive benefits against AmB-associated toxicity, although, further trials to unravel the clinical relevance will be explored.

INTRODUCTION

Amphotericin B (AmB), a polyene macrolide, is one of the oldest first-line drugs used for severe systemic mycoses and it is well-known nephrotoxic drug (Houšť *et al*., 2020). Clinically, AmB has been recognized to cause serious complications and other fatal consequences including tubular damage, hypokalemia, and hypomagnesaemia, renal tubular acidosis, inability to concentrate urine and subsequently acute renal failure (Scorzoni *et al*., 2017; Itoh *et al*., 2021). Several potential pharmacological mechanisms suggested vasodilation achieved through the antioxidant defense system within the renal system can help cushion its nephrotoxicity effect (Dabak and Kocaman, 2015; Sottero *et al*., 2019; Itoh *et al.*, 2021). Several potential pharmacological mechanisms have been proposed to ameliorate AmB adverse drug effects (Giacobbe *et al.,* 2021). There is growing evidence that antioxidant agents may offer such chemo preventive benefits (Silva *et al*., 2021). Antioxidants are biological agents whose primary function is to scavenge and stabilize free radicals which cause oxidative damage in a biological system (Sottero *et al*., 2019; Nury *et al*., 2021). Silymarin, a flavonolignans, was synthesized from the seeds and fruits of the milk thistle *Silybumm arianum* (Abenavoli *et al*, 2018). Silymarin acts in different ways, the most important being to prevent lipid peroxidation formation through the antioxidant system activation (Soleimani *et al*., 2019; Silva *et al*., 2021). Silymarin was approved for use in humans and it is very effective in combating hepatic related diseases (Abenavoli *et al*, 2018). Thus, silymarin is currently indicated for alcoholic hepatitis, acute and chronic hepatitis, fatty liver disease and has demonstrated chemoprotective potentials for renal diseases (Dabak and Kocaman, 2015; Guzel *et al*., 2019). Additionally, silymarin has strong affinity for mouse testis specific cytochrome c (T-Cc) when docked into its binding pocket which has attracted scientific interests in recent times (Delmas *et al*., 2020). Thus, we found in the database T-Cc heme with a greater resistance against H_2O_2 degradation to be suitable for silymarin in the database and this was used as docking molecule against the AmB-induced toxicity. There is paucity of information on possible molecular insight through which silymarin can act to reverse the lipid peroxidation products released by AmB*in*silico. Understanding the modulatory effect of silymarin during AmB nephrotoxicity would enhances our knowledge of the possible molecular interaction between silymarin and AmB. Therefore, this study investigated the effects of silymarin during AmB induced renal toxicity in male Wistar rats.

MATERIALS AND METHODS

Chemicals

Amphotericin-B (AMFOCARE^{TM}) was purchased from Rajasthan Antibiotics Limited, RIICO Industrial Area (Rajasthan, India). Silymarin (Silybon-140) was purchased from Micro-Labs Limited, (Sipcot, Hosur, India). All other chemicals used in this study were of the highest purity available and analytical grade.

Experimental animals

Forty-two (42) male albino Wistar rats (140 - 200 g) were purchased from Ibadan, Oyo State, Nigeria, and were acclimated for 14 days. The animals were housed under a 12-h lighting/dark period (light on 6:00 a.m. - 9:00 p.m.). Beddings were changed on alternate days. All animals were allowed free access to rat pellets (Grower Mash, Oyo State, Nigeria) and water *ad* libitum (Babcock University tap water via an automatic water supplying system) throughout the period of this experiment.

Ethics

All experimental procedures were conducted after approval of the study by the Babcock University Health Research Ethics Committee (BUHREC/247/19) and the Institutional Animal Care and Use Committee of Benjamin S. Carson (Snr.) School of Medicine, Babcock University, Ilishan and followed the procedures of the ARRIVE guidelines as documented by Kilkenny *et al*. (2010) for reporting animal research. Following the acclimatization period, rats were weighed and randomly divided into six groups of seven/group.

Experimental design

This study was a 7-day repeated-dose experiment. The AmB was administered during the last four days following the method of Tonomura *et al*. (2009) with slight modifications. Treatments with silymarin was given for the first 3 days and continued from day 4 through day 7. AmB powder was dissolved in a 0.9 % normal saline solution at the required concentration. The following treatment groups were employed:

Group 1: Control (0.9 % normal saline,10 mL/kg, p.o.),

Group 2: AmB (2.5 mg/kg i.p.),
Group 3: Silymarin (2 mg/kg p.o.),
Group 4: AmB (2.5 mg/kg, i.p.) + silymarin (0.5 mg/kg p.o.,),
Group 5: AmB (2.5 mg/kg, i.p.) + silymarin (1 mg/kg, p.o.,),
Group 6: AmB (2.5 mg/kg, i.p.) + silymarin (2 mg/kg p.o.,).

Silymarin was administered to rats 1 hour prior to AmB. Silymarin was administered at the sub-therapeutic, and therapeutic doses and calculated based on 70 kg body weight for a physiological man. AmB was given by injection in order to allow for proper absorption. In addition, during this study, animal weight for all groups was monitored on days 1, 3 and 7 respectively.

Necropsy

All the rats in each group were euthanized humanely on day 8, twenty-four hours after the last administration. Animals were sacrificed by cervical dislocation. The blood samples were kept in plain bottles and serum was separated by centrifugation at 3500 × g for 7 min with cooling. The left and right kidneys of the rats were carefully excised and cleared of any adhering tissue, weighed and expressed in grams per kilogram body weight. The right kidney was processed for homogenization and used for oxidative stress assay while left kidney was fixed in 10% formol saline, dehydrated in graded alcohol, sectioned and was stained with hematoxylin and eosin (H&E).

Biochemical assays

Creatinine, urea, uric acid, total bilirubin, total cholesterol (TC) and high-density lipoprotein (HDL) levels were assessed using commercial kits obtained from Randox Laboratories Ltd. (Crumlin, UK) and following procedures described by the manufacturers. Renal lipid peroxidation (malondialdehyde) and reduced glutathione (GSH) levels were estimated following the methods of Shahidi and Zhong (2010) and Rahman *et al*. (2006) respectively. The method described by Iwase *et al*. (2013) was used to determine the activity of catalase (CAT).

Ligand selection, accession and preparations of the target protein and standard

The chemical structure of silymarin was obtained from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov). The Molfiles and structure-data file (MOL SDF) format of this ligand was converted to PDBQT file using PyRx tool to generate atomic coordinates and the energy was minimized by optimization using the optimization algorithm at force field set at mmff94 (required) on PyRx.The protein, mouse testicular cytochrome C (T-Cc) at 1.6 Angstrom was prepared by retrieving its three-dimensional crystal structure (PDB: 2AIU) from RCSB PDB (http://www.rcsb.org/pdb/home/home.do).
Subsequently, the bound complex molecules with the proteins were removed. The non-essential water molecules and all heteroatoms were detached using Pymol tool and Discovery studio 2017R2. The crystallized ligand was extracted from the active site and it grid coordinate was revealed around the binding pocket when viewed on pymol and Discovery studio 2017R2 visualizer. The standard compound used in the present study was the co-crystalized ligand of the mouse testicular cytochrome C (PDB: 2AIU). The structure of the standard (Protoporphyrin IX containing Fe) (PDB Ligaind ID: HEM) extracted from the receptor's active site was converted to PDBQT file using PyRx tool to generate atomic coordinates and energy was minimized by optimization using the optimization algorithm at force field set at mmff94 (required) on PyRx.

Molecular docking using pypx

Subsequent to receptor and ligands preparations, molecular docking analysis was performed using PyRx, Auto DockVina option based on scoring functions and analysed using the same exhaustively. Following minimization process, the grid box resolution was centered at -0.0705 * 14.3637 * 6.1324 along the x, y and z axes respectively at grid dimension of 25 * 25 * 25 Å to define the binding site (Figure 1a and 1b). The standard was first docked within the binding site of T-Cc and the resulting interaction was compared with that of silymarin into the similar active sites using the same grid box dimension (Tuttle *et al*., 2005).

Statistical analysis

All results were expressed means ± standard error of the mean. Differences between groups were determined by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, version 20.0) software for windows and Post hoc test for intergroup using the least significant difference, followed by Dunnett's test. Significance was considered at $p < 0.05$.

RESULTS

Biochemical indices

AmB (2.5 mg/kg) administration produces elevated serum creatinine, urea and uric levels by 18 7%, 100.2 % and 58.4 % respectively compared with the control normal saline group (Table 1). Animals that

received AmB + silymarin (1 mg/kg) and AmB + silymarin (2 mg/kg) had lowered creatinine levels by 37.4 % and 60.9 % compared with control AmB group. Similarly, therapeutic doses of silymarin (1 mg/kg and 2 mg/kg) evoked reduced abnormal urea levels by 43.4 % and 53.9 % compared with the AmB untreated group. The co-administrations of silymarin with AmB non significantly decreased uric acid levels at all doses used in this study ($p > 0.05$). Administration of AmB (2.5 mg/kg) produced elevated serum total cholesterol level by 83.5 % and lowered HDL levels by 42.1 % when compared with the control normal saline group (Table 1). Therapeutic dose of 1 mg/kg was more effective in lowering the TC level by 41.6 % in the treated rats, whereas all the silymarin doses used improved HDL levels when compared with the control.

Lipid peroxidation

The administration of AmB (2.5 mg/kg) produced an increased MDA levels by 145.5 % compared with the control normal saline group (Table 2). Significant ($p < 0.05$) reduction was obtained in all the animals that received Silymarin intervention before AmB.

Antioxidants

Reduced glutathione (GSH) level was lowered by 61.6 % in AmB (2.5 mg/kg) intoxicated rats compared with control normal saline group (Table 2). However, the levels of GSH remained elevated in all treated rats that received AmB + silymarin combinations ((93.7 %, 124.2 % and 140.2 %). Additionally, silymarin 1 and 2 mg/kg caused improved CAT activities animals by 48.3 % and 125.4 % respectively.

Organ weight

Kidney weight relative to body weight remained significantly unchanged in all rats, although, AmB (2.5 mg/kg) when administered alone causes a slight reduction by 7.3 % (Table 2).

In silico study

The binding potential of Silymarin and HEM within T-Cc binding pocket showed target Silymarin as the lead compound with the binding energy of -5.2 kcal/mol while that of the co-crystalized ligand, target HEM, was -4.9 kcal/mol (Figure 2, 3). Also, silymarin demonstrated effective interactions at the three (3) hydrogen bonds that involved CYS18 and HIS19 residues; and eight (8) hydrophobic interactions that involved PHE47, TYR68, PRO31, MET18, LYS80, ILE82, and LEU33 residues (Figures 2, 4). More so, silymarin had a higher binding affinity to the binding pocket of T-Cc when compared with the co-crystallized ligand due to the hydrophobic bonds present in luecine (8) (Figures 4 and 5).

Table 1. Effect of Silymarin on Biochemical Parameters in Normal and AmB-treated male rats.

Treatment	Control	AmB (2.5 mg/kg)	Silymarin (2 mg/kg)	AmB + Silymarin (0.5 mg/kg)	AmB + Silymarin (1 mg/kg)	AmB + Silymarin (2 mg/kg)
Creatinine	1.03±0.03	2.89±0.06* (-187.6) [a]	1.13±0.01 (-9.7) [a]	1.85±0.01 (35.9) [b]	1.81±0.04† (37.4) [b]	1.13±0.02† (60.9) [b]
BUN	9.35±0.17	18.72±0.55* (-100.2) [a]	9.05±0.23 (3.2) [a]	13.00±0.42 (30.6) [b]	10.59±0.54 (42.4) [b]	8.63±0.12† (53.9) [b]
UA	14.12±0.42	22.37±0.37* (-58.4) [a]	14.53±0.36 (-2.9) [a]	17.22±0.28 (23.0) [b]	15.42±0.51† (31.1) [b]	14.28±0.12† (36.2) [b]
TC	0.96 ± 0.08	1.73 ± 0.04 (-80.2) [a]	1.04 ± 0.03 (-8.3) [a]	1.16 ± 0.02 (32.9) [b]	1.01 ± 0.05 (41.6) [b]	1.06 ± 0.05† (38.7) [b]
HDL	1.90 ± 0.02	1.10 ± 0.07* (42.1) [a]	1.70 ± 0.04 (10.5) [a]	1.20 ± 0.01 (-9.1) [b]	2.10 ± 0.05† (-90.9) [b]	2.22 ± 0.02† (-101.8)[b]

n = 7. Uric acid (UA): mg/dl; Blood Urea Nitrogen (BUN): mg/dl; Total Cholesterol (TC): mg/dl; High density lipoprotein (HDL): mg/dl. Values in parentheses represent % change; (–) = increase, (+) = decrease. [a]% change relative to control normal saline group. [b]% change relative to AmB (2.5 mg/kg) group. $^{*}p < 0.05$ or $^{**}p < 0.01$ compared with control (normal saline group), $^{\dagger}p < 0.05$ or $^{\dagger\dagger}p < 0.01$ compared with control AmB (2.5 mg/kg) group.

Table 2. Effect of Silymarin on Lipid Peroxidation and Antioxidant Indices in Normal and AmB-treated male rats.

Treatment	Control	AmB (2.5 mg/kg)	Silymarin (2 mg/kg)	AmB + Silymarin (0.5 mg/kg)	AmB + Silymarin (1 mg/kg)	AmB + Silymarin (2 mg/kg)
MDA	9.30 ± 0.02	22.83 ±0.03** (-145.5) [a]	9.19 ±0.04 (1.2) [a]	13.17 ± 0.02† (42.3) [b]	10.57 ± 0.02† (53.7) [b]	9.47 ± 0.06† (58.5) [b]
GSH	3.31 ± 0.04	1.27 ± 0.03* (61.6) [a]	4.54 ±0.06 (-37.1) [a]	2.46 ± 0.04 (-93.7) [b]	2.86 ± 0.04† (-125.2) [b]	3.05 ± 0.05†† (-140.2) [b]
CAT	3.11 ± 0.06	2.40 ± 0.07 (22.8) [a]	2.63 ± 0.09 (15.4) [a]	2.56 ± 0.04 (-6.7) [b]	3.56 ± 0.06† (-48.3) [b]	5.41 ± 0.07† (-125.4) [b]
Kidney (g/kg BW)	3.66 ± 0.02	3.92 ± 0.03 (-7.1) [a]	3.09 ± 0.02 (15.6) [a]	4.15 ± 0.03 (-5.9) [b]	3.29 ± 0.02 (16.1) [b]	3.14 ± 0.01 (19.9) [b]

n = 7. (MDA) (nmol/mg protein); GSH (µmol/mg protein); Catalase (µmol/ml/mg protein). Values in parentheses represent % change; (–) = increase, (+) = decrease. [a]% change relative to control normal saline group. [b]% change relative to AmB (2.5 mg/kg) group. $^{*}p < 0.05$ or $^{**}p < 0.01$ compared with control (normal saline group), $^{\dagger}p < 0.05$ or $^{\dagger\dagger}p < 0.01$ compared with control AmB (2.5 mg/kg) group.

Table 3: Lipinski's drug-like properties of Silymarin

Molecular Properties	Lipinski's rule of Five	Silymarin drug-like properties
Molecular Mass	<500KDa	488.44
Hydrogen bond Acceptor	<10	10
Hydrogen bond Donor	<5	5
ClogP	<5	2.13
Topological Polar Surface Area	<140A^{o2}	155.14

cLogP: Calculated lipophilicity, A^{o}:Amstrong**.** The rule describes drug candidate's pharmacokinetics in the human body which also including their absorption, distribution, metabolism, and excretion (ADME) using DataWarrior.

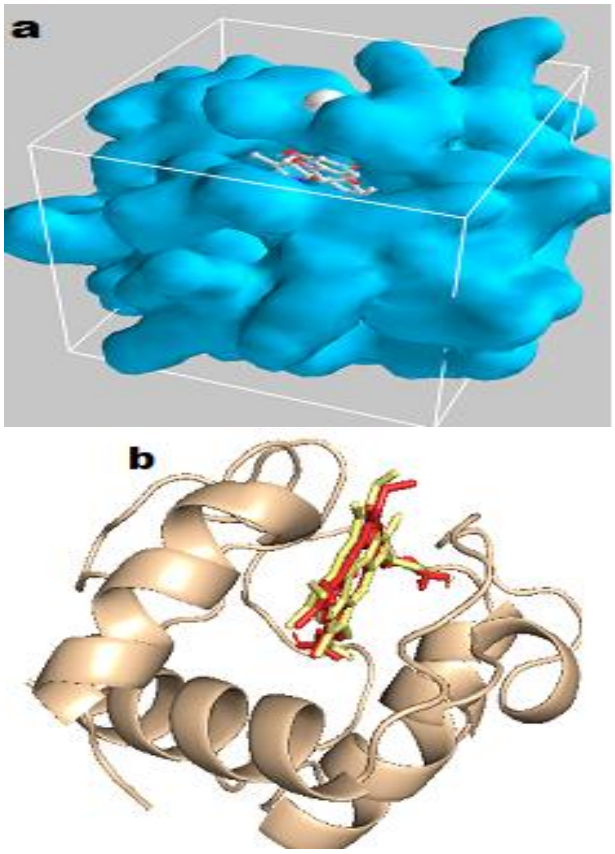

Figure 1: (a) Grid box within which the ligand binds -0.0705 * 14.3637 * 6.1324 along the X, Y, Z-axis (b) Validation of docking: Comparability of the re-docked binding mode and the co-crystallized pose of HEM with the accompany residues of T-Cc binding pocket (a snapshot from PyRx).

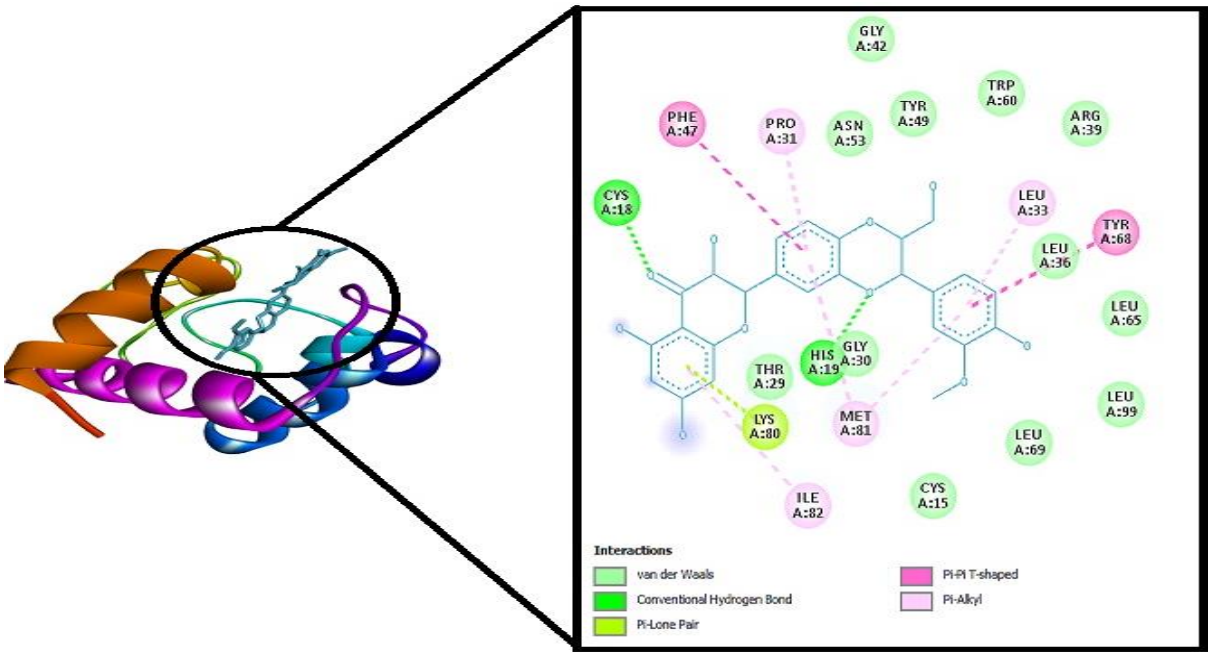

Figure 2: The 3D and 2D interactions of Silymarin within the binding site of T-Cc.

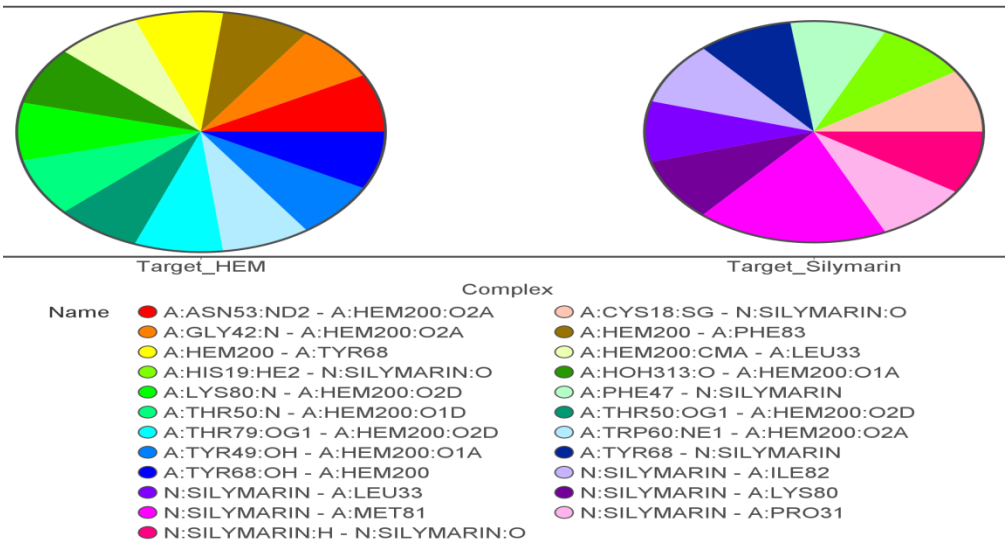

Figure 3: Interaction associated with both silymarin and HEM within T-Cc binding pocket.

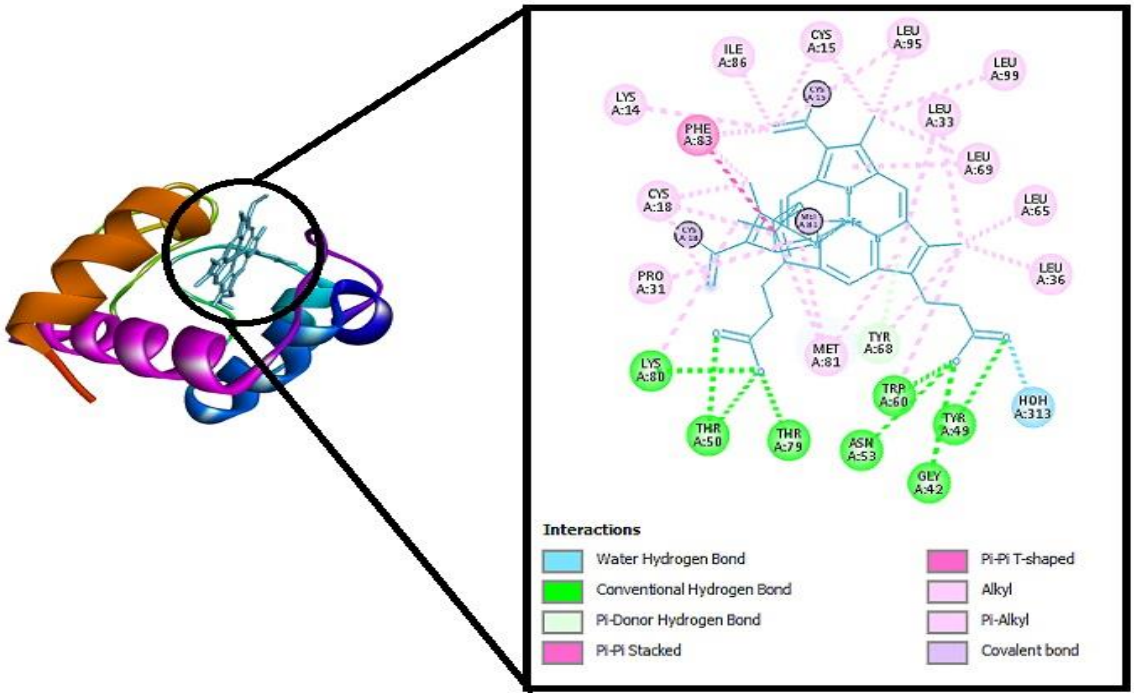

Figure 4: The 3D and 2D interactions of HEM within T-Cc binding site.

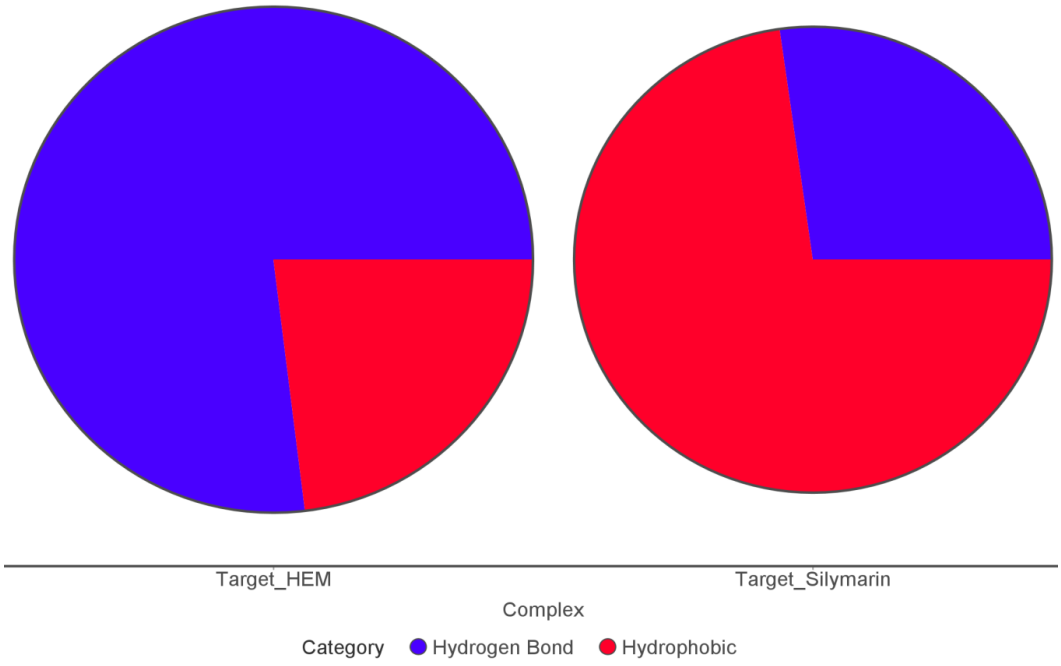

Figure 5: Comparison of both Hydrogen bonds and hydrophobic interactions associated with Silymarin and HEM within T-Cc binding pocket.

Histology

The kidney of AmB (2.5 mg/kg) and AmB + silymarin (0.5 mg/kg) treated animals showed normocellular glomerular tufts disposed on a background containing viable tubules with congested blood vessels (Figure 6). Renal abnormality was restored in treated rats that received 1 mg/kg and2 mg/silymarin.

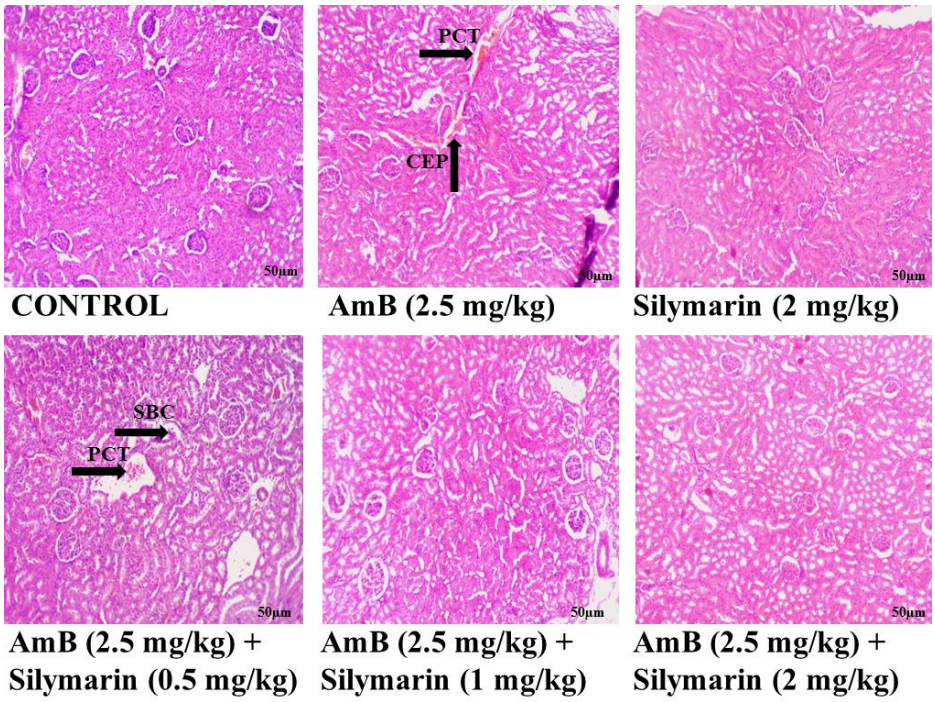

Figure 6: Histology sections of kidney tissue of normal and amphotericin (AmB) treated male rats. Control, Silymarin (2 mg/kg), AmB + Silymarin (1 mg/kg) and AmB + Silymarin (2 mg/kg) showed normocellular glomerular tufts disposed on a background containing renal tubules with no abnormality seen (normal kidney). AmB (2.5 mg/kg) and AmB + Silymarin (0.5 mg/kg) showed normocellular glomerular tufts disposed on a background containing viable tubules with congested blood vessels seen (Vascular Congestion). SBC: Space of Bowman's Capsule, C(EG): Congested Erythrocytes in Glomerular Capillaries, PCT: Proximal Convoluted Tubule (H & E stain, mag. × 100).

DISCUSSION

The study on the modulatory effects of Silymarin during renal toxicity induced by AmB in male rats correlated with the predicted molecular fingerprint *in* silico. AmB is still regarded as a life-saving drug in curing severe fungal infections since it has no direct replacement (Houš *et al*., 2020). But, down casting the natural antioxidant system by AmB could be a dilemma. Nephrotoxicity associated with AmB is strongly limiting its applications (Loo *et al*., 2014; Itoh *et al*., 2021). So far, N-acetyl cysteine, pentoxifylline, coated chitosan, electrolyte supplementations, immunoadjuvant have been demonstrated in preclinical studies of AmB-associated toxicity but not all were suitable for bioavailability and renoprotection (Singh *et al*., 2017; Giacobbe *et al.,* 2021). Some medicinal plant derived antioxidants attenuated the products of AmB-induced oxidative stress and are currently undergoing clinical trials (Soleimani *et al*., 2019; Nury *et al.,* 2021). Silymarin comprises of silibinin, silydianin, and silychristin and it is a very popular antioxidant with documented evidence for safety and toxicity profile (Soleimani *et al*., 2019; Nury *et al.,* 2021). In this study, therapeutic doses of silymarin ameliorated AmB-induced elevated renal function test of serum creatinine, urea, and uric acid levels respectively. This is in line with the results of other authors that reported the potential roles silymarin could play in the vicinity where chemically-induced nephrotoxicity thrives (Prabu and Muthumani, 2015; Al-Sa'aidi, 2019). We further confirmed the AmB-induced elevated renal biomarker function test which was accompanied by an increased MDA levels in rats. In this study the therapeutics dose of Silymarin effectively mopped up the elevated MDA and this agrees with a study by Jalali *et al*. (2009) were silymarin combined with deferoxamine protected against iron overload in the kidneys of animals. Silymarin has been shown to boosts antioxidant enzyme release and attenuates elevated MDA, H_2O_2 and advanced oxidation protein products respectively (Abdel-Magied and Elkady, 2019; Guzel *et al*., 2019; Silva *et al*., 2021). Interestingly, from the result obtained in this study, Silymarin demonstrated the potential for antihyper cholesterolaemia and improved HDL levels in the treated rats. This agrees with other reports that the nephrotoxicity effect of AmB is enhanced in lipid

membrane-mediated hypercholesterolemic animals (Kamiński, 2014). Similarly, antioxidants GSH and CAT level remained elevated in all treated rats that received Silymarin treatments in this report. Further correlation was obtained *in* silico, in which silymarin was docked into the binding pocket of T-Cc for its T-Cc activation (agonistic) property (Delmas *et al*., 2020). In this study, Silymarin was discovered as the lead compound with the binding energy of -5.2 kcal/mol while that of the co-crystalized ligand was -4.9 kcal/mol. We validated the accuracy of our docking protocols by re-docking the co-crystallized ligand (PDB Ligand ID: HEM) back into the binding pocket of the T-Cc (PDB: 2AIU). As observed in this study, the re-docked pose overlapped almost totally with the experimental orientation, indicating that Auto dockvina on PyRx re-docked the co crystallized ligand, with a very high accuracy, back into the binding pocket of the T-Cc. This was used to ensure the reliability of our docking methodology and the docking scores obtained (Figure 1b). And when subjected to the Lipinski's rule of five, to assess the drug-likeness of silymarin, silymarin violated none of these rules. The highest binding energy (-5.2 kcal/mol) attributed to silymarin in this regard was believed to be as a result of its chemical interactions at the receptor's active site (Figures 3 and 4). The results showed three (3) hydrogen bonds involving CYS18 and HIS19 residues plus eight (8) hydrophobic interactions involving PHE47, TYR68, PRO31, MET18, LYS80, ILE82, and LEU33 residues were obtained. This defines the importance of hydrophobic interactions in the design of drugs. Previous study has shown that hydrophobic interactions can increase the binding affinity between target drug interfaces (Delmas *et al*., 2020). This explains why silymarin had a better agonistic effect when compared with the co-crystalized ligand as seen in our results. It may therefore suggest that the higher binding affinity of silymarin to the binding pocket of T-Cc when compared with that of the co-crystallized ligand may be attributed, in part, to the number of hydrophobic bonds present in luecine (eight) as compared to the standard (three) (Figures 5 and 6). The chemo preventive role of Silymarin received further support from its ability to abrogate the morphological injuries associated with changes in the AmB untreated rat kidneys. These results suggest the involvement of Silymarin in modulating AmB-induced renal toxicity which informed our consent for further studies involving clinical and experimental researches.

CONCLUSION

The protective role of silymarin against AmB-induced damages might result from its anti-oxidative effects, anti-hypercholesterolemia and the ability to protect kidney damage. Also, study of *in* silico further supports its ability to scavenge AmB-associated lipid peroxidation molecules. This positions Silymarin as a potential adjuvant that may help protects against AmB-induced renal toxicity.

ABBREVIATIONS

AmB: Amphotericin-B; LPO: Lipid peroxidation; T-Cc: Testis specific cytochrome c; H_2O_2: Hydrogen peroxide; ROS: Reactive oxygen species; MDA: Malondialdehyde; GSH: Reduced glutathione; CAT: Catalase; S-Cc: somatic cells Cytochrome c; PDB: Protein databank; mmff94: Merck molecular force field 94; SDF: Structure-data File; H-acceptors: Hydrogen acceptors; H-donors: Hydrogen donors; cLogP: Calculated lipophilicity

COMPETING INTEREST

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