Indigenous Indian Guggul extract augments saxagliptin effect against diabetes-induced complications

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Abstract

Type 2 Diabetes Mellitus is an endocrine metabolic disorder leading to impaired glucose utilization. This disease is managed by both commercial drugs and herbal products eliciting various interactions. The evaluation of one such interaction between saxagliptin (SAXA, substrate of CYP3A4), and guggul extract (GE) from *Commiphora wightii* is done in this present study. The concomitant administration of the SAXA with GE had restored otherwise increased blood glucose and biochemical parameters' levels more effectively as compared to the solely consumed SAXA or GE. These results were corroborated by histopathological evaluation where the combination treatment showed better mitigation of hepatic, renal and pancreatic tissue damage that occurred due to induced diabetes. Furthermore, CYP3A11 mRNA (murine homolog to human CYP3A4) expression levels were observed to be reduced to non-diabetic levels in combination therapy which was otherwise unachievable. The molecular docking studies predicted improved interaction between CYP3A4 and bioactive content of GE, i.e., guggulsterone E-Z (G E&Z), possessing binding energy = -9.96 kcal/mol along with SAXA, confirming the co-existence of both the ligands at different binding sites of the metabolizing enzyme. The synergistic interactions of GE and SAXA helped in better restoration of tissue damage and CYP3A11 mRNA expression levels caused by induced diabetes.

Introduction

Type 2 Diabetes mellitus (T2DM) is a chronic metabolic disease that occurs when the body is incapable of making good use of the produced insulin [1]. Several oral anti-diabetic drugs (OADs) are being used to treat T2DM. These drugs vary in their mechanism of action but the prime aim is to lower the blood glucose level by enhancing insulin activity. Saxagliptin (SAXA) belongs to the class gliptin of OADs and has been recently introduced in pharma markets. The gliptins inhibit the activity of DPP-4 (dipeptidyl peptidase-4), an enzyme that inactivates and degrades incretin hormones, cytokines, and other peptides. SAXA is the only drug of this class that is metabolized by CYP3A4 (a major drug-metabolizing hepatic enzyme). Although effective, this medication has been reported to have certain associated adverse effects. Moreover, the high cost of this drug makes space for alternative options for the treatment of diabetes [2,3].

Patients relied heavily on herbal remedies to complement conventional therapies for the treatment of diabetes. A good number of plant species are being used as hypoglycemic agents as are involved in the regulation of carbohydrate metabolism by various mechanisms thus, improving glucose uptake and β-cell regeneration along with control in insulin release [4]. Herbal products are preferred over allopathic medicines because of their low cost, easy accessibility, fewer side effects, and lower dependency among patients. Guggul has been used in Ayurveda for centuries as a treatment for diabetes [5–7]. There are several sources of Guggul and one of them is a small tree, *Commiphora wightii*. The oleo-gum resin (Guggul gum) of this plant exudes out as a result of injury and has enormous benefits, making it a quintessential part of the traditional medicine system. Guggulsterone is the major bioactive compound

found in the gum resin and it has shown antioxidant, hypo-lipidemic, immune-modulatory, and other significant activities [7–9].

Despite several benefits, herbal supplements cannot manage T2DM alone. In such cases when a herb is knowingly or unknowingly co-administered with a drug, it may have certain interactions with the latter. The co-administration of herbs can augment or inhibit the drug's pharmacological activity through additive, synergistic, or antagonistic actions. For instance, *Aloe vera* has been reported to show an additive effect when given in combination with repaglinide, and glibenclamide [10]. Similarly, the combined treatment of *Panax ginseng* and metformin has been shown to elicit an additive antidiabetic effect in comparison to the sole treatment [11,12]. On the other hand, an antagonistic interaction between *Gymnema sylvestre* and metformin has also been reported [13]. Phytochemically, medicinal plants are known to possess a repository of complex mixtures of bioactive compounds that can alter the absorption rate or the oxidative metabolism of the co-administered drug [14].

Considering the various interactions of herbs and drugs concomitantly being administered, our study focuses on the interaction between SAXA and Guggul extract (GE) in an induced diabetic mice model. The effect of combination therapy was evaluated *in vivo* by serum biochemical parameter studies, total glycogen content evaluation of the liver, and histological analysis of liver, kidney and pancreatic tissues. As SAXA is the only gliptin that is metabolized by cytochrome P450 3A4 (CYP3A4), the hepatic mRNA expression levels of CYP3A11 (murine homolog of human CYP3A4) of all the treatment groups were also evaluated and compared. This study was done to understand the mechanism of reversible tissue damage by GE in induced diabetes. The study was further strengthened by predicting a probable interaction of the major phytoconstituent of GE with CYP3A4 through molecular docking studies [15].

Results

Combination treatment restored body weight, fasting blood glucose level, serum biomarker levels, and total glycogen content

In the present study, the diabetic control group showed a reduction in average body weight (19.66 ± 1.15 gms) at the end of the treatment period. The alone and combination treatments showed gain in weight with the highest gain in the SAXA + GE treatment group (32.33 ± 0.57 gms). The fasting blood glucose levels (BGL) rose dramatically after the last dose of STZ (0th day of experimental study) in diabetic control and the three treatment groups (Table 1). However, these increased glucose levels were significantly restored to normal values after four weeks of treatment of SAXA, GE and their combination in comparison to the diabetic control where the 28th -day BGL was 302.66 ± 2.08 mg/dl. In this case, also, the combination dose showed better restoration of increased glucose levels among all three treatment groups (91.33 ± 1.52 mg/dl).

Table 1
Effect of the treatments on body weight and blood glucose levels

Body weight (gms)					
Groups →	**Vehicle control**	**Diabetic control**	**SAXA**	**GE**	**SAXA + GE**
Day ↓					
0th Day	26.66 ± 0.57 ^{ns}	24.33 ± 0.57	23.33 ± 0.57 ^{ns}	22.33 ± 0.57 ^{ns}	23.66 ± 0.57 ^{ns}
28th Day	33.33 ± 0.57 **	19.66 ± 1.15	30 ± 1.0 **	29.33 ± 0.57 **	32.33 ± 0.57 **
Blood glucose level (mg/dl)					
0th Day	129.66 ± 0.57 **	495.66 ± 3.51	484.66 ± 2.51 ^{ns}	462.33 ± 2.51 ^{ns}	479 ± 3.60 ^{ns}
28th Day	115.33 ± 1.57 **	302.66 ± 2.08	115 ± 3.0 **	128 ± 2.64 **	91.33 ± 1.52 **

Here, ** = highly significant ($p < 0.01$), * = significant ($p < 0.05$) and ns = non-significant when compared with diabetic-control (STZ-treated group)). Analyzed by one-way ANOVA followed by Dunnett's test. n = 5.

In the case of biochemical parameters, the diabetic control group showed significantly high levels of albumin, uric acid, creatinine, ALP, AST, ALT, total cholesterol, and triglycerides. However, a significant reduction in serum biomarker levels & lipid profile was observed in all the treatment groups with the combination group showing the most remarkable decrease in the levels as compared to the diabetic control group (summarised in Table 2). Among the three treatment groups, the combination treatment group showed a highly significant decrease in the kidney function tests (Albumin- 1.54 ± 0.06 g/dl; Uric acid- 4.78 ± 0.45 mg/dl and Creatinine- 2.95 ± 0.729 mg/dl) and liver function tests (alkaline phosphatase (ALP)- 141.88 ± 3.19 IU/L and aspartate transferase (AST)- 130.83 ± 0.816 IU/L) when compared to only SAXA treatment group. Also, a significant reduction was observed in the combination group in the case of alanine transaminase (ALT) and lipid profiles in comparison to only drug treatment. On the other hand, the treatment groups receiving only GE treatment showed a significant or highly significant increase in biochemical parameters when compared to the groups receiving only SAXA as a treatment. The glycogen content in the STZ-treated (diabetic control) group is low (51.55 ± 3.85 µg/ml) whereas, the levels were elevated in groups that received a combination dose (110.44 ± 3.85 µg/ml) much more in comparison to the groups where only SAXA (69.33 ± 3.33 µg/ml) or only GE (93.78 ± 3.85 µg/ml) was administered (Table 2).

Table 2
Effect of different treatments on serum biomarkers level, lipid profile & total glycogen content

S. No.	Biochemical parameters	Vehicle control	Diabetic control	SAXA	GE	SAXA + GE
Kidney Function Tests (KFTs)						
1	Albumin (g/dl)	1.66 ± 0.08	5.08 ± 0.05$^{##}$	2.26 ± 0.04**	3.15 ± 0.03*^^	1.54 ± 0.06**^^
2	Uric acid (mg/dl)	4.54 ± 0.049	9.06 ± 0.41$^{##}$	5.80 ± 0.36**	6.34 ± 0.35*^	4.78 ± 0.45**^^
3	Creatinine (mg/dl)	2.67 ± 0.923	7.47 ± 0.923$^{##}$	3.73 ± 0.923**	4.27 ± 0.923*^	2.95 ± 0.729**^^
Liver Function Tests (LFTs)						
1	ALP (IU/L)	145.57 ± 3.19	280.08 ± 3.19$^{##}$	162.15 ± 3.20**	176.90 ± 5.53*^	141.88 ± 3.19**^^
2	SGOT/AST (IU/L)	117.87 ± 0.968	294.67 ± 0.620$^{##}$	167.37 ± 0.815**	185.05 ± 1.388*^	130.83 ± 0.816** ^^
3	SGPT/ALT (IU/L)	154.40 ± 4.08	307.63 ± 3.54$^{##}$	175.62 ± 2.04**	201.55 ± 3.54*^^	156.76 ± 2.04**^
Lipid profile						
1	Total Cholesterol (mg/dl)	74.95 ± 1.81	210.65 ± 2.37$^{##}$	96.65 ± 1.37**	119.92 ± 2.46*^	86.39 ± 2.05**^
2	Triglycerides (mg %)	110.58 ± 3.99	248.67 ± 3.99$^{##}$	128.57 ± 4.20**	148.15 ± 2.42*^	113.76 ± 3.66**^
Total glycogen content						
1	Glycogen concentration (ug/ml)	109.33 ± 3.33	51.55 ± 3.85$^{##}$	69.33 ± 3.33*	93.78 ± 3.85**^	110.44 ± 3.85**^^

Here, ** = highly significant ($p < 0.01$), * = significant ($p < 0.05$) and ns = non-significant when compared with diabetic-control (STZ-treated group)). $^{##}$ = highly significant ($p < 0.01$) when compared with vehicle control. ^ = significant ($p < 0.05$) and ^^ = highly significant ($p < 0.01$) when compared with drug only (SAXA). Analyzed by one-way ANOVA followed by Dunnett's test. n = 5.

Combination treatment restored diabetes-induced tissue damage

The H& E stained liver tissue from diabetic mice showed a congested central vein and focal necrosis (Fig. 1b) along with a clear sign of steatosis when compared to the treatment groups as well as vehicle control (where no diabetes was induced). Similarly, as shown in Fig. 2b, the kidney tissue showed diabetes-induced alterations like diabetic nephropathy with glomerular congestion and sclerosed glomeruli. To an extent, hepatic and renal damage was also observed in the group administered with drug only (Fig. 1c & 2c) which was found to be recovered if GE treatment was given along with SAXA (Fig. 1e & 2e). The liver tissues of the GE only (Fig. 1d) and combination treatment showed a proper arrangement of hepatocytes which were devoid of necrosis. Similarly, the distractedly arranged nephrons showed signs of recuperation in the kidney tissues. These were even properly arranged into glomeruli (Fig. 2d & 2e**)** The hypolipidemic activity of the extract has also helped in the reduction of fat accumulation in the liver tissues of both groups. The histological study of the pancreatic tissues of vehicle control mice unveiled that the tissues had a normal architecture of islets of Langerhans. The β-cells were intact and appeared granulated and darker in shade. The cells in the islets were densely packed (Fig. 3a). On the contrary, in the STZ-treated (diabetic control) pancreatic tissues, the cell density diminished (Fig. 3b). The pancreatic tissues of SAXA-treated showed improved β-cell architecture however the normal architecture was not restored (Fig. 3c). The tissues receiving GE treatment (Fig. 3d & 3e**)** showed diminishing empty spaces in Langerhans and the cells even regained their compactness.

GE in combination with SAXA reduced hepatic CYP3A11 mRNA expression levels

The quantified mRNA levels were normalized to β-actin (housekeeping gene). In control conditions (i.e., non-diabetic liver), the expression of CYP3A11 was normalized. When diabetes was induced in the mice using STZ, the expression of CYP3A11 increased multifold (~ 7 folds) indicating hepatic damage. This increased response was non-significantly altered by the drug (i.e., SAXA) that was being used as an OAD strategy. The expression in this case decreased to around ~ 3 folds in comparison to the normal control. The GE supplementation (alone and in combination with SAXA) showed restored mRNA expression levels near control conditions.

Different binding pockets of SAXA and GE confirm synergism

The *in silico* study was conducted to predict the predominant binding orientation of the human CYP3A4 (the protein) to the ligands (SAXA and guggulsterone E &Z (G E&Z)) and to further confirm the synergistic role of GE and saxagliptin. The 3-dimensional structures (Figs. 4 & 5) of the best pose of docking revealed that the position of both the ligands on the active site of the target protein is different. The ligands lie in proximity forming either hydrogen, π-alkyl, or alkyl bonds with different amino acids of the protein. The binding energy for ligand-protein interaction was lowered from − 10.27 kcal/mol (when only saxagliptin was docked) to -11.16 kcal/mol (when both saxagliptin & guggulsterone E&Z were docked). Also, the inhibition constant *(Ki*) value showed a notable decrease from 29.41 nM to 6.64 nM, respectively in the case when only saxagliptin was bound and when both saxagliptin & guggulsterone were docked to

CYP3A4 (Table 3). The hydrogen and other hydrophobic bonds involved in the interaction of saxagliptin (a substrate of CYP3A4) with the target protein remained unchanged when G E&Z was docked to saxagliptin-bound CYP3A4. However, one hydrophobic interaction with ALA:370 was lost in the case when G E&Z was docked to saxagliptin-bound protein.

Table 3
Molecular docking statistics of human CYP3A4 enzyme with SAXA and G E&Z (alone and in combination)

Ligand bound with CYP3A4	Free binding energy (kcal/mol)	Inhibition constant (Ki)	No. of hydrogen bonds	H-bond interaction	H-bond length (Å)	Hydrophobic bonds
Saxagliptin	-10.27	29.41 nM	2	GLY: 298, GLY: 444	1.85, 2.89	π-alkyl- PHE: 302, PHE: 447, PHE: 271, PHE: 137 Alkyl- ILE: 184, ALA: 448, ALA: 305, ILE: 301 ILE: 443
Guggulsterone E&Z	-10.61	16.59 nM	2	ARG: 105, GLU: 374	3.50, 1.86	Alkyl- ALA: 370, LEU: 94, LEU: 373, ARG: 440, ARG: 105
Saxagliptin + Guggulsterone E&Z	-11.16	6.64 nM	4	GLY: 298, GLY: 444, ARG: 105, GLU: 374	1.85, 2.89, 3.50, 1.86	π-alkyl- PHE: 302, PHE: 447, PHE: 271, PHE: 137 Alkyl- ILE: 184, ALA: 448, ALA: 305, ILE: 301 ILE: 443, ALA: 370, LEU: 94, LEU: 373, ARG: 440, ARG: 105

Discussion

STZ is a glucose analogue and it accumulates in β-cells of the pancreas forming an alkylating agent which brings out the diabetogenic action of this compound [16]. The uncontrolled high blood sugar levels due to STZ-diabetes induction lead to increased urination, excessive thirst, increased metabolism, protein catabolism, and muscle oxidative damage resulting in unintended weight loss [17]. In the present study, a sharp decline in the weight of the diabetic control group could be attributed to the unavailability of glucose to the cells which compels the body to burn fat causing a reduction in body weight. This loss in weight was regained in all three treatment groups. Saxagliptin belongs to a class of OAD that has the added advantage of being weight-neutral without causing hypoglycemia [3]. Hence this OAD was able to restore the lost weight without any detrimental effects. Also, being a glucagon suppressor, SAXA treatment results in improved fasting hyperglycemia. This restoration in body weight and BGL was further enhanced with GE supplementation as it contains phytoconstituents like guggulsterone which improves

the activity of the peroxisome proliferator-activated receptor (PPAR)-γ gene involved in the metabolism of glucose and lipid [18].

Oxidative stress is the primary cause of the onset and progression of long-term diabetic complications. Persistent hyperglycemia may induce oxidative stress and cause liver, kidney, and pancreas (β-cell destruction) tissue damage [19,20]. In the present study, the increase in specific biomarker levels in the case of diabetic control suggests organ damage. The restored levels of biochemical parameters, when GE was administered alone and in combination with SAXA, could be due to the numerous compounds present in GE. Guggul has been reported to contain 38% oleo-gum resin [18]. Although this resin constitutes many compounds (such as steroids, flavones, lignans, tannins, cembrenoids and terpenes), it is now accepted that its pharmacological action can be owed to guggulsterone [18,21]. The two isomers E & Z guggulsterone have pronounced anti-inflammatory properties. These phytosterols can neutralize the generated reactive oxygen species (ROS), inhibit lipid peroxidation, and even induce metal chelation [22]. Both phytoconstituents are even antagonists for the farnesoid X receptor (FXR, a bile receptor), which is an important regulator of bile/cholesterol homeostasis [23]. Hence, the hypolipidemic activity and ability to reverse tissue damage can be attributed majorly to these two phytosterols.

The diabetes induction using STZ leads to progressive hyperglycemia with normal non-fasting insulin levels and damage to the preserved β-cell mass. The blood glucose level in T2DM rises due to insulin resistance and hence excess glucose is not converted into glycogen [24]. Hence the diabetic control showed low levels of total glycogen content. Guggulsterone, an active constituent of GE, possesses antioxidant properties that can inhibit the lipid peroxidation reaction and thus restore β- cell damage[6]. In the case groups treated with GE, the antioxidants present in the extract have reversed the tissue damage of the pancreas which may lead to enhanced production of insulin. Hence, GE-treated groups have higher glycogen content than drugs. In the case of a combination dose, as both GE and SAXA are insulin enhancers, this treatment showed the highest glycogen content among all three treatment groups.

The liver has a major role in the maintenance of blood glucose levels and the regulation of glucose supply to various other organs. The intolerance to blood glucose may lead to liver tissue damage [25]. Moreover, renal failure is yet another complication of DM. The high blood glucose level leads to the formation of ample reactive oxygen species (ROS) leading to the deterioration of renal tissue [26]. A similar type of damage in kidney and liver tissues is reflected in the induced-diabetic (diabetic control) group in the study. The induction of diabetes using STZ also led to β-cell necrosis [27] (shown by empty spaces) (Fig. 3b). Although saxagliptin has been reported to attenuate liver inflammation and improve liver steatosis conditions, there is a case report stating saxagliptin-induced hepatotoxicity where steatohepatitis and intra-hepatic cholestasis were reported after consumption of Kombliglyze (Saxagliptin + Metformin) [28]. The drug is also reported to reduce renal tubulointerstitial inflammation but not glomerulosclerosis in *in vivo* mice models [29]. Hence, the SAXA-treated liver and kidney samples showed signs of damage and thus altered levels of biochemical parameters. The gliptins represent the only class of drugs that have the potency to improve β-cell health [3]. This drug is not only efficacious but

also safe as it does not cause significant hypoglycemia. So, the β-cell architecture was restored in SAXA-treated pancreatic tissue but not the normal architecture was not reattained (Fig. 3c). However, GE supplementation mitigated these histological changes, preserving the structural integrity of hepatic, nephrotic and pancreatic cells. A previous study had reported ample amounts of phenolic compounds in the Guggul extract like sesamin (a lignan), classified as pro-antioxidant, which has shown anti-inflammatory effects [30]. Another known phytoconstituent, cadinene (bicyclic sesquiterpene) is a natural antioxidant with potent ROS quenching activity [31]. Apart from this guggulsterone E & Z, myrrhanol A, myrrhanone A, mansumbinone and mansumbinoic acid are also reported as potential candidates for the anti-inflammatory activity of guggul extract [32–34]. Hence, the extract's ability to reverse the tissue damage can be attributed to these phytochemicals.

The GE also helped in the restoration of raised mRNA expression CYP3A11 levels in the induced diabetic group. The STZ-induced diabetes results in an elevation in hepatic bile acids (due to liver damage) which further leads to indirect activation of *constitutive androstane receptor (CAR)*. CAR activation, in turn, mediates the induction of major drug-metabolizing enzymes including CYP3A11 (murine homolog of CYP3A4) [35]. The GE, when given in combination with SAXA, not only helped in controlling the BGL compared to the drug alone, but it also reduced the hepatic mRNA expression of CYP3A11. The alone treatment of SAXA, on the other hand, could not significantly reduce these mRNA expression levels. Hence, it can be concluded that saxagliptin although controls the levels of serum biomarkers and BGL, cannot aid in the reversion of liver tissue damage caused by STZ (as shown in Fig. 1b) [36].

The molecular docking studies further corroborate the results of biochemical parameters, histopathological analysis, and mRNA expression levels. CYP3A4 is responsible for the metabolism of almost all xenobiotics making both saxagliptin and GE its ligands. Saxagliptin is a known substrate for CYP3A4 and the phytochemicals of GE might compete with this drug when given concomitantly. The primary active constituent of GE is guggulsterone E & Z (G E&Z) and the antidiabetic potential of these phytochemicals has already been reported in previous studies [37,38]. Hence, this phytochemical was predominantly chosen over others to dock with CYP3A4 alone and in combination with SAXA. Ahmed and coworkers reported that if the binding energy of a docked pose is less than − 4.7 kcal/mol then there's an efficient binding of any ligand with interacting protein [39]. The lower binding energy of all three docked poses confirms their efficient binding. The free binding energy became more negative when SAXA and G E &Z were bound together to the binding pocket of CYP3A4 which infers that guggulsterone might be responsible for the enhanced binding of saxagliptin to CYP3A4. This was supported by a low *Ki* (inhibition constant) value which depicts a stronger affinity of both the ligands with the protein when docked together. Also, as the pockets for binding are different for both ligands, neither of them compete for binding. Therefore, it can be concluded that the activity of the drug might be elevated due to the ameliorative effect of guggulsterone for it reduces the free activation energy.

Conclusions

The GE can synergistically aid in the improvement of the allopathic treatment of diabetes and its related secondary complications in the induced T2DM mice model. The combined GE + SAXA treatment showed a significant reduction in blood glucose levels and appropriate serum biomarkers along with an improved total lipid profile. Moreover, the antioxidant activity of the phytoconstituents of the extract has reduced the diabetes-induced oxidative stress in the liver, kidney and pancreatic tissues as observed in histological studies. The increased expression levels of hepatic CYP3A11 (murine homolog of human CYP3A4) mRNA (in the case of diabetic control) were also lowered in the combination treatment depicting tissue damage reversal activity of the extract. The docking studies have further shown that both saxagliptin and guggulsterone E&Z lie in proximity but have different pockets of CYP3A4 protein suggesting the inability of the latter to compete with the former when administered concomitantly.

Methods

Reagents and extract

All the materials and reagents used are of analytical grade procured from HiMedia Laboratories Pvt. Ltd, India, and Sigma Aldrich ®, St. Louis, Missouri, USA. Onglyza 5 mg (saxagliptin, AstraZeneca Pharma India Ltd.) was purchased in Jaipur, Rajasthan, India. The commercial kits used for the evaluation of serum biochemical parameters were from either Erba Diagnostics Manheim GmbH, Germany, or Accurex Biomedical Pvt. Ltd., Maharashtra, India. The ethyl acetate extract of oleo-gum of *Commiphora wightii* (Indian bdellium-tree/ guggul/) containing 2.5% w/w guggulsterone was procured from Sv Agrofood, Maharashtra, India (Batch no. SVA/GE/202010, manufacturing date: October 2020).

Experimental animals

Healthy adult male Swiss albino mice (*Mus musculus*) weighing 24–28 g and of age 4–6 weeks were maintained under standardized conditions at the animal house of the institute. The mice were provided with standard pellet food and had free access to purified drinking water. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and ethical clearance was sought from the Institutional Animal Ethics Committee for the conduction of the study (1689/PO/Re/S/13/CPCSEA). All the points relevant with the designing of the study were followed in accordance with ARRIVE guidelines 2.0.

Experimental protocol

Diabetes was induced by intraperitoneally injecting streptozotocin (55 mg/kg b.w./day) freshly prepared in cold sodium citrate buffer (0.1 M, pH 4.5) for 5 consecutive days in overnight fasted mice. The threshold value of fasting blood glucose level to diagnose diabetes was considered as > 180 mg/dL [40]. The mice were divided into five different treatment groups with five mice in each group. The type of dosing and other details are listed in *Supplementary File 1*. Based on the earlier literature review, the therapeutic dose for SAXA (Onglyza) is 10 mg/kg b.w./day [41]. The therapeutic dose for the extract was

chosen to be 200 mg/kg b.w./day i.e., containing 5 mg/kg b.w. of guggulsterone [42]. Both SAXA and GE were dissolved in water and administered through oral gavage daily for 4 weeks.

Estimation of body weight and fasting blood glucose level

The blood glucose levels were evaluated weekly, i.e., on the 7th, 14th, 21^{st,} and 28th day by tail tip amputation process using a glucometer. The body weight of mice was also recorded at the same interval.

Estimation of serum biomarkers level and lipid profile

On the last day of the experiment, blood was collected through a heart puncture. Isolated serum was analyzed for varying levels of kidney and liver function parameters like albumin, uric acid, creatinine, urea, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The serum was also analyzed to evaluate lipid profiles like total cholesterol and triglyceride levels. The estimation of different biomarkers and lipid profiles was done by using commercial kits for kidney and liver function tests.

Estimation of total glycogen content

For estimation of total glycogen content in liver samples, 50 mg of the dissected tissue was digested in hot 30% KOH. In the tissue homogenate obtained, ethanol was added at a final concentration of 55%, mixed, and centrifuged at 1700 x g for 10 minutes. The pellet obtained was resuspended in 2 ml of distilled water. This suspension was used for the estimation of total glycogen content by using a modified phenol-sulfuric acid microassay method suggested by Rasouli et al. [43]. For this method, glucose-water (10 mg/dl), 6.5% phenol-water, and 85% concentrated sulfuric acid were mixed in a ratio of 1:1:5. The standard was replaced with the digested liver sample from each group and a blank without glucose was set for each sample. The absorbance was read at 492 nm via UV-Vis spectroscopy.

Histopathological analysis

Following euthanasia, liver, kidney and pancreatic tissues were fixed in a 10% formalin solution at room temperature. The tissues were later embedded in paraffin wax, sectioned, and mounted on glass slides. The sections were stained using Hematoxylin and Eosin (H & E) stains and examined using a light microscope for diabetes-induced pathological alterations.

cDNA synthesis and quantification of mRNA expression by qPCR

Total RNA was isolated from 100 mg of frozen mouse liver tissue using TRIzol reagent (Ambion, Life Technologies) as per the manufacturer's protocol. The isolated RNA was resuspended in diethylpyrocarbonate (DEPC, Sigma) treated water and quantified spectrophotometrically at 260 nm and 280 nm, respectively. All the RNA samples were considered pure as they had 260:280 ratios between 1.8 and 2.0. The integrity of the RNA samples was electrophoretically determined using ethidium bromide-stained agarose gel. cDNA was prepared from 0.5 µg of RNA by reverse transcriptase using RevertAid

First Strand cDNA Synthesis Kit (ThermoFisher). Gene-specific primers, chosen to amplify CYP3A11 (an equivalent of CYP3A4 of humans in mice) [44] and β-actin [45] were synthesized from Eurofins Scientific, India. *Gene Runner* (Ver. 6.5.2) was used to check the primer dimerization. qPCR was performed using QuantiTect SYBR Green PCR Kit (Qiagen, Denmark). The PCR cycles for cyp3a11 and β-actin were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C (1 min), annealing at 55°C (1 min) and extension at 72°C (5 min) with a final extension at 72°C (1 min). The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold gene expression level of CYP3A11 relative to the control and the housekeeping gene (β-actin) [46].

Molecular docking studies

In the current study, molecular docking was used to evaluate the *in silico* interaction of human CYP3A4 (protein target) with the ligands (*viz. saxagliptin and guggulsterone E-Z)*. The X-ray crystal structure of the target protein was obtained from the RSCB protein data bank (PDB) with a PDB ID: 4NY4. The structures for the ligands were retrieved from the PubChem database (*saxagliptin- CID: 11243969, guggulsterone E-Z- CID: 13873622*). Before docking, the downloaded protein was fixed by removing ligands, water molecules, and hetatoms. Energy minimization was done for the protein and the ligand using the YASARA minimization server and OpenBabel 3.1.1-x64 respectively. AutoDock Tool (ADT) 1.5.7 was used to add polar hydrogens and charges to the fixed protein. Molecular docking was performed using AutoDock 4.2 [47]. The putative active sites were chosen as the grid centers and the grid box, of size 72 x 87 x 62 and 0.375 Å spacing, was set at -19.194, -21.962, and − 9.714 Å (for x, y, and z). The results were visually analyzed using Discovery Studio 2021 version v21.1.0.20298.

Statistical analysis

Data were expressed as mean ± SD. One-way ANOVA followed by Dunnett's test was performed by Statistical Package for the Social Sciences 23 software (SPSS, USA).

Declarations

Data availability

The generated and analyzed data are included in this published article.

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Authors' contribution

SJ: Data curation; Investigation; Formal analysis; Methodology; Software; Writing - original draft; **MKS**: Project administration; Formal analysis; Resources; Supervision; **NG**: Conceptualization; Investigation; Project administration; Validation; Writing - review & editing; **SC**: Conceptualization; Supervision; Visualization; Writing - review & editing.

Ethics declarations

Competing interests

The authors declare that there are no competing interests whatsoever in the publication of the research article.

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Figures

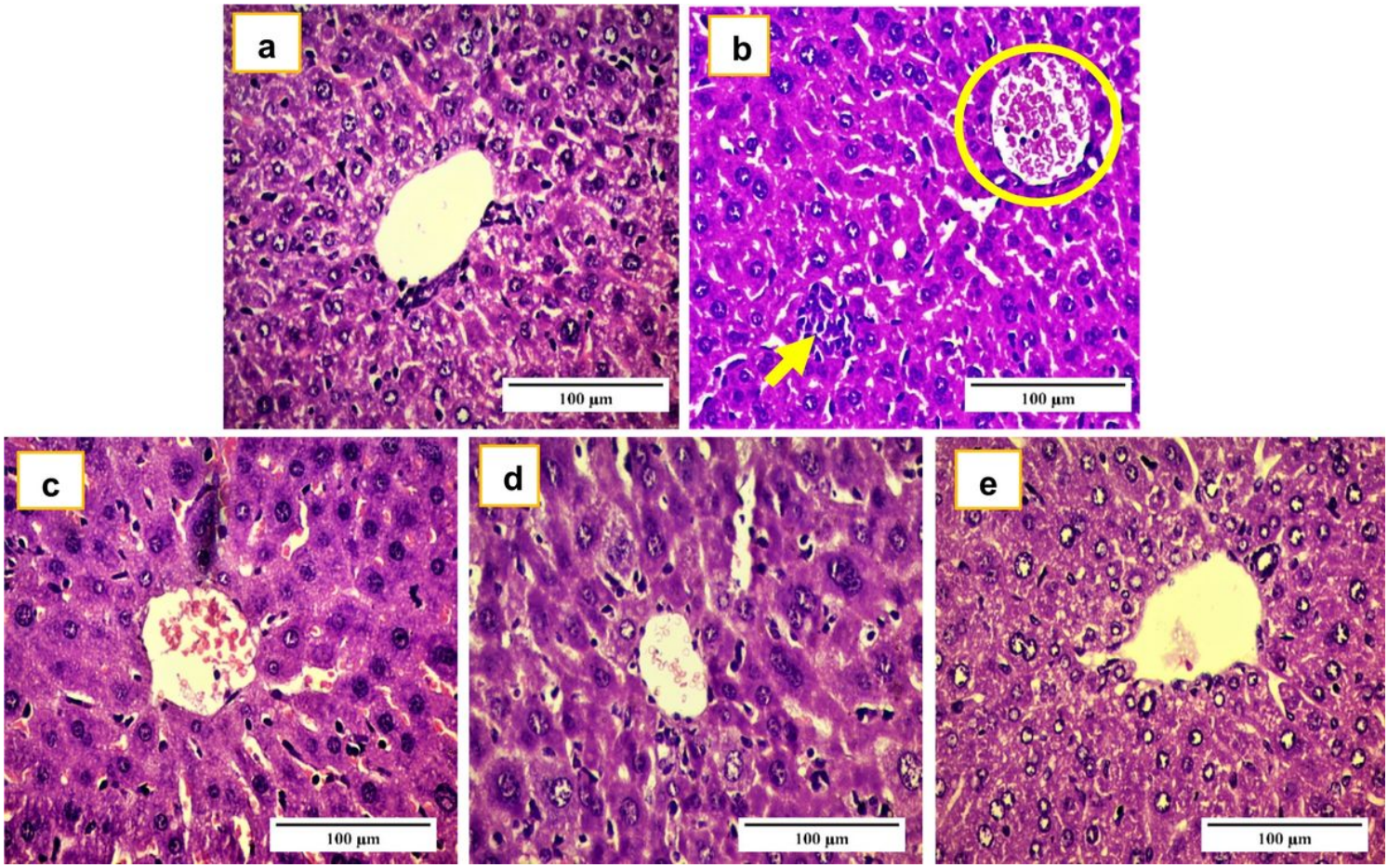

Figure 1

Liver Histopathology: (a) Vehicle control liver with intact central vein & portal veins (**b)** Diabetic control liver showing congested central vein (circle) and focal necrosis (arrow). Treated groups: **(c)** SAXA (10 mg/Kg b.w./day) **(d)** GE (200 mg/Kg b.w./day) **(e)** SAXA + GE. H & E magnification 400X.

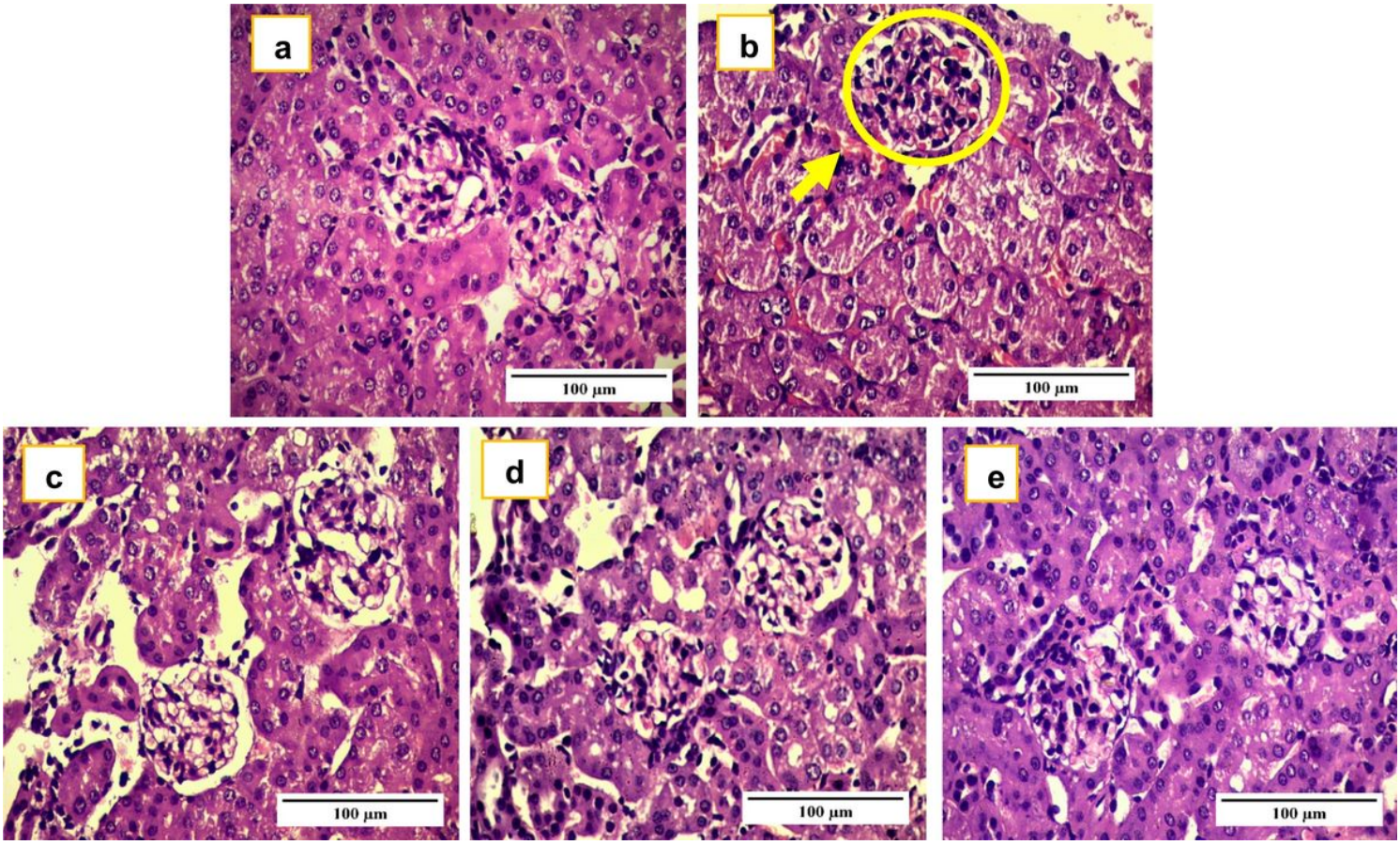

Figure 2

Kidney Histopathology: (a) Vehicle control kidney with intact glomerulus **(b)** Diabetic control kidney showing diabetic nephropathy with glomerular congestion (circle) and sclerosed glomeruli (arrow). Treated groups: **(c)** SAXA (10 mg/kg b.w./day) **(d)** GE (200 mg/kg b.w./day) **(e)** SAXA + GE. H & E magnification 400x.

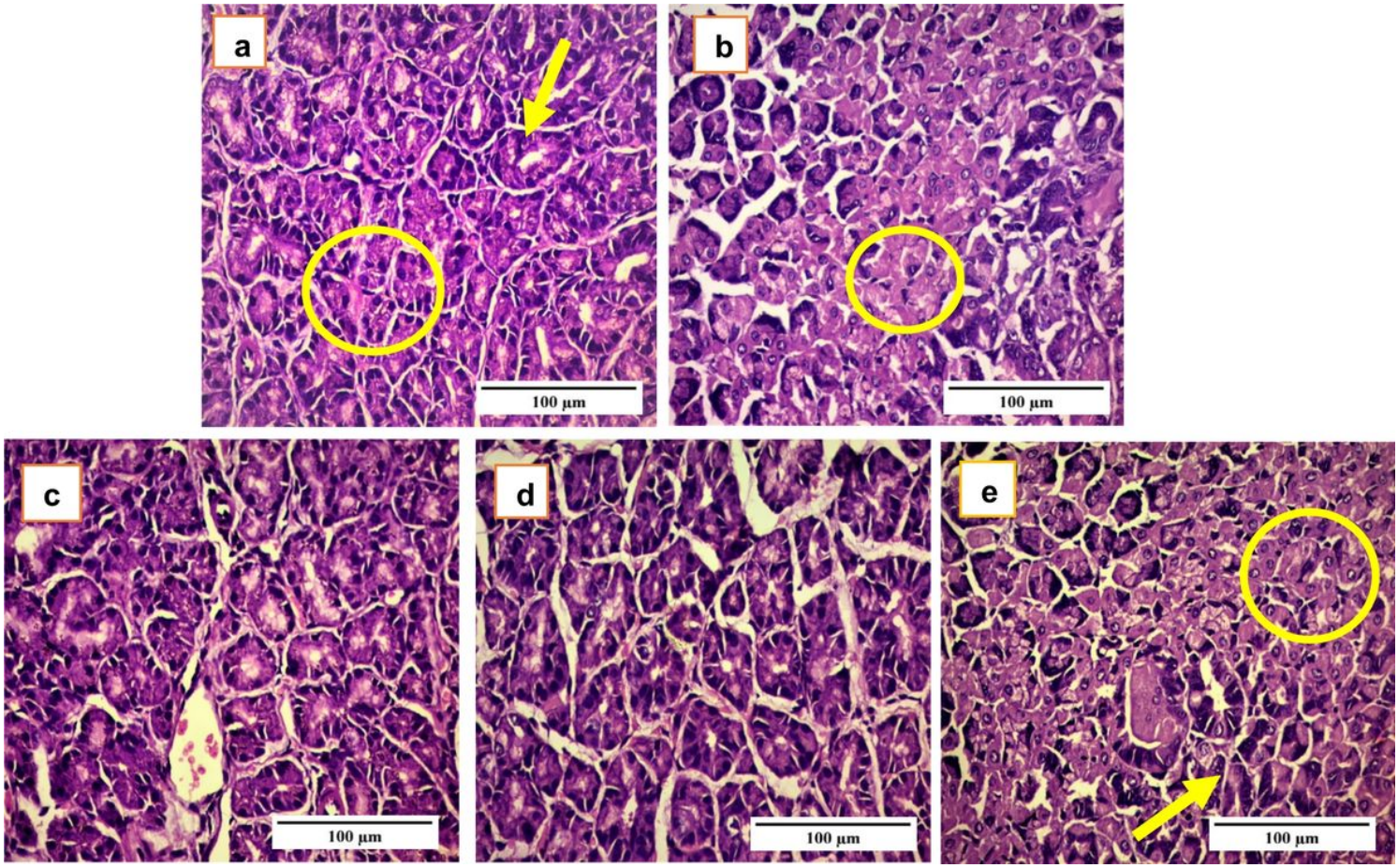

Figure 3

Pancreas Histopathology: **(a)** Vehicle control pancreas with intact islets of Langerhans (circle) and granulated β-cells (arrow) **(b)** Diabetic control pancreas showing diminishing cell density (circle) Treated groups: **(c)** SAXA (10 mg/kg b.w./day) **(d)** GE (200 mg/kg b.w./day) **(e)** SAXA + GE. H & E magnification 400x.

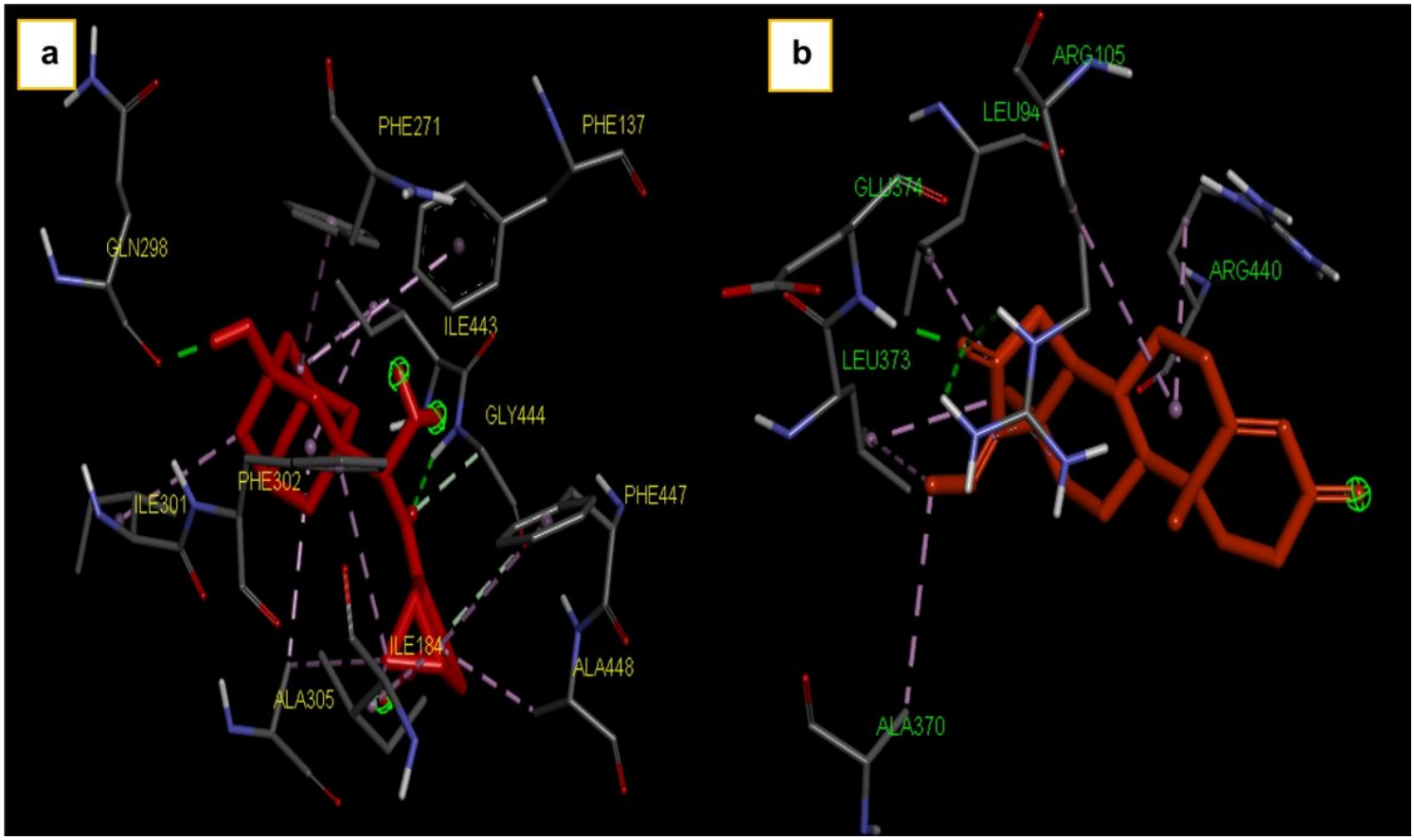

Figure 4

3D-structure of Human Cytochrome P450 3A4 (CYP3A4) docked individually to **(a)** Saxagliptin, **(b)**Guggulsterone E-Z. Images are retrieved from Discovery Studio 2021 (version v21.1.0.20298) post-docking.

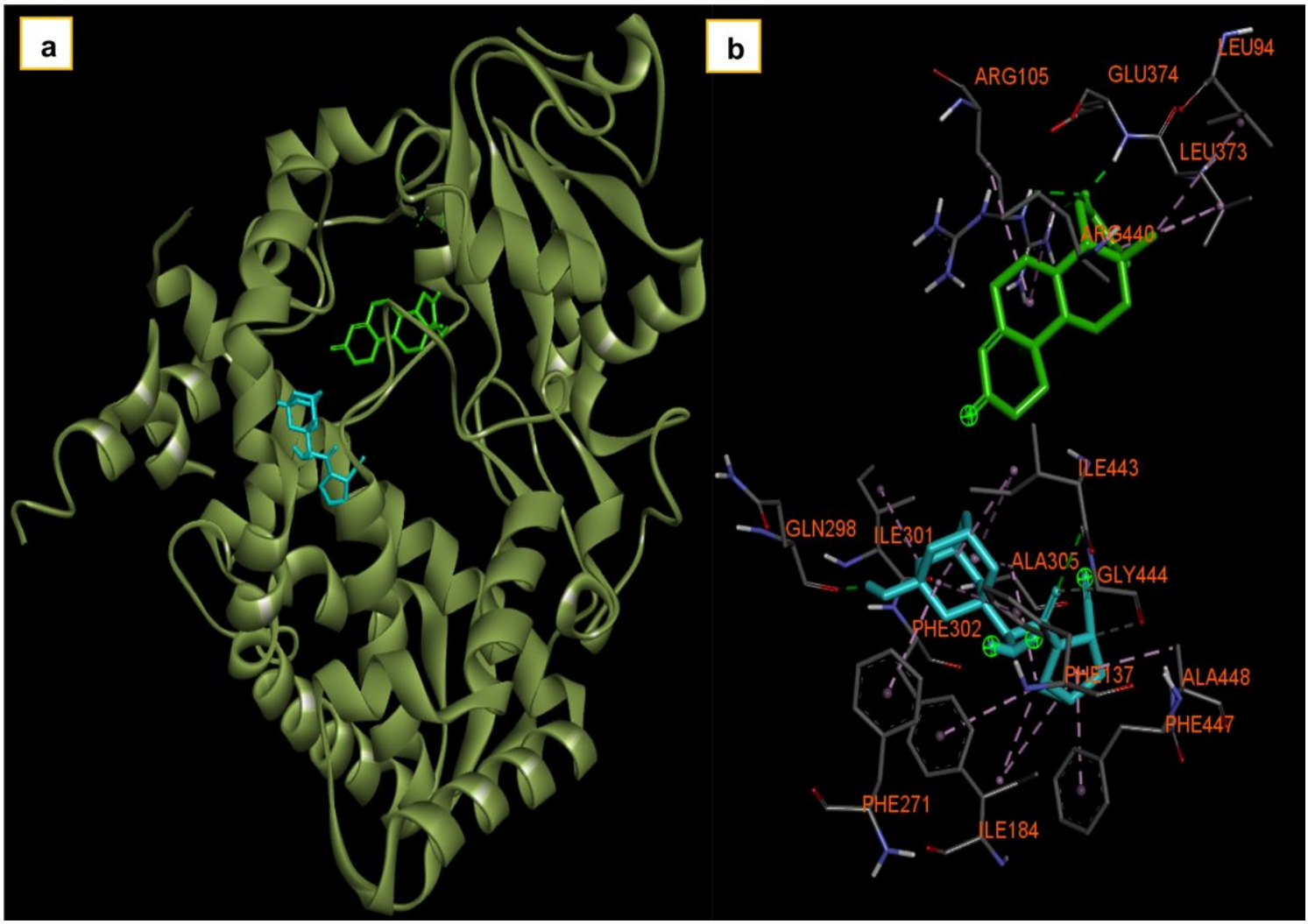

Figure 5

(a) 3D-structure of Human Cytochrome P450 3A4 (CYP3A4) docked firstly to saxagliptin and later to guggulsterone E-Z. **(b)** Ligand interactions with specific amino acids of CYP3A4. Saxagliptin (blue), Guggulsterone E-Z (green). Images are retrieved from Discovery Studio 2021 (version v21.1.0.20298) post-docking.

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