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In silico exploration of the mechanistic insights: interactions of curcumin, quercetin, and ethephon with estrogen receptors and steroidogenic enzymes

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ABSTRACT

Background: Ethephon is a widely used plant growth regulator in agriculture to promote fruit ripening, abscission, flower induction and seed germination which hold chances of mammalian toxicity due to environmental and food contamination. Curcumin, a polyphenolic compound obtained from turmeric and quercetin, a flavonoid present in several vegetables and fruits can influence pituitary and ovarian hormones and mediate benefits on reproductive processes. In this context, the present study was undertaken with the objectives of determining whether curcumin and quercetin have an ameliorative effect on probable adverse effects of ethephon in reproductive system by *in silico* analysis. Method: To identify mechanism of ethephon, quercetin and curcumin, molecular docking has been performed. Three dimensional structures of ER α (PDB Id: 1A52), ER β (PDB Id: 1X7B), CYP19A1 (PDB Id: 3S79), CYP11A1 (PDB Id: 3NA1), CYP17 (PDB Id: 3RUK), 17βHSD (PDB Id: 1BHS), StAR proteins (PDB Id:3P0L) were retrieved from RCSB and docking was done with AutoDock V 4.0 using three ligands. Results: The binding energies of curcumin and quercetin showed more binding energy ranging from -7.97 to -3.12 where as that of ethephon was from -4.44 to -1.56 with all the proteins under study. Curcumin and quercetin showed no affinity towards ER α. Ethephon showed more affinity to ER α than curcumin and quercetin (217.20 and 32.87 kcal/mol respectively). Conclusion: From this study, it could be concluded that curcumin and quercetin can provide an ameliorative effect on reproductive toxicity that can be caused by ethephon.

Keywords: Ethephon, Curcumin, Quercetin, Docking, *In silico*.

INTRODUCTION

Chemical messengers, that influence the development or metabolism of plant tissues are known as plant growth regulators [1]. They encourage plant growth and development, manage fruit ripening and extend shelf life, control plant height and improve plant structure, root development and transplant success, while lessening the effects of environmental pressures on crops, whereby increasing crop productivity [2]. A common organophosphorous ethylene-releasing plant growth regulator used in agriculture, ethephon [3] encourage seed germination, abcission, flower induction, and fruit ripening [4]. Ethephon is frequently applied on wheat, coffee, tobacco, cotton, pineapple and rice to hasten the ripening of fruit. In cotton, it encourages early concentrated boll opening, increases defoliation, and starts fruiting over a period of several which raise the quality of harvest [5].

These chemicals contaminate soil during agricultural applications, infiltrate ground water and hence their careless use can have detrimental consequences on the environment and living things [6]. Additionally, through the food chain, they can cause cumulative harmful effects [2]. Many investigators studied on the residue levels of ethephon its conjugates, the metabolite 2-hydroxyethyl phosphonic acid (HEPA) and found them in the edible portion (grain) of wheat and cereals, however it was less in tomato fruit whereas the majority of residues in cotton came from parent ethephon [7]. The residue may cause serious toxicity in human beings and animals due to the potent oxidant activity. Male albino rats treated with ethephon at 200 mg/kg demonstrated increased level of malonyldialdehyde (MDA), ALT and AST and decreased concentration of superoxide dismutase (SOD), catalase (CAT) glutathione (GSH) albumin with degeneration of hepatocytes and inflammatory cell infiltration [8]. At 150 mg/kg there was increased serum urea level, creatinine, Blood urea nitrogen (BUN) and microscopy revealed degeneration of epithelium of renal tubules, mononuclear cell infiltration and cytoplasmic vacuolation, enlarged glomeruli and tightly filled bowmans capsular spaces [9]. Animals intoxicated with ethephon also experienced haematological alterations and plasma cholinesterase inhibition [10] and immunotoxicity [4]. Treatment of female mice with ethephon decreased serum level of progesterone after 20- and 40-days post concomitant to the impairment of the hypothalamo-gonadal regulation of hormonal homeostasis, resulting in decreased GnRH release and pituitary responsiveness to GnRH. Albino male rats demonstrated decreased serum level of reproductive hormones like FSH, LH, testosterone compared to

control [11]. By changing neurotransmitter levels and affecting the hypothalamo-pituitary-gonadal regulation of reproduction, organophosphorous chemicals can disrupt the hormonal balance and affect vertebrate reproductive capabilities. Treatment of cell line with increasing concentrations of ethephon resulted in the increased cell proliferation with decreased proliferation at high doses.

Consuming dietary antioxidants is thought to be essential for a healthy life, particularly plant phenolics, flavonoids, and carotenoids that have the ability to scavenge reactive oxygen species (ROS). One polyphenolic component that may be extracted from turmeric, curcumin, has several pharmacological actions and is a great antioxidant [12]. It is also known for its antiapoptotic and proliferative [13-14], anti-inflammatory [15-16] antihyperglycaemic [17-18], antihypercholesterolaemic [19], anti-inflammatory [20], antibacterial and antifungal properties [21] among many. Curcumin can affect ovarian and pituitary hormones and mediate positive effects on the reproductive system by affecting the hypothalamo-hypophysial-ovarian axis by interacting with steroid hormones and their receptors. Reproductive disorders were treated with curcumin, which controlled the anterior pituitary's secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), signalling the ovaries to make oestrogen and inhibin [22].

A flavonoid found in a number of fruits and vegetables, quercetin also has antioxidant properties, enhances follicular development, and lowers granulosa cell mortality in test subjects. It is also known for its anticancer [23-24], antidiabetic [25], antibacterial [26], antioxidant, antiinflammatory [27] properties. It is well known for its protective role in toxicities of reproductive system [28-29] and enhancement of female reproduction [30].

The biological activity and toxicity of chemicals, including their interactions with receptors and physicochemical characteristics, can be studied using a variety of computational techniques. Predicting whether and how strongly a particular molecule will bind to a target location is the ultimate goal of in silico docking studies [31]. In this context, the present study was undertaken with the objectives of determining whether curcumin and quercetin have an ameliorative effect on reproductive toxicity by ethephon by *in silico* analysis.

MATERIALS AND METHODS

Preparation of Macromolecule

Three dimensional structures of estrogen receptor alpha (ERα, PDB Id: 1A52), estrogen receptor beta (ERβ, PDB Id: 1X7B), CYP19A1 (PDB Id: 3S79), CYP11A1 (PDB Id: 3NA1), CYP17 (PDB Id: 3RUK), 17βHSD (PDB Id: 1BHS), Star proteins (PDB Id:3P0L) were retrieved from RCSB (http://www.rcsb.org). The structure was prepared for docking using Discovery studio 3.5. The standard protocol and parameters recommended by AutoDock Tools tutorial were followed for preparation of ligand and receptor molecules [32].

Preparation of Ligands

Ethephon, Curcumin, Quercetin (Figure 1) were used for docking studies as ligands. Chemical structure of ligands were downloaded in the Spatial Data File (.SDF) format from the PubChem Compound Database (National Center for Biotechnology Information; https://pubchem.ncbi.nlm.nih.gov/). The structure was modified in Marvin View and converted to Tripos Mol 2 file format. Ligand modifying tools of ADT was used to prepare ligand structures in terms of root detection, the expansion of root as well as to choose the number of rotatable bonds. After the initial preparation, the ligand was converted to PDBQT format enabling its use with AutoDock4 [33].

Docking

The docking studies were conducted using Autodock4. Binding energy and ligand efficiency are the most indicative of the overall strength of a given predicted interaction calculated by Auto Dock. Auto dock generates the ligand molecule in ten different conformation, orientations, and positions as well as performs the docking of the ligand to a set of grids describing the target protein. The grid map for docking studies was computed using AutoGrid4 included in the Autodock4 package. The prepared file was saved in the grid parameter file. The docking parameter file was generated with optimized parameters as recommended by the Auto Dock Tool. The Lamarckian genetic algorithm was used for all docking runs, and the dock scores achieved were reported in kcal/mol. The docking procedure used in the study included 10 independent Genetic algorithm runs. The post docking analysis was visualized using Discovery Studio Visualizer.

Estimation of Binding Energy

The binding energy for each molecule were obtained from the RMSD table in the docking log file and was expressed in kCal/mol. Binding energy is defined as the sum of all intermolecular forces acting upon the receptor-ligand complex. If the binding energy is low for a docked compound, it indicates that those compounds have a higher affinity [33].

RESULTS

The docking results of ethephon, curcumin and quercetin with different receptors of oestrogen and critical enzymes in the steroidogenic pathway is depicted in table 1 as glide score. It was seen that there was very high binding energy (positive) for Curcumin (217 kcal/mol) and quercetin (32.87 Kcal mol) for ERα whereas the glide score of curcumin with ERβ, CYP19A1, CYP11A1, CYP17, 17βHSD and StAR were -4.88, -7.87, -7.48, -3.59, -6.64, -7.15 kcal/mol respectively. The glide energy for quercetin for these ligands were -5.23, -8.49, -6.79, -4.64, -7.97, -7.61 kcal/mol respectively. The affinity of ethephon was more towards ERβ, 17βHSD, CYP11A1 and CYP19A1.

Table 1: Binding energy in kcal/mol of ligands with macromolecules

	Ethephon	Curcumin	Quercetin
ERα (1A52)	**-2.93**	**217.29**	**32.87**
ERβ (1X7B)	**-4.44**	**-4.88**	**-5.23**
CYP19A1(3S79)	**-3.27**	**-7.87**	**-8.49**
CYP11A1(3NA1)	**-3.12**	**-7.48**	**-6.79**
CYP17(3RUK)	**-1.56**	**-3.59**	**-4.64**
17βHSD(1BHS)	**-3.68**	**-6.64**	**-7.97**
StAR (3P0L)	**-2.75**	**-7.15**	**-7.61**

Figures 1 to 7 represents the interaction of the ligands with the active amino acid sites of different ligands used in the study.

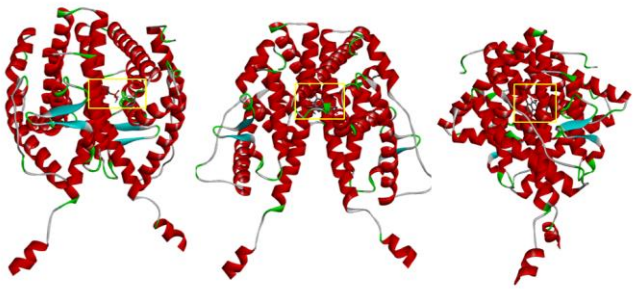

Figure 1: Post docking analysis of ethephon, curcumin, quercetin with estrogen receptor alpha

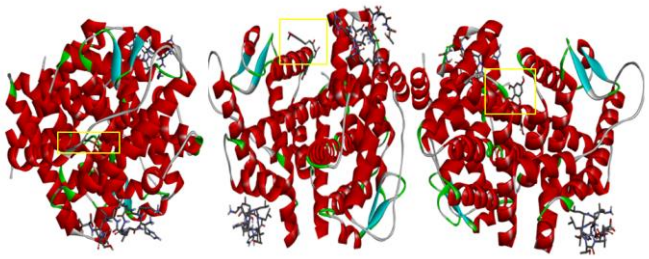

Figure 2: Post docking analysis of ethephon, curcumin, quercetin with estrogen receptor beta

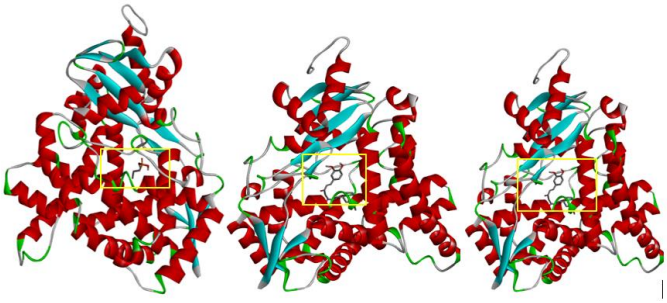

Figure 3: Post docking analysis of ethephon, curcumin, quercetin with CYP19A1

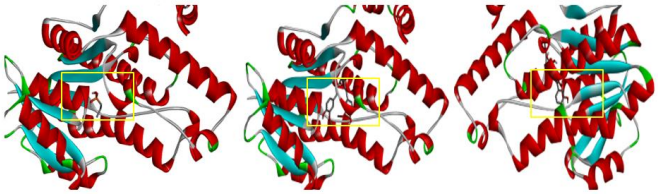

Figure 4: Post docking analysis of ethephon, curcumin, quercetin with 17βHSD

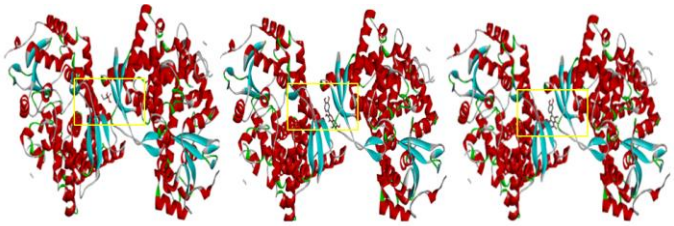

Figure 5: Post docking analysis of ethephon, curcumin, quercetin with CYP11A1

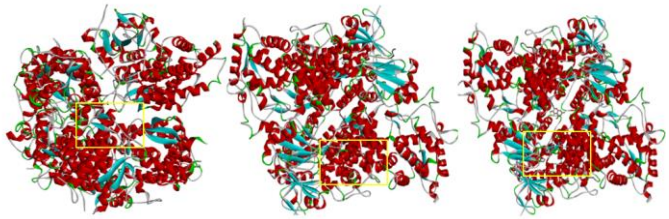

Figure 6: Post docking analysis of ethephon, curcumin, quercetin with CYP17A1

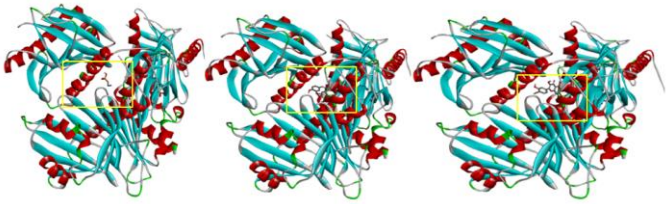

Figure 7: Post docking analysis of ethephon, curcumin, quercetin with StAR

DISCUSSION

In the present study, we analysed the *In silico* binding affinities of ethephon, an organophosphate plant growth regulator, curcumin and quercetin to oestrogen receptors ERα, ERβ and various key enzymes in the synthesis of oestrogen including CYP19A1, CYP11A1, CYP17, 17βHSD and steroidogenic acute regulatory protein (StAR). The aim was to find out the affinity of the possible effects/ toxicity of ethephon in female reproduction and the ameliorative potential of the phytochemicals in the toxicity.

Plant growth regulators are used in agriculture for increasing crop production with minimising the loss during various agricultural practices, by influencing the ripening, storage and fruit size. They also aid in the control of pests and diseases that helps in increasing the yield per plant or cropping area. However, farmers may use these inadvertently in order to increase their profit, producing undesirable residue levels in the produce leading to their entry in food chain and causing non target system toxicities. One of the major concerns with almost all agrochemicals is the endocrine disruption and female reproductive system may be affected badly. The major concern is in the synthesis of oestrogen as well as in its action which may affect fertility, reproduction as well as form cause for different malignancies.

From the results of the study, it is clear that quercetin and curcumin have more negative binding energy and affinity with estrogen receptor beta (ERβ), and proteins CYP19A1, CYP11A1, CYP17, 17βHSD, StAR except alpha estrogen receptor (ERα). Ethephon have more negative binding energy than curcumin and quercetin with ERα. Oestrogen exerts its genomic effects through the classical nuclear estrogen receptors The biosynthesis of steroid hormones starts with the mobilisation of cholesterol to the mitochondrial inner membrane, facilitated StAR, which is the rate-limiting step [34]. Once cholesterol enter the inner mitochondrial membrane, it is converted into pregnenolone by the enzyme CYP11A1 [35]. Pregnenolone is converted to androstenedione by enzymes such as CYP17A1 and 3β-HSD, and androstenedione is converted to oestrone by the enzyme CYP19A1, also known as aromatase, and subsequently to oestradiol by 17β-HSD [36].

Higher ERα:ERβ ratio was found in the tumour when compared to normal tissue [37], and this difference is the result of increased ERα expression in the tumour compartment and, presumably, decreased Erβ expression [38]. ERα participates in cell proliferation and cell survival, whereas ERβ modulates ERα activity when both receptors are co-expressed in the normal tissue and in cell lines of breast cancer [39]. So, High binding affinity of ethephon with ERα can be considered to affect proliferation whereas curcumin and quercetin having high affinity to other receptors and less affinity to estrogen alpha receptor resulting in antiproliferative effect. So, use of curcumin and quercetin may reduce the effect of ethephon in the proliferation of oestrogen responsive cells indicating a preventive/curative effect on cancer. Curcumin and quercetin showed high affinity binding with steroidogenic enzymes than ethephon. Hence curcumin and quercetin may have a beneficial role in the modulation of fertility and increased enzyme activity would lead to enhanced 17β-estradiol metabolism resulting in decreased cell proliferation [40].

Various phytochemicals were shown to possess oestrogenic or antioestrogenic activity. Genistein and coumestrol, displayed oestrogenic activity [41] whereas genistein showed positive or negative modulation of oestrogen [41,42]. Phytochemicals may affect steroid hormone synthesis by modulating the activity of aromatase or replacement of substrates for hormone synthesis [43]. The alcoholic extract of *B. diffusa* was found to increase progesterone and decrease oestrogen secretion from MCF-7 cells [44] whereas extract of tubers of *A. racemosus* caused a dose and time dependent increase in the secretion of oestrogen with enhancement of progesterone at higher doses only [45]. It is also reported that genistein, daidzein, equol and liquiritigenin, recruited the coactivator SRC3 to ERβ significantly

more effectively than to ERα [46] however the affinity of quercetin for ERα and ERβ was 10^5 to 10^6 times lower than the affinity of 17β-oestradiol for ERα and ERβ [47].

CONCLUSION

From this study, it could be concluded that ethephon is having high affinity to ERα that can result in cell proliferation whereas curcumin and quercetin having high affinity to other protein and less affinity to ERα results in antiproliferative effect and these compounds can counteract all the ill effects of ethephon.

Conflict of interest

The authors declared no conflict of interest.

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