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In Silico Toxicological Assessment and Efficacy of Activated Carbon Derived from Low-Quality Face Tissues for Eosin Dye Adsorption

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Abstract

Eosin dye is an efficient qualitative and quantitative chemical through various scientific and biological uses but it was demonstrated as a lethal substance to animal or human lung, liver, eye, kidney, Central Nerve System (CNS) and parts of gastrointestinal system. In many published studies, it removed via clay/carbon composite, lemon peel, or fly ash adsorption beside oxidation, filtration, photocatalysis, ion exchange, sonochemistry, nanotechnology, and others. Here, is another attempt to prepare an activated carbon from carbon-based material (low quality face tissue) where its capacity was determined by toxic anionic dye (Eosin B) – spectroscopic investigation. In this attempt, Eosin B showed an excellent removal and adsorption capacity starting from first 5 mL eluted from this prepared adsorbent. Removal of Eosin is important to human and environment where present *In Silico* study confirmed that Eosin B was safe to embryo or foetus health (during pregnancy), Cardio- topic including: Arrhythmia, Cardiac Failure, Heart Block, hERG Toxicity, Hypertension, and Myocardial Infarction, toxic to avian with high Rat Acute Toxicity (LD_{50}) lowest probability of lung, prostate, and renal cell lines even with its water solubility in aqua environment, Yes response towards P-glycoprotein substrate or multidrug resistance (MDR1) function in cancer pharmacotherapy. Also, Eosin B had BBB or CNS permeability as well as fish (Minnow) toxicity. It is safe for Renal OCT2 substrate, besides hERG I & II inhibitors, Hepatotoxicity, and skin sensitization, Cytochromes P450 under testing. Therefore, Eosin B is structurally toxic to human and environment in short – or long – term period.

1. Introduction

Eosin is a synthetic organic dye and it is capable to generate singlet oxygen in photo-sensitizing synthetic chemistry (photo-catalysis). Recent published reviews highlighted Eosin in various subjects like catalyzing organic redox reactions such as C-C, C-X formation or cyclization, transition metal reaction, dehydrogenation, and / or oxidation [1, 2].

There are two compounds have Eosin first naming that differ in chemical formula and structures besides related physical, chemical, and biological properties as shown in Figure (1) and Table (1).

Both fluorescent dyes are water - soluble artificial derivatives used for biological staining or counterstaining for general demonstration of cell or tissue like distinguishing between cytoplasm in various cells in red or pink colour. Also, these xanthene dyes can distinguish between muscle and collagen [3].

Various research articles reported using Eosin Y in quantitative analysis of metals or pharmaceuticals by forming an associated coloured complex depending upon pH where this coloured probe has various acidic pK values ranged from 2.1 to 4.95 by ionization of hydroxyl or carboxylic group. The cationic, neutral, monovalent anion and di-valent anion forms are highly affected by the presence of strong electron withdrawing group (four bromo- groups in Eosin) while Eosin B has (two bromo- groups and two nitro- groups) with the assistance of oxygen atom in hydroxyl and carboxylic groups. In drug and metal (as active basic centres) determinations, binary or ternary system is formed with Eosin (acidic centre) resulting remarkable changing in adsorption, fluorescence quenching, and Rayleigh scattering [4-10].

Although, Eosin dye is an efficient qualitative and quantitative chemical with different scientific and biological uses but a systemic review [11] and similar reference [12] demonstrated its toxicity to the health of human or animals. From this important point, Guruge et al. presented a first investigation of 72 materials used in pharmaceutical and personal care sectors in Sri Lankan and became an environmental risk source in surface water including Eosin [13]. Here, toxicity represented stability of aromatic ring in Eosin structure against degradation towards cancer or in lung, liver, eye, kidney, Central Nerve System (CNS) and parts of gastrointestinal system.

For these toxicological influences, many published articles participated in Eosin pollution and removal approaches such as using clay/carbon composite [14], lemon peel [15], and fly ash [16]. Eosin effect like other pollutants in aqua medium can be minimized by several chemical, physical, and biological techniques such as oxidation, filtration, photocatalysis, ion exchange, adsorption, sonochemistry, nanotechnology, and others [17-21].

In adsorption, low cost and effective adsorbent was main target in many research papers especially natural base such as starch, lignin, chitosan, waste biomaterials, … etc. where these biomaterials offered many influenced characters such as physicochemical properties (surface area, porosity, availability of hydrogen bonding, besides structural network), compatibility, and degradability [22-27].

From toxicological points and availability, no previous research papers were directed to search using soft cleaning tissues as a possible goal in Eosin adsorption as a bio-based material combined by *In Silico* study. Here, *In Silico* study is another motive in looking for more subjective substance in Eosin removing from aqua medium.

It is known that *In Silico* studies are computerized approaches providing time, cost, and laboratory studies in **A**dsorption, **D**istribution, **M**etabolism, **E**xcretion, and **T**oxicity (ADMET) studies. Machine learning methods particularly in toxicological studies concentrated on discovering trusted clinical outcomes. They are very supportive in numerical definition of molecular actions avoiding external complications of test tube, petri dish, and animal testing. These computer–assistance methods released potential results in therapeutics and toxicity basing on molecular structure for future biotechnology sectors [28-31].

In this research paper, online prediction approach of Eosin (both Y & B) offers the evidence about their toxicity that led to necessity of removal for human and other living creatures in the earth planet. Here, online biosig websites linked to the lab in Brisbane (Queensland) were used to predict Eosin (Y & B) toxicity against Embryo, Cardio, bee, avian, minnow, Ames, Rat Acute Toxicity (LD_{50}), Rat Chronic Toxicity (LOAEL) beside Cardio-toxicity that includes Arrhythmia, Cardiac Failure, Heart Block, hERG Toxicity, Hypertension, and Myocardial Infarction.

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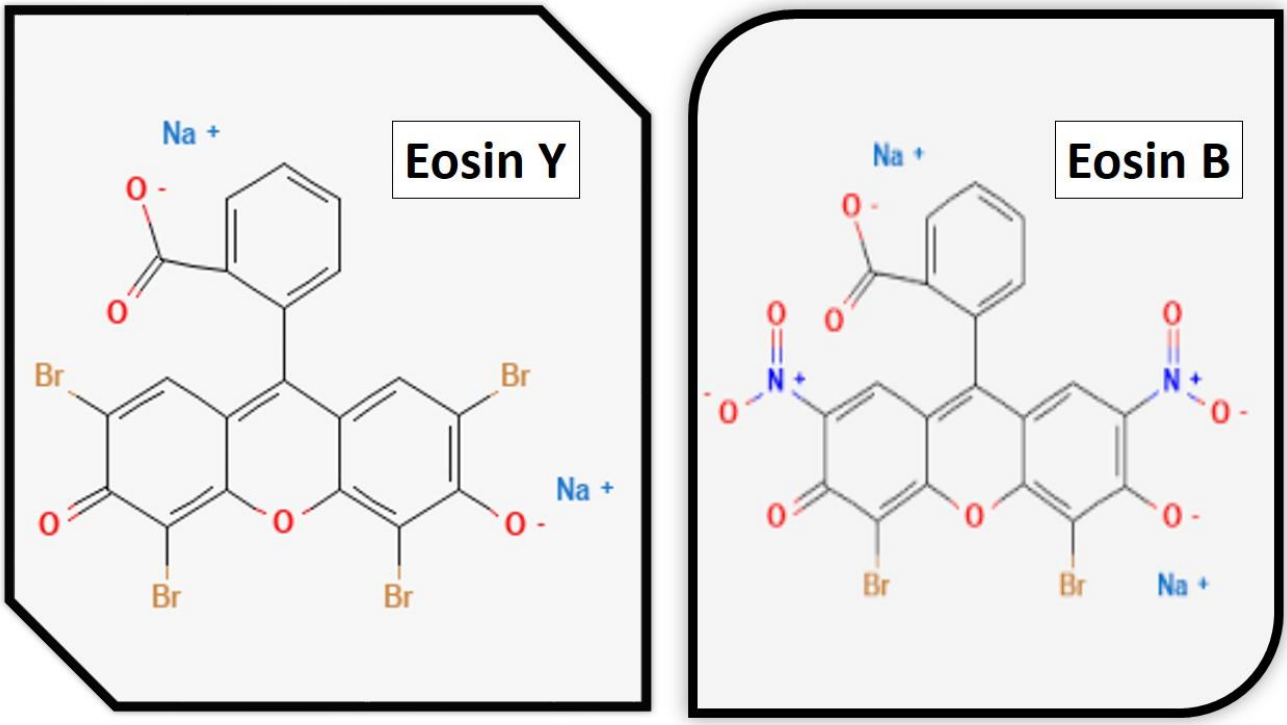

Figure (1): Chemical structure of Eosin Y & B.

Table (1): Eosin Y & B in general characterization.

Name	IUPAC name	SMILES	Formula	General remarks
Eosin Y	disodium;2-(2,4,5,7-tetrabromo-3-oxido-6-oxoxanthen-9-yl)benzoate	C1=CC=C(C(=C1)C2=C3C=C(C(=O)C(=C3OC4=C(C(=C(C=C24)Br)[O-])Br)Br)Br)C(=O)[O-].[Na+].[Na+]	$C_{20}H_6Br_4Na_2O_5$	Other names: Eosine Yellowish C.I. Acid Red 87 Fluorochrome dye deprotonated both COOH and OH then neutralized by sodium ion. Sodium salt of 2', 4', 5', 7'- tetrabromo fluorescein. Using this red dye for textiles, tissue and vital staining with special medium, cosmetics, pharmaceuticals,
Eosin B	disodium;2-(4,5-dibromo-2,7-dinitro-3-oxido-6-oxoxanthen-9-yl)benzoate	C1=CC=C(C(=C1)C2=C3C=C(C(=O)C(=C3OC4=C(C(=C(C=C24)[N+](=O)[O-])[O-])Br)Br)[N+](=O)[O-])C(=O)[O-].[Na+].[Na+]	$C_{20}H_6Br_2N_2Na_2O_5$	Using in paper, cotton, and wool dyeing. Staining of nuclei, muscular fiber, and epithelia. **Eosin B** (C.I. 45400) Acid Red 91

2. Experimental Procedure

2.1. Chemicals:

Eosin Bluish (B) dye, sulphuric acid, and sodium carbonate were obtained from well-known chemical companies.

2.2. Instruments

UV-VIS spectrophotometer, Shimadzu, Japan was used for qualitative and quantitative analysis of Eosin dye in its aqueous solution (standard solution (1-12) ppm and after adsorption steps by prepared activated carbon. While, FTIR spectrophotometer, Shimadzu, Japan was used for qualitative analysis of prepared activated carbon before and after adsorption steps.

2.3. Methods

Two samples of low-quality face tissues purchased from local Baghdad market (56.4320, 58.6100) grams were soaked in 150 mL of concentrated sulphuric acid for several days until all tissues turned to black colour. One gram of sodium carbonate was added to the mixture as a further activation step. The resulted activated carbon was washed with deionized water until pH7 then dried at 70^{o}C.

Conversion quantity of the produced activated carbon was more than 30% where 56.432 grams produced 18.52 grams of the active carbon (conversion quantity = 32.8183%) while 58.61 grams of face tissues gave 20.31 grams (conversion quantity = 34.6528%).

2.3.1 Standard Solutions of Eosin and Estimation of the Trend Line Characters

Aqueous Eosin B solutions (1-12) ppm that prepared and their absorbance were measured by UV-Vis spectrophotometer (Shimadzu, Japan) for qualitative and quantitative standard curve estimation beside curve equation and linear regression (R^2).

2.3.2. Adsorption Steps

Aqueous Eosin B solution (500 ppm, 1L) at room temperature was subjected to adsorption step with (5 grams) activated carbon that prepared as mentioned above. To evaluate adsorption efficiency, 5 mL of the first, final, and all sample were selected where the prepared activated carbon (adsorbent) represented a simple filter. Second 1L was added to the same adsorbent with the same collections (first, last, mixture) were evaluated by UV-Vis instrument after first (1L) adsorption and spectral measurements were completed (Figure 2, Table 2).

To quantify adsorption capacity (Q, mg/g) and Removal efficiency (R, %), Equations (1 & 2) were applied and the resulted were tabulated (Table 2) where C_o, C_i, m, and V are initial concentration (ppm), concentration (ppm) at specific step, mass of the adsorbent (gram), and solution volume (litter) respectively.

$$\text{Removal efficiency (R\%)} = [(C_o - C_i)/C_o] \times 100 \quad \ldots. \ (1)$$

$$\text{Adsorption capacity (Q)} = [(C_o - C_i)/ m] \times (V) \quad \ldots. \ (2)$$

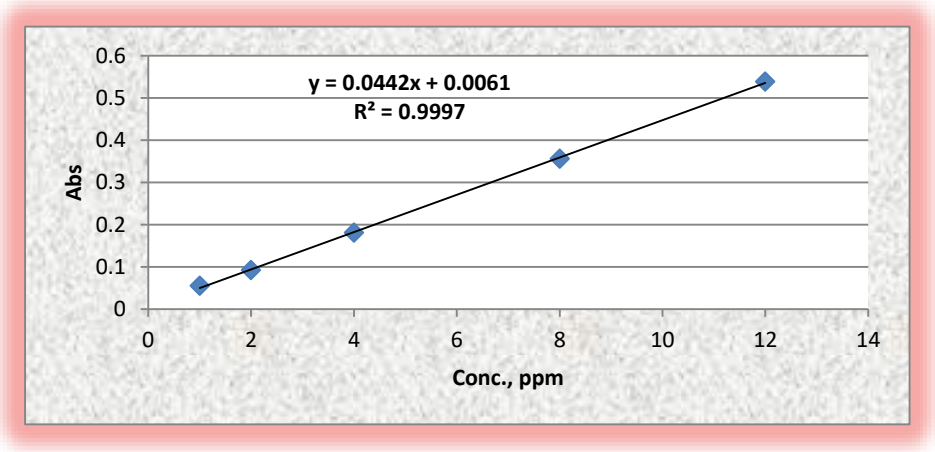

Figure (2): Standard calibration curve of **Eosin B**.

Table (2): Quantitative evaluation of Eosin B removal by the prepared activated carbon.

	abs at 517 nm	conc., ppm	R%	Q, mg/g
First 5 ml	0.763	17.12443	96.57511	96.57511
First 5ml, Second try	2.709	61.15158	87.76968	87.76968
Last 5ml	3.436	77.59955	84.48009	84.48009
Last 5ml second try	2.737	61.78507	87.64299	87.64299
Mixture	2.767	62.4638	87.50724	87.50724
Mixture, second try	2.737	61.78507	87.64299	87.64299

2.3.3. Toxicity Prediction

Online websites were the prediction base for Eosin (Y & B) toxicity evaluation against Embryo, Cardio, bee, avian, minnow, Ames, Rat Acute Toxicity (LD_{50}), and Rat Chronic Toxicity (LOAEL) by general internet location biosig websites linked to the lab in Brisbane (Queensland). Also, ADMET characterization and Cardio-toxicity includes Arrhythmia, Cardiac Failure, Heart Block, hERG Toxicity, Hypertension, and Myocardial Infarction were additional *In Silico* testing as in Tables (3, 4, 5, & 6).

Table (3): Prediction of Eosin (Y & B) toxicity against Embryo, bee, avian, minnow, LD_{50}, and LOAEL.

Name	Embryotox Safety profile	*Herbicide*	*Honey Bee*	*Avian*	*Minnow*	*Ames*	*Rat Acute Toxicity (LD_{50})*	*Rat Chronic Toxicity (LOAEL)*
Eosin Y	Safe	No		No	1.638	No	678.4	9.3
Eosin B				Yes	1.505	Yes	1020.0	138.4

Table (4a): Prediction of Eosin (Y & B) toxicity against breast, CNS, colon, leukaemia, and melanoma cancer cell lines.

Anticancer activity	Name	Breast	CNS	Colon	Leukemia	Melanoma
Mini.	**Eosin Y**	4.584	4.374	4.635	4.569	4.322
	Eosin B	4.451	4.529	4.567	4.555	4.493
Max.	**Eosin Y**	5.355	4.739	4.418	5.231	5.044
	Eosin B	5.484	4.808	5.411	5.613	5.069

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Table (4b): Prediction of Eosin (Y & B) toxicity against Lung, Ovarian, Prostate, and Renal cancer cell lines.

Anticancer activity	Name	Small cell Lung	Non- small Lung	Ovarian	Prostate	Renal
Mini.	Eosin Y	4.457	4.434	4.451	4.667	4.295
	Eosin B	4.436	4.321	4.386	4.179	4.216
Max.	Eosin Y	5.077	5.461	4.879	4.501	5.445
	Eosin B	5.146	5.384	4.945	4.557	5.432

Table (5): Cardio-toxicity prediction of Eosin (Y & B).

Name	*Arrhythmia*	*Cardiac Failure*	*Heart Block*	*hERG Toxicity*	*Hypertension*	*Myocardial Infarction*
Eosin Y or B	Safe					

Table (6): ADMET prediction of Eosin Y & B.

Property	Character	Eosin Y	Eosin B
Absorption	Water solubility, (log mol/L)	**-3.298**	-3
	Mol/L	(0.0005035)	(0.001)
	Caco2 permeability, (log Papp in 10^{-6} cm/s)	**0.515**	**-0.282**
	Intestinal absorption (human), (% Absorbed)	**45.724**	**37.932**
	Skin Permeability, (log Kp)	**-2.735**	**-2.735**
	P-glycoprotein substrate	**No**	**Yes**
	P-glycoprotein I inhibitor		**No**

Property	Character	Eosin Y	Eosin B
	P-glycoprotein II inhibitor		
	BBB permeability, (logBB)	**-1.127**	**-1.809**
	CNS permeability, (logPS)	**-3.022**	**-3.787**
Metabolism	CYP2D6 substrate	**No**	
	CYP3A4 substrate	**Yes**	
	CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4 inhibitior	**No**	
Excretion	Total Clearance, (log ml/min/kg)	**-2.133**	**-1.872**
	Renal OCT2 substrate	**No**	
Toxicity	AMES toxicity	**No**	**Yes**
	Max. tolerated dose (human), (log mg/kg/day)	**0.317**	**0.225**
	hERG I inhibitor	**No**	
	hERG II inhibitor		
	Oral Rat Acute Toxicity (LD_{50}), (mol/kg)	**2.357**	**2.464**
	Oral Rat Chronic Toxicity (LOAEL), (log mg/kg_bw/day)	**1.379**	**0.732**
	Hepatotoxicity	**No**	**Yes**
	Skin Sensitisation		**No**
	T.Pyriformis toxicity, (log ug/L)	**0.285**	**0.285**
	Minnow toxicity, (log mM)	**-1.426**	**-1.974**

3. Results and Discussion

Pollution is highly dangerous subject in earth life progress now and for the next generations. Many organic pollutants can be easily removed by several approaches such as adsorption where adsorption material can be regenerated and pollutant can be recovered again. Many adsorption studies focused upon using natural materials or their wastes from in nature and from production routes [15, 16, 17, 32]. Lemon peel utilized for removal of Eosin dye in an aqueous medium that affected by adsorbent dose, temperature, contact time, isothermal models (Freundlich and Langmuir). Adsorption capacity was 8.240 mg/g at 30 ^{o}C, exothermic, and pseudo-second-order kinetics [15].

Also, biosorption of Eosin (Y & B) using *Saccharomyces cerevisiae* was investigated through time, initial concentration, pH, temperature, and adsorbent dosage parameters by batch method where (200 and 100) mg/g

for Eosin Y and B adsorption respectively. This material was fitted with Langmuir and Temkin models and presented a spontaneous, endothermic, and pseudo-second order kinetic model for both anionic dyes [33].

In another published study, effect of Hexadecyl trimethyl ammonium salts on Eosin adsorption by natural clay as a stable adsorbent in various pH media was investigated after instrumental characterization including Thermal Gravimetric Analysis (TGA) and in-situ X-rays Diffraction (XRD). Experimental results confirmed that endothermic removal with max. 55mg /g that decreased to (25 mg/g) after surface active material under study were added. Regeneration recycles reduced efficiency from 20% to 30% [34].

Cellulose in its base is a natural substance can be obtained from plant or micro-organisms such as bacteria or fungi. Soft cleaning tissues are important material manufactured from cellulose and used in cleaning and safety sections where their daily usages become a critical issue. In Covid -19 period, using this substances especially by medical sectors provided biological pollution zones exceedingly spread all over the world. With the base of solution results from problem, conversion of this bio-based material to activated carbon was a good choice in solution strategy.

In this research paper, soft cleaning tissues (face tissue or Kleenex as a known trade mark of this material) were converted to activated carbon via chemical method with using concentrated sulphuric acid and sodium carbonate. Conversion percentage was more than 30% as mentioned in experimental section. Each unit in this natural polysaccharide base material ($(C_6H_{10}O_5)_n$) may be considered as $5H_2O$ molecules and six carbon atoms. So, our experimental chemical transformation was with in remarkable chemical changing from low quality soft cleaning tissues to healthier material. Standard curve linearity at 517 nm controls concentration range and its trending line results (Lambert – Beer range, intercept, linear regression (R^2), molar absorptivity, and trend line equation) (Figure 2, Table 7).

Table (7): Linearity results at 517 nm.

Lambert – Beer range	(1-12) ppm (1.60236E-06 to 1.92283E-05) Mole / L
Trend line equation	y = 0.0442x + 0.0061 (ppm range) y = 27565x + 0.0061 (mole/L range)
Linear regression (R^2)	0.9997
Molar absorptivity, L $mol.^{-1}$ cm^{-1}	27565

Eosin B contains C=O, N=O, C-O, C-N, C-Br, C-C, and aromatic and non-aromatic C=C bonds having $\sigma\rightarrow\sigma^*$, $\pi\rightarrow\pi^*$, $n\rightarrow\sigma^*$, and $n\rightarrow\pi^*$ transitions. Here, $\pi\rightarrow\pi^*$ transition is a favourable shift occurred at low frequency when energy absorption of this anionic dye provides a strong interaction with solvent through hydrogen bonding.

Bromo, carboxylate and nitro groups encourage basic property and availability of hydrogen bonding interaction between solvent (water) and solvate (Eosin B). Also, maximum wavelength (517 nm) of Eosin B works in the visible region ((380-780) nm) with Energy: $\Delta E=hc/\lambda$, 1.027 $x10^{-22}$ kJ where charge transfer requires high energy based on unsaturated and aromatic bonds.

Prepared activated carbon exhibited good adsorptive performance with Eosin B dye at all studied situations. Adsorption capacity and removal efficiency gave similar numbers because of using (1L) and (5 grams) (Equations 1 & 2, Table 2). It can be concluded that repeating step viewed adsorbent and eluent interaction from the first 5 mL to the end. In solution, Eosin B molecule is adjacent by water molecules (solvation) that must be occupied in the accessible activated carbon pores where water losing can be happened through drying by time. So, changing of (R% and Q) in the repeated adsorption revealed solvated Eosin B molecules influenced by the formed interactions (Eosin B in pores and in aqueous solution) as an expected explanation.

Here, removal efficiency (or adsorption capacity) of this anionic material were slightly increased in the repeating step similar to the usual water filtration. In conclusion, soft tissue - activated carbon adsorbed most of 500 ppm

of Eosin B solubilized in 1L through 5 gm by simple filtration technique was excellent adsorbent for continuous adsorption (or dye removal).

At this point, Eosin Y & B were introduced in *In Silico* evaluation that broadly performed in various fields by collective online biosig websites linked to the lab in Brisbane (Queensland) having varius pharmacokinetic-toxicity tools such as cancer, embryo, crop-, besides water solubility, Human Intestinal absorption (%), permeability of BBB and CNS, Hepatotoxicity, skin sensitization, Cytochrome P450 Family by both substrate and inhibition, and more others. SMILES specification is the base in prediction approaches.

Enzyme expression and protein transportation of human colon cancer cell line known as (*cancer coli*-2, Caco2) is thoroughly studied where chemical under study is diffused by intestinal pathway(s) owing to system simplicity and reproducibility. It influenced by hydrophilic (or hydrophobic) characters wherever high hydrophilic results a significance low Caco-2 monolayer as *in vitro* model [35].

Various *in vitro* and *in vivo* studies of ADMET were managed to estimate human and environment hazards particularly in oral absorption and related issues. Outputs of safety assessment in protection of human or animal according to scientific methodologies related to Reduction-Refinement and Replacement (AHE-3Rs] associated to molecular descriptions [36 - 41].

According to Tables (3, 4, 5, & 6), Eosin Y & B dyes had distinguishable characters can be summarized as below:

- Both dyes were safe to embryo or foetus health (during pregnancy) where Embryo or fetus is oversensitive to all chemical kinds where direct / or indirect exposure through placental membrane and pointers irregular growth demonstrated by deterioration or death [42].
- Also, they were safe in Cardio- topic including: Arrhythmia, Cardiac Failure, Heart Block, hERG Toxicity, Hypertension, and Myocardial Infarction. Any chemical substance may influence heart and / or blood vessels and their functions for short- or long –term. Heart toxicity can be classified to Rhythm disturbances (Calcium or sodium ion, hERG channel blockade), Myocardial ischemia (endogenous catecholamines, nitrates, β-blockers, anticancer drug), heart failure or Left ventricular (LV) dysfunction (Calcium or sodium ion channel blockade), Induction of pericardial disease (immune action), Impairment of cardiac valves, arterial blood pressure as systematic hypertension or Pulmonary hypertension, and Thromboembolic complications (Arterial or venous) [43].
- Both works with same behaviour in Skin Permeability. Skin is a key wall against the external environment constitutes of multiple layers: epidermis, dermis, and subcutis with multiple constructs, morphology, and functions. It is a preferred route in therapy due to its non- aggressive nature and safety for chemical transport such as insulin or vaccine especially in pandemic times. Skin permeability is an essential in pharmaceutical and cosmetic hazard and risk evaluation via *in vitro, in vivo, ex vivo*, and *In Silico* assays in chemical assessment under deep control of ethical issues. *In Silico* or computerized based modeling is an interesting evaluation in skin permeability with reduction of time and economic effects with absence of ethical issues as it has been published [44-47].
- Eosin Y safe to avian but had a mixed repose in Ames test. *Salmonella typhimurium* reverse mutation assay abbreviates as Ames test as a DNA mutation term located in *S. typhimurium* (bacterium in this test) and identified as carcinogenic materials.
- Eosin Y had the minor Rat Acute Toxicity (LD_{50}) and Rat Chronic Toxicity (LOAEL) in website that varied in response in website.
- Eosin Y had the lower quantity required for minimum probability in cancer cell lines of CNS and Melanoma. So, it is less harmless in these types of cancers.
- In contrast, Eosin B had the lowest probability of lung, prostate, and renal cell lines. So, it is safer than Eosin Y in these cancer cell lines.
- Eosin B had the highest values of water solubility in aqua environment, Caco2 permeability, human intestinal absorption (% Absorbed), Total Clearance**.** Caco-2 permeability is known *in vitro* evaluation of human intestinal absorption [48].

- Eosin Y presented No response towards P-glycoprotein substrate and inhibition. P-glycoprotein has a multidrug resistance (MDR1) function representing Adenosine Triphosphate Binding Cassette Transporter (ABCB1) in cancer pharmacotherapy where any compound can be classified as modulator, substrate, inhibitor, or inducer to this protein [49].
- Eosin Y had the highest BBB and CNS permeability in addition to Minnow toxicity compared to Eosin B.
- Both had No response towards CYP2D6 substrate, and CYP1A2, besides CYP2C19, CYP2C9, CYP2D6, or CYP3A4 inhibitor, in addition to Renal OCT2 substrate, besides hERG I & II inhibitors, Hepatotoxicity, and skin sensitization.

4. Conclusions

Another attempt to prepare an activated carbon from carbon based material where its capacity was determined by toxic anionic dye (Eosin B). In this attempt, Eosin B showed an excellent removal and adsorption capacity starting from first 5 mL eluted from this prepared adsorbent. Removal of Eosin is important to human and environment where present *In Silico* study confirmed that Eosin B was safe to embryo or foetus health (during pregnancy) , Cardio- topic including: Arrhythmia, Cardiac Failure, Heart Block, hERG Toxicity, Hypertension, and Myocardial Infarction.it was toxic to avian with high Rat Acute Toxicity (LD_{50}). Also, it was with the lowest probability of lung, prostate and renal cell lines even with its good water solubility in aqua environment. Yes response towards P-glycoprotein substrate or multidrug resistance (MDR1) function in cancer pharmacotherapy was noticed. Also, Eosin B had BBB or CNS permeability as well as fish (Minnow) toxicity. It is safe for Renal OCT2 substrate besides hERG I & II inhibitors, Hepatotoxicity, and skin sensitization, Cytochromes P450 under testing. So, Eosin B is structurally toxic to human and environment in short – or long – term period.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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