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# Zebrafish shares common metabolic pathways with mammalian olanzapine-induced obesity

Pukar Khanal<sup>1\*</sup> , B. M. Patil<sup>1\*</sup> and Banappa. S. Unger<sup>2</sup>

## Abstract

**Background:** Olanzapine is one of the most prescribed antipsychotic agents in the pharmacotherapy of psychiatric illness; however, it is associated with multiple side effects primarily obesity. Multiple investigations have been made to model the olanzapine-induced obesity in rodent models which was found to be dose-dependent, gender-dependent, and species-dependent. *Danio rerio* is a choice of an animal model to understand the pathogenesis of multiple diseases. The present study dealt to understand the olanzapine-associated obesity in zebrafish using in silico and wet-lab experimental protocols by performing gene set enrichment analysis, phylogeny comparison of receptors, and assessing the effect of olanzapine on metabolic rate, lipid metabolism, body weight, and food intake in zebrafish.

**Results:** The metabolic pathway was predicted to be majorly modulated by olanzapine in human, rat, mouse, and zebrafish. A clade of receptors of rat, mouse, and human receptor for feeding and satiety center was found similar to zebrafish. The decrease in lipid metabolism was observed in zebrafish larvae if exposed to olanzapine solution. Similarly, there was a significant decrease in metabolic rate in 200  $\mu$ M and 400  $\mu$ M concentration of olanzapine.

**Conclusion:** Enrichment analysis predicted the probable modulation of metabolic pathways in zebrafish if exposed to olanzapine. Further, olanzapine was identified to play a prime role in decreasing lipid metabolism and metabolic rate and increasing food intake and weight gain in zebrafish which suggests the validation of this model for olanzapine-induced obesity.

**Keywords:** *Danio rerio*, Lipid metabolism, Metabolic rate, Olanzapine-induced obesity, Zebrafish

## Background

The current pharmacotherapy of psychiatric illness utilizes many atypical antipsychotic molecules in which olanzapine reserve its own importance in the major portion of prescription due to its high efficacy. However, the molecule is associated with multiple side effects, i.e. diabetes, dyslipidemia, and cardiovascular system (CVS) complications including obesity [1–3]. Many attempts have been made to understand the mechanism for olanzapine-induced weight gain in various rodent models. However, the effects of olanzapine observed in rodent

models were gender-dependent, dose-dependent, and duration-dependent [4–8] reflecting the lack of an adequate model to represent the pathogenesis of olanzapine-induced obesity. Further, rodent models conserve the property of being unwieldy, expensive, and are allied with ethical issues for their utilization in research. This limitation kindled us to identify a new simple and alternative animal model for olanzapine-induced obesity.

*Danio rerio*, commonly identified as zebrafish, is an alternative animal model that has been extensively utilized in biomedical research [9] due to its similarity in physiological and metabolic pathways with *Homo sapiens* and other rodent animal models. Further, it has got popularity in lipid research, mainly for lipoprotein metabolism, lipid absorption and deposition, hepatic

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steatosis, obesity, diabetes, and other metabolic disorders [10]. Further, these vertebrates are also reported to share parallel pathophysiological pathways to mammals for diet-induced obesity [11]. These reports kindled us to investigate if olanzapine-induced abnormalities primarily lipid metabolism, metabolic rate, food intake, and weight gain can be expressed in zebrafish or not. To fulfill this hypothesis, we utilized the cheminformatics tools to predict the olanzapine-induced gene expression profile in *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, and *Danio rerio*, similarity index of the receptors available in the feeding, and satiety center. Further, the effect of olanzapine on lipid metabolism, metabolic rate, food intake, and weight gain was evaluated via experimental protocols.

## Methods

### Olanzapine-induced gene expression and gene set enrichment analysis

Canonical SMILES of olanzapine was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and queried for the probable mRNA-based gene expression using DIGEP-Pred [12]. All the expressed genes with probable activity (Pa) greater than probable inactivity (Pi) were queried in STRING [13] database to evaluate the protein-protein interaction for *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, and *Danio rerio* with reference to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (<https://www.genome.jp/kegg/pathway.html>) database.

### Phylogenetic comparison of olanzapine-modulated receptors

Proteins modulated by the olanzapine were identified from BindingDB [14] which works based on the principle “similar compounds tend to bind the same proteins” by querying canonical SMILES. The amino acid sequences of protein molecules for *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, and *Danio rerio* were retrieved from the NCBI database (<https://www.ncbi.nlm.nih.gov/>). The accession number of protein molecules subjected to phylogenetic comparisons is summarized in Table 1. Amino acid sequences were aligned using multiple sequence alignment, and the phylogenetic tree was constructed using the UPGMA clustering method. The bootstrap score was used to assess the similarity score between the protein molecules.

### Ethical approval and housing of zebrafish

The experiment was performed after receiving ethical clearance from the IAEC, accession number KLECOP/CPCSEAReg.No.221/PO/Re/S/2000/CPCSEA Res.25-09/09/2017. Adult wild zebrafish of 2.5–3.5 cm were purchased from Fish-O-fish aquarium, Nehru Nagar Kurla (E) Mumbai and were housed under 14:10 light/dark

photoperiod in a re-circulating aqueous system (water temperature  $26 \pm 1^\circ\text{C}$ , dissolved oxygen (measured using dissolved oxygen kit, HighMedia)  $> 4\text{ mg/L}$ , conductivity  $500\text{--}650\ \mu\text{S cm}^{-1}$  (maintained using Red Sea Salt), pH 6.8–7.4, and total dissolved salt (TDS) 250 to 325 ppm) and fed with dry flakes (once) and brine shrimps (twice) daily. Ten percent of total system water was replaced with new RO-purified water to maintain the water condition.

### Breeding and maintenance of fertilized embryos

Adult zebrafish were allowed to mate in the ratio of 2:3. Initially, before the beginning of the dark photoperiod, the animals were separated and mixed together just before the beginning of light photoperiod, and fishes are allowed to mate. After 30 to 45 min, the embryos were collected, washed, and maintained in embryo medium (HBSS Sigma). The fish were returned to their cages allowing a week of resting period before two successive breedings. Embryos were washed at 6 and 24 hpf to remove the coagulated embryos; a percentage of coagulated embryos and hatched larvae were recorded at 24 and 72 h, respectively.

### Selection of dose

OECD 236 guidelines were followed to perform the fish embryonic toxicity (FET) which identified lethal concentration ( $\text{LC}_{50}$ :64  $\mu\text{M}$ ) and maximum non-lethal concentration (MNLC:50  $\mu\text{M}$ ) of olanzapine in 72 hpf larvae. Hence, five different concentrations of olanzapine in geometric series were chosen to evaluate the effect of olanzapine on lipid metabolism and metabolic rate. Further, metabolic rate was evaluated in a broader range of drug concentrations, i.e. below and above the MNLC since the change in metabolic rate was not observed in lower concentrations.

### Effect of olanzapine on lipid metabolism in zebrafish larvae

The effect of olanzapine on lipid metabolism was assessed in 72 hpf larvae [15]. Zebrafish larvae were exposed to multiple concentrations of olanzapine for 24 h at  $28^\circ\text{C}$ . After 24 h, larvae were fixed in 4% paraformaldehyde, and Oil Red O (ORO) imaging procedure was performed under a dissecting stereoscopic microscope (Olympus Co., Tokyo, Japan). ORO was then quantified to determine the accumulation of lipids in different parts of larvae. The amount of lipid was quantified using the Fiji (Image J) software under red, green, and blue channels.

### Effect of olanzapine in metabolic rate in zebrafish larvae

Whole-body metabolic rate assay [16] was performed to evaluate the effect of olanzapine on metabolic rate with minor modifications. Briefly, 72 hpf zebrafish larvae were

**Table 1** Accession number of proteins retrieved from NCBI

Receptor	Accession number of receptor molecule of organisms			
	<i>Homo sapiens</i>	<i>Rattus norvegicus</i>	<i>Mus musculus</i>	<i>Danio rerio</i>
5-HT 2A	AAB31320.1	NP058950.1	NP766400.1	XP689300.7
5-HT2C	AAB40898.1	NP036897.2	NP032338.3	ABP04055.1
$\alpha$ -2A	AAK51162.1	NP036871.3	NP03144304	NP997520.3
$\alpha$ -2C	NP_000674.2	NP612515.1	NP031444.2	NP997522.1
D4	NP_000788.2	AAA18588.1	AAC52190.1	NP001012638.2
D2	AAC78779.1	NP036679.1	NP034207.2	NP898891.1
D3	AAB08750.1	AAA41076.1	NP031903.1	AA87173.1
SERT	P31645.1	NP037166.2	NP034614.2	NP001170930.1
NTS1	AAR07901.1	NP001102437.1	EDL07336.1	XP021329279.1
H1	NP_001091683.1	NP058714.1	NP001304053.1	NP001036196.1
M1	NP_000729.2	NP542951.1	NP001106167.1	XP001334664.4
M2	NP_001006633.1	NP112278.1	NP987076.2	NP840086.1
5-HT 1A	NP_000515.2	NP036717.1	NP032334.2	NP001116793.1
5-HT 7	AAH47526.1	NP075227.1	CAQ76702.1	XP003199632.2
$\alpha$ -1A	AAA35496.1	AAA63477.1	AAC02658.1	XP001922013.3
5-HT 6	AAA92622.1	NP077341.2	NP067333.1	XP009295353.1
D1	AAB26273.1	AAA70428.1	NP001278730.1	ACI42369.1

exposed to five different concentrations (200  $\mu$ M, 100  $\mu$ M, 50  $\mu$ M, 25  $\mu$ M, and 12.5  $\mu$ M) of olanzapine and assay buffer (0.01% w/v of phenol red in sodium bicarbonate solution at pH 8.0 and conductivity 350  $\mu$ M) in 96-well microtiter plate, and absorbance was recorded for 180 min at 570 nm at 10 min interval. The amount of acid produced over a period of time was quantified by using a linear regression curve.

#### Effect of olanzapine in food intake and weight gain

Bodyweight was recorded at the interval of 3 days, and food intake (daily) for 21 days using thirty adult female zebrafish weighting  $229 \pm 20$  mg. The study design included five groups, each containing six zebrafish in each: Group 1, exposed to system water; Group 2, to 0.001% DMSO in system water; Group 3, exposed to 16.66  $\mu$ M olanzapine; Group 4, exposed to 5  $\mu$ M olanzapine; and Group 5, exposed to 0.5  $\mu$ M olanzapine. After the completion of the study, all adult zebrafish were allowed to rehabilitate separately with the adequate conditions and not utilized for any other experiments.

#### Statistical analysis

Results were expressed as mean  $\pm$  SEM. The significant difference among data was determined using one-way ANOVA followed by the Tukey test using the GraphPad Prism software version 5.0. The difference between mean was considered significant if  $p$  value  $< 0.05$ . The network of gene-pathway

interaction was interpreted by edge count. The similarity index between proteins was compared via the bootstrap score using MEGA7.

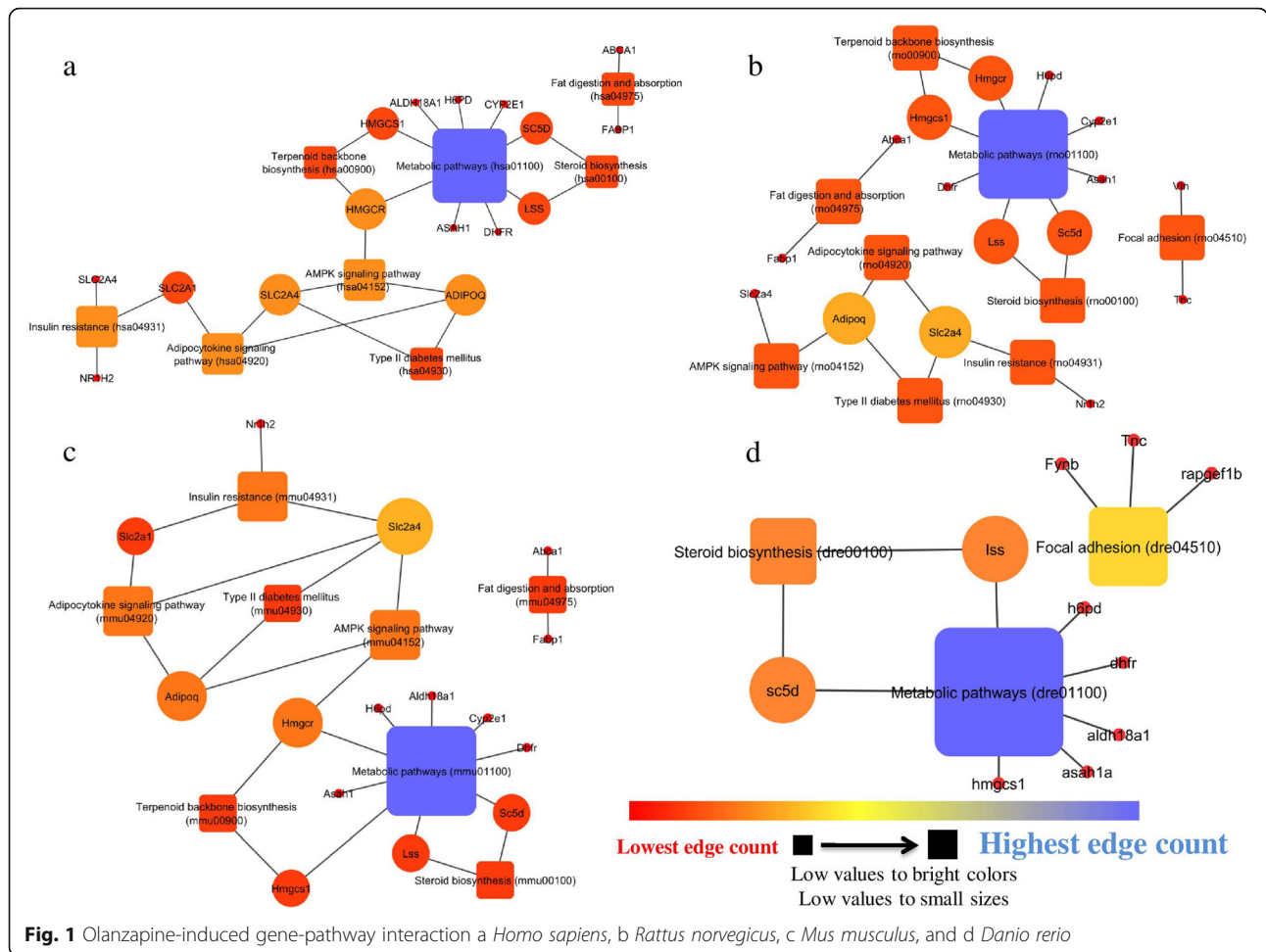
## Results

### Olanzapine-induced gene expression and gene set enrichment analysis

Based on mRNA-based prediction, forty-five genes were predicted to be expressed; among them, twenty were downregulated and twenty-five were upregulated. Gene set enrichment predicted forty-five proteins to be modulated in *Homo sapiens*, forty-three in *Rattus norvegicus* and *Mus musculus*, and thirty-nine in *Danio rerio*. Similarly, SERPINA3 and C10ORF118 were not detected in *Rattus norvegicus* and *Mus musculus* and EVI2B, FNDC4, SERPINA3, CYP2E1, LST1, and NR1H2 in *Danio rerio*. Further, KEGG pathway analysis identified eight pathways to be modulated in *Homo sapiens*, nine in *Rattus norvegicus*, eight in *Mus musculus*, and three in *Danio rerio*. Among all, the metabolic pathway was common and was majorly modulated in all species. Figure 1 summarizes the detail pathway-gene interaction, and Table 2 summarizes the gene set enrichment of individual pathways of each species.

### Phylogenetic comparison of olanzapine-modulated receptors

Among *Rattus norvegicus*, *Mus musculus*, and *Danio rerio*, receptors of *Rattus norvegicus* and *Mus musculus*



**Fig. 1** Olanzapine-induced gene-pathway interaction a *Homo sapiens*, b *Rattus norvegicus*, c *Mus musculus*, and d *Danio rerio*

were more similar to *Homo sapiens* compared to *Danio rerio*. However, M2, 5HT-1A, and alpha 1A receptors of *Rattus norvegicus* were more similar to *Homo sapiens* compared to *Mus musculus*. The phylogenetic representation including a bootstrap score of the *Danio rerio* branch with a clade of *Homo sapiens*, *Rattus norvegicus*, and *Mus musculus* is presented in Fig. 2.

**Effect of olanzapine on lipid metabolism**

In 50 μM olanzapine, the natural death of larvae occurred at 192 h of exposure (Fig. 3). Fish embryonic toxicity (FET) also reflected 50 μM olanzapine as a maximum non-lethal concentration. Hence, multiple concentrations of olanzapine, i.e. 50 μM, 25 μM, 12.5 μM, 6.25 μM, and 3.125 μM, were chosen to expose 72 hpf larvae. Accumulation of lipid increased significantly from lowest concentration (3.125 μM, *p* < 0.05) to the highest concentration (50 μM, *p* < 0.001) (Fig. 4) which was quantified by integrated optical density (IOD) of stain; represents the total amount of lipid present in the whole body of larvae.

**Effect of olanzapine on metabolic rate in zebrafish larvae**

Empirical titration was performed by adding a known amount of acid (H<sup>+</sup>) in each well of 96-well microtiter plates and titrated with 50 μl assay buffer (0.01% w/v phenol red). The linear equation  $y = -0.3342x + 2.3768$  was obtained with *R*<sup>2</sup> value of 0.997. Larvae exposed to the range of concentrations of olanzapine modulating lipid metabolism, body weight, and food intake had no significant effect to change the metabolic rate. However, exposure of larvae at higher concentration, i.e., 200 μM and 400 μM of olanzapine, showed a significant change in acid production at different time intervals (Fig. 5). Toxicity of phenol red was evaluated by exposing 72 hpf zebrafish larvae to buffer at pH 8.0 containing phenol red (0.01% w/v). Death of larvae in assay buffer began after the 4<sup>th</sup> day of exposure. However, log-rank (Mantel-Cox) test showed in significant difference in death in assay buffer compared to system water suggesting that phenol red did not cause any toxic effect during the experiment (Fig. 6).

**Table 2** Olanzapine-induced gene set enrichment analysis in *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, and *Danio rerio*

Organism	Pathway	Description	Gene count	Genes	False discovery rate	
<i>Homo sapiens</i>	hsa04975	Fat digestion and absorption	2	FABP1, BCA1	0.0428	
	hsa04931	Insulin resistance	3	SLC2A1, SLC2A4, NR1H2	0.0428	
	hsa04920	Adipocytokine signaling pathway	3	SLC2A1, SLC2A4, ADIPOQ	0.0428	
	hsa04152	AMPK signaling pathway	3	SLC2A4, ADIPOQ, HMGCR	0.0428	
	hsa01100	Metabolic pathways	9	ALDH18A1, DHFR, H6PD, LSS, SC5D, HMGCS1, CYP2E1, HMGCR, ASAH1	0.0428	
	hsa00900	Terpenoid backbone biosynthesis	2	HMGCS1, HMGCR	0.0428	
	hsa00100	Steroid biosynthesis	2	LSS, SC5D	0.0428	
	hsa04930	Type II diabetes mellitus	2	SLC2A4, ADIPOQ	0.0482	
<i>Rattus norvegicus</i>	rno04920	Adipocytokine signaling pathway	3	Adipoq, Slc2a4	0.0222	
	rno04931	Insulin resistance	3	Slc2a4, Nr1h2	0.0242	
	rno04152	AMPK signaling pathway	3	Adipoq, Slc2a4	0.0242	
	rno01100	Metabolic pathways	8	Cyp2e1, Hmgcr, Sc5d, Hmgcs1, Lss, H6pd, Dhfr, Asah1	0.0242	
	rno00900	Terpenoid backbone biosynthesis	2	Hmgcr, Hmgcs1	0.0242	
	rno00100	Steroid biosynthesis	2	Sc5d, Lss	0.0242	
	rno04975	Fat digestion and absorption	2	Fabp1, Abca1	0.0251	
	rno04930	Type II diabetes mellitus	2	Adipoq, Slc2a4	0.0315	
	rno04510	Focal adhesion	3	Vtn, Tnc	0.0428	
	<i>Mus musculus</i>	mmu04975	Fat digestion and absorption	2	Abca1, Fabp1	0.0283
		mmu04931	Insulin resistance	3	Nr1h2, Slc2a4, Ic2a1	0.0283
		mmu04920	Adipocytokine signaling pathway	3	Adipoq, Slc2a4, Slc2a1	0.0283
mmu04152		AMPK signaling pathway	3	Adipoq, Slc2a4, Hmgcr	0.0283	
mmu01100		Metabolic pathways	9	Asah1, Cyp2e1, H6pd, Hmgcr, Dhfr, Aldh18a1, Hmgcs1, Lss, Sc5d	0.0283	
mmu00900		Terpenoid backbone biosynthesis	2	Hmgcs1, Hmgcr	0.0283	
mmu00100		Steroid biosynthesis	2	Sc5d, Lss	0.0283	
mmu04930		Type II diabetes mellitus	2	Adipoq, Slc2a4	0.0374	
<i>Danio rerio</i>	dre00100	Steroid biosynthesis	2	sc5d, lss	0.0114	
	dre01100	Metabolic pathways	7	sc5d, hmgcs1, lss, h6pd, dhfr, aldh18a1, asah1a	0.0320	
	dre04510	Focal adhesion	3	fynb, tnc, rapgef1b	0.0453	

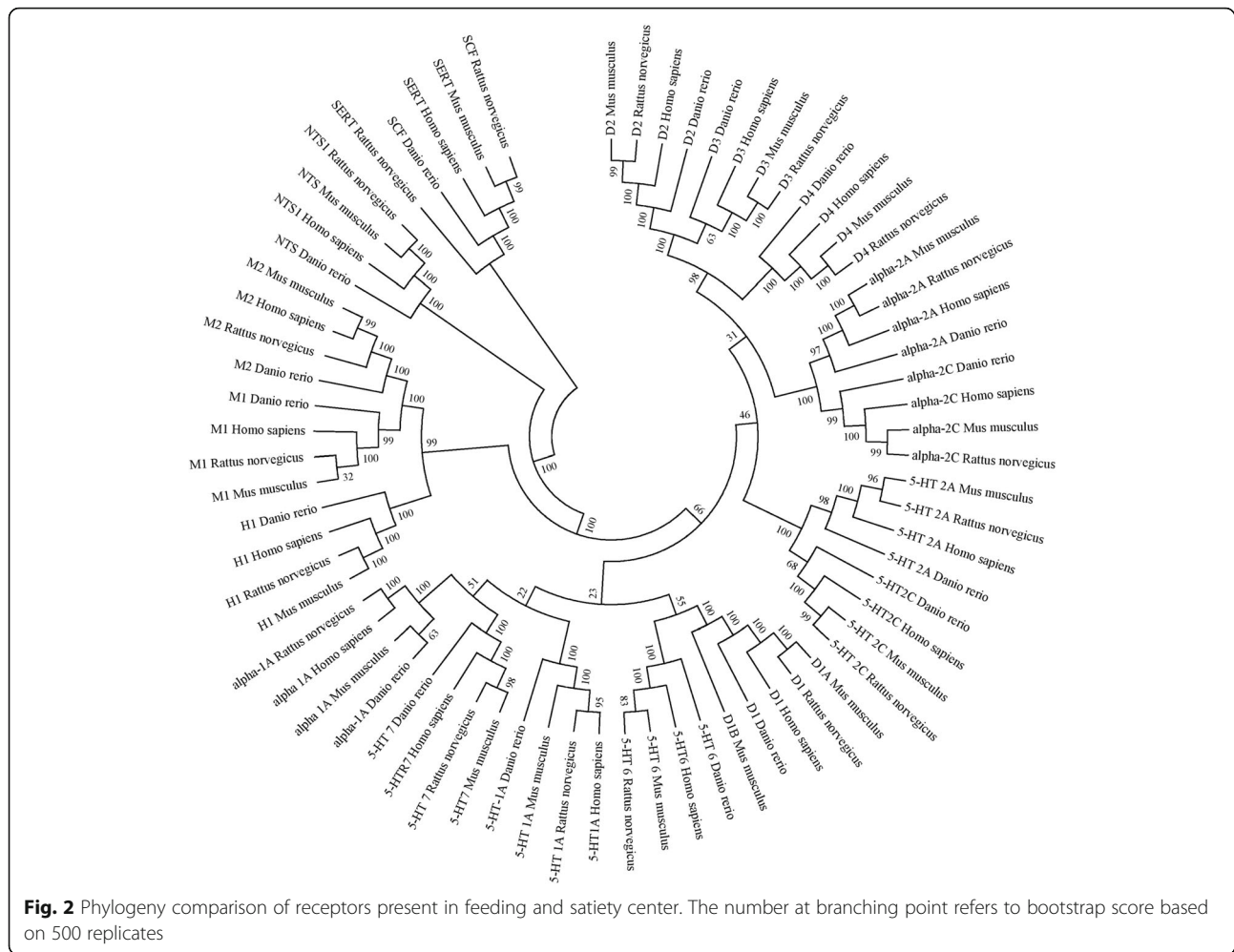
### Effect of olanzapine on food intake and weight gain

Hyperphagia was observed from the 7<sup>th</sup> day and continued till the 20<sup>th</sup> day in adult zebrafish exposed to 0.5  $\mu$ M olanzapine. Similarly, zebrafish exposed to 0.5  $\mu$ M showed a significant increase in weight gain on the 12<sup>th</sup> day and continued until the 20<sup>th</sup> day (Fig. 7).

### Discussion

The present study dealt to investigate whether olanzapine-induced metabolic changes can be demonstrated in zebrafish or not which can be further employed as an alternative experimental model. Before the performance of wet-lab experiments, we predicted the olanzapine-induced gene expression in four



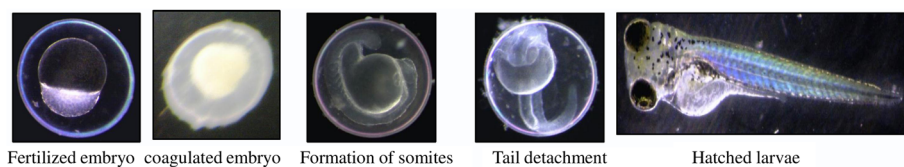


different species. Interestingly, we identified a metabolic pathway to be common in all, modulating the highest number of gene sets. Further, a clade of proteins involved in feeding and satiety center of *Danio rerio*, *Rattus norvegicus*, and *Mus musculus* was found to be similar with *Homo sapiens*. This reflected that the receptors present in hunger and satiety center are closely related among four different species; suggested the feeding behavior of *Danio rerio* could be similar to *Homo sapiens*, *Rattus norvegicus*, and *Mus musculus*. These two observations further kindled us to evaluate the effect of olanzapine in lipid metabolism, metabolic rate, food intake, and weight gain in zebrafish larvae.

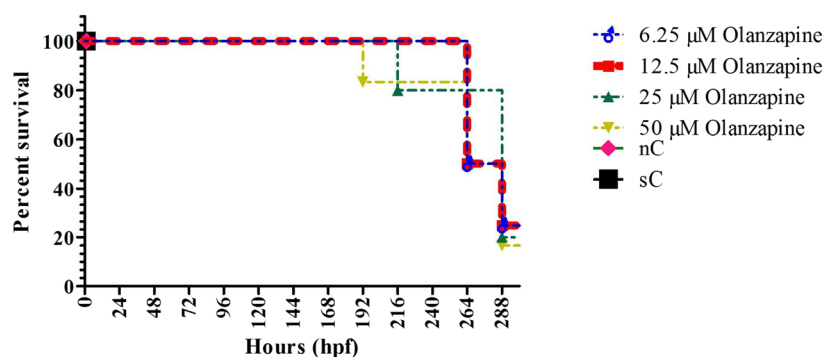
Literature reflects that olanzapine-induced obesity is associated with hyperphagia [17] leading to weight gain. This hypothesis of olanzapine-induced obesity has been successively demonstrated in rodent models [4–8]. In the present study, exposure of adult zebrafish in olanzapine showed an increase in food intake

and weight gain in 0.5 μM concentration. Hence, the study was further designed to understand the effect of olanzapine on lipid metabolism in zebrafish larvae.

Further, a correlation has been made between olanzapine-induced obesity and anomalous metabolism of the lipids [18, 19]. An elevated level of free fatty acids, TGs, total cholesterol, LDL, and low level of HDL have been reported in olanzapine-induced obesity directing towards dyslipidemia and its associated complications [20, 21]. Clinical case for dyslipidemia condition has been reported in the patients under the pharmacotherapy of olanzapine which is additionally demonstrated in the rodent models by numerous investigators [4–8]. Similarly, in the current study, exposure of 72 hpf larvae in the solution of olanzapine for 24 h showed a decrease in lipid metabolism which was confirmed via the Oil Red O staining technique. This altered lipid metabolism in olanzapine-exposed larvae reflects “dwindle lipid metabolism” as one of the important reasons for olanzapine-induced obesity.



**Kaplan-Meier Curve for Maximum Non Lethal Concentration**



**Fig. 3** Survival curve (Kaplan-Meier curve) of larvae exposed to olanzapine. nC, negative control, sC, solvent control. Total number of larvae exposed to each concentration ( $n$ ) = 6

The decrease in lipid metabolism is linked with repression in energy expenditure [22] which occurs due to variation in basal metabolic rate [23–25]. This variation of basal metabolic rate is closely related to abnormal metabolism of lipids [26]. Total respiratory compliance is decreased in the obese population compared to the normal population. This reduction in total respiratory compliance is due to increased pulmonary blood volume, decrease in chest wall, and accumulation of fat in and around the ribs, diaphragm, and abdomen. Interestingly, this observation was also recorded in the present study during the assessment of lipid metabolism in zebrafish larvae. Reduction in total respiratory compliance causes an increase in respiratory resistance [26–28] and high anaerobic respiration [26, 29] leading to lactic acidosis reflecting the decreased metabolic rate. Metabolic rate was assessed in zebrafish larvae by utilizing an anaerobic system which is based on the principle of accumulation of lactic and carbonic acid [16]. Larvae exposed to olanzapine showed increased acid production ( $H^+$ ) leading to a decrease in basal metabolic rate.

Further, it has been predicted that a single compound can bind/modulate multiple proteins and regulate pathways associated with them [30, 31]. So, there is a possibility that olanzapine can also bind or regulate multiple proteins/pathways related to the homeostasis of appetite and energy expenditure. Hence, further investigation needs to assess the role of olanzapine-modulated

proteins/pathways in the development and progression of obesity and other side effects.

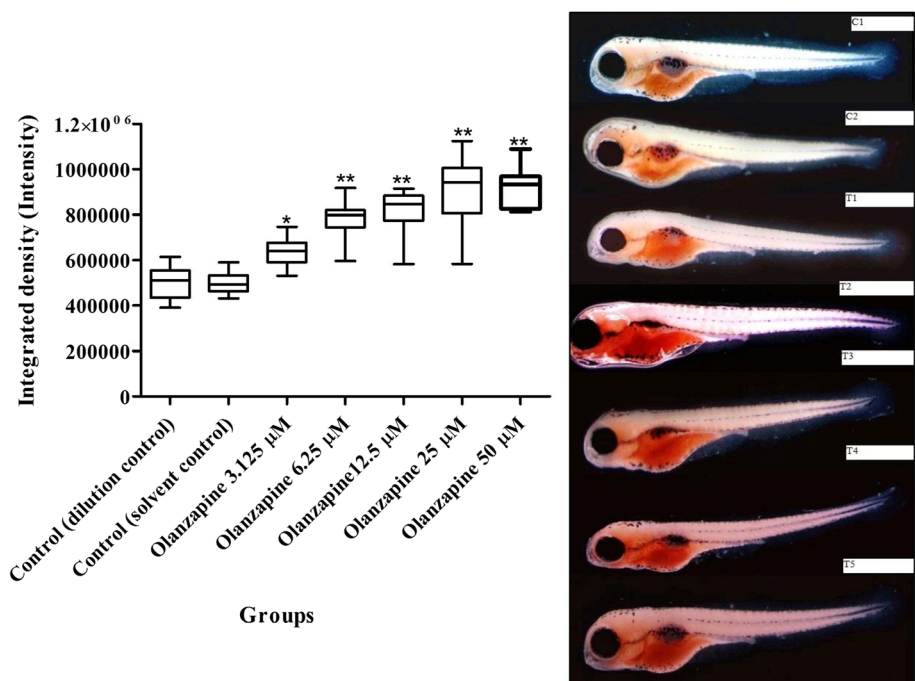
### Conclusion

The gene-set enrichment analysis identified five common genes which are also expressed in human and rodents. However, the role of each gene, i.e. *h6pd*, *dhfr*, *aldh18a1*, *asah1a*, and *hmgcs1*, needs to be extensively investigated for olanzapine-induced metabolic abnormalities in zebrafish and other rodent models. However, this prediction helped us to identify olanzapine-induced metabolic abnormalities, i.e., decreased lipid metabolism and metabolic rate and increased food intake and body weight. Limiting to the present study, change in the metabolic rate occurred in the toxic concentration of olanzapine, i.e. 400  $\mu$ M and 200  $\mu$ M making suspicious for change in metabolic rate due to energy expenditure. Further, approaches should be framed to evaluate biochemical parameters and assess gene expression profiles related to olanzapine-induced obesity in zebrafish using well-designed wet-lab protocols to confirm the current findings.

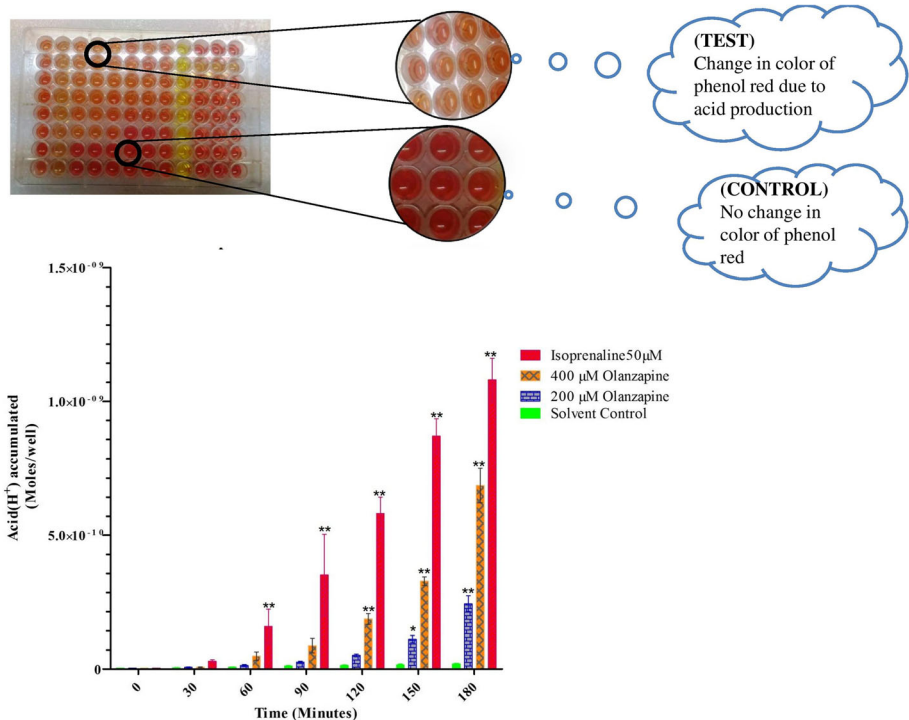
### Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s43094-020-00049-7>.

Additional file 1. ARRIVER1

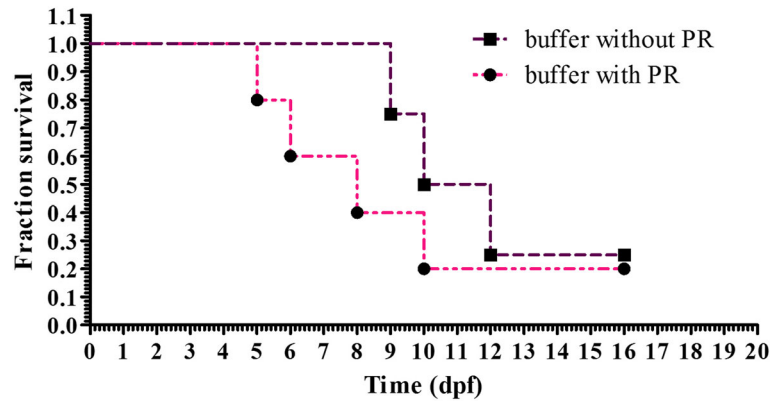


**Fig. 4** Effect of olanzapine (50 μM, 25 μM, 12.5 μM, 6.25, and 3.125 μM) in lipid metabolism in zebrafish larvae. Zebrafish at 72 hpf were exposed to C1, system water; C2, 0.0001% v/v DMSO; T1, 3.125 μM; T2, 6.25 μM; T3, 12.5 μM; T4, 25 μM, and T5, 50 μM of olanzapine. Lipids of zebrafish gut and vasculature increased with increase in the concentration of olanzapine. Scale 100 μm. Number of larvae in each groups (n) = 10. All the data are expressed in mean ± SEM. \*p < 0.05, \*\*p < 0.01 compared to solvent control

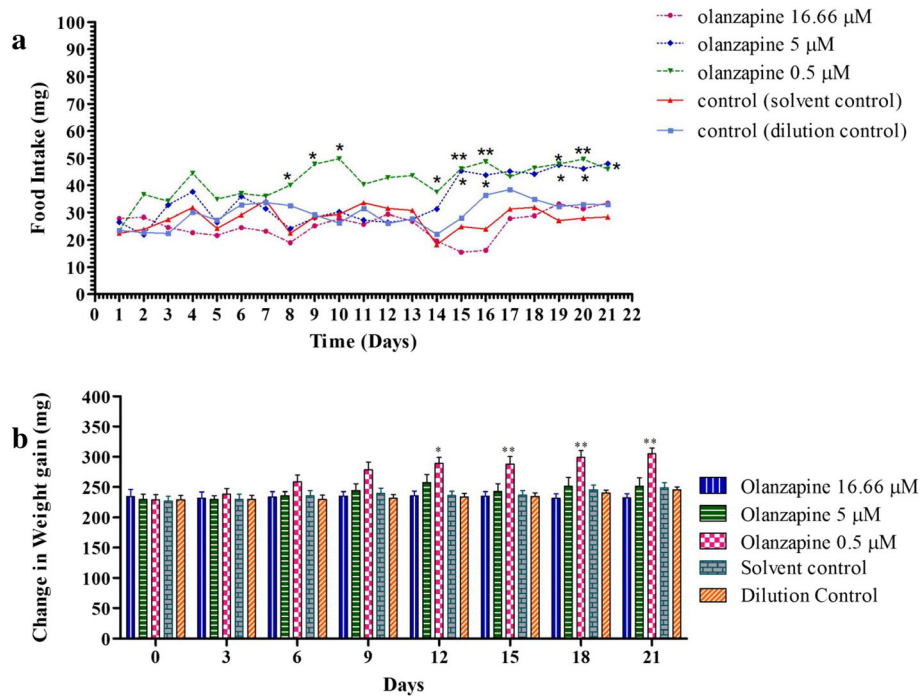


**Fig. 5** Effect of olanzapine (400 μM and 200 μM) on metabolic rate. Data are shown in 30 min interval. All the data are expressed in mean ± SEM. Number of larvae in each group (n) = 8. \*p < 0.05, \*\*p < 0.001 significantly different versus solvent group





**Fig. 6** Survival curve of larvae exposed to buffer in the presence and absence of phenol red. PR, phenol red



**Fig. 7** Effect of olanzapine on **a** food intake and **b** body weight. All the data are expressed in mean  $\pm$  SEM. Number of adult zebrafish in each group ( $n$ ) = 6. \* $p$  < 0.05, \*\* $p$  < 0.001 significantly different versus solvent group

### Abbreviations

ANOVA: Analysis of variance; CVS: Cardiovascular system; FET: Fish embryonic toxicity; HDL: High-density lipoprotein; IAEC: Institutional Animal Ethics Committee; KEGG: Kyoto Encyclopedia of Genes and Genomes; MNLC: Maximum non-lethal concentration; NCB: National Center for Biotechnology Information; OECD: Organisation for Economic Co-operation and Development; ORO: Oil Red O; Pa: Probable activity; Pi: Probable inactivity; SMILES: Simplified molecular-input line-entry system; TDS: Total dissolved salt; UPGMA: Unweighted pair group method with arithmetic mean

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### Authors' contributions

PK contributed in experimental design for in silico study, performed the wet-lab experiment, and drafted the manuscript. BMP introduced a concept for utilizing zebrafish in olanzapine-induced obesity in zebrafish and reviewed the manuscript. BSU provided the necessary facilities for the experimental work, trained PK in developing the skills in zebrafish experiments and contributed in drafting the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

The data for this work are available with Pukar Khanal upon request.

### Ethics approval and consent to participate

The experiment was performed after receiving ethical clearance from Institutional Animal Ethical Committee (IAEC) at KLE College of Pharmacy, Belagavi, KAHER, Belagavi accession number KLECOP/CPCSEA-Reg. No.221/PO/Re/S/2000/CPCSEA Res.25-09/09/2017.

### Consent for publication

Not applicable.

### Competing interests

The authors declared no conflict of interest.

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