SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Effect of anti-diabetic drugs on bone: a systematic literature review and meta-analysis of animal studies	
2.	Authors (names, affiliations, contributions)	**Mohammad Adil**, School of Pharmaceutical Education and Research, Department of Pharmacology, Jamia Hamdard (Hamdard University), New Delhi-110062, India **Pooja Verma**, School of Pharmaceutical Education and Research, Department of Pharmacology, Jamia Hamdard (Hamdard University), New Delhi-110062, India **Shiva K. Venkata**, Poona College of Pharmacy, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Pune-411038, India **Amit D. Kandhare**, Poona College of Pharmacy, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Pune-411038, India **Pinaki Ghosh**, Poona College of Pharmacy, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Pune-411038, India **Manju Sharma**, School of Pharmaceutical Education and Research, Department of Pharmacology, Jamia Hamdard (Hamdard University), New Delhi-110062, India	
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5.	Funding sources/sponsors	None	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration		
8.	Registration number (if applicable)		
9.	Stage of review at time of registration		
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Diabetes mellitus is associated with increased fracture risk [1, 2], and the mechanisms behind deleterious effects of diabetes on bone health are not well explored. Increasing evidence suggested that anti-diabetic drugs might have significant role on the skeletal system [3]. For instances, thiazolidinediones increases the bone loss and risk of fracture possibly through PPARγ activation in bone marrow cells and hamper the osteoblastogenesis via decreasing Runx2 transcription factor, IGF-1 and Wnt signalling pathways (4, 5]. On the other hand, metformin and sulfonylureas shows neutral or positive effect on bone health and reduced risk of fracture [6,7]. In addition,	

		results from the animal and human studies create controversy over insulin safety profile on bone health. Incretin based therapy (GLP-1 receptor agonist and DPP-4 inhibitors) and SGLT2 inhibitors are currently available marketed anti-diabetic drugs. Data from animal studies suggested that incretin based therapy play an important role in the regulation of bone turnover [8, 9]. SGLT2 inhibitors may cause bone loss or increased risk of fracture might be due to decrease bone mineral density (BMD), altered calcium, phosphate and sodium concentration [10, 11]. Therefore, aim of this systematic literature review is to accumulate data from animal studies and provide better information about the safety concern of anti-diabetic medication.	
	Research question		
11.	Specify the disease/health problem of interest	Diabetes mellitus	
12.	Specify the population/species studied	All animal models with experimental diabetes	
13.	Specify the intervention/exposure	Any anti-diabetic drugs	
14.	Specify the control population	Diabetic animals	
15.	Specify the outcome measures	Blood glucose level and bone (osteoblast and osteoclast) biomarkers	
16.	State your research question (based on items 11-15)	What are the effect of anti-diabetic drugs on bone and its association with bone biomarkers?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	☒MEDLINE via PubMed ☒Web of Science ☐SCOPUS ☐EMBASE ☐Other, namely: ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [insert file name]	
19.	Identify other sources for study identification	☒Reference lists of included studies ☐Books ☒Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	**Search string for bone:** "Skeleton"[Mesh] OR bone[Title/Abstract] OR "osteogenesis"[MeSH Terms] OR ("osteogenesis"[MeSH Terms] OR "osteogenesis"[All Fields] OR ("bone"[All Fields] AND "formation"[All Fields]) OR "bone formation"[All Fields]) OR (("osteogenesis"[MeSH Terms] OR "osteogenesis"[All Fields] OR ("bone"[All Fields] AND "formation"[All Fields]) OR "bone formation"[All Fields])	

		AND ("Markers"[Journal] OR "markers"[All Fields])) OR "Bone Resorption"[Mesh] OR (("bone resorption"[MeSH Terms] OR ("bone"[All Fields] AND "resorption"[All Fields]) OR "bone resorption"[All Fields]) AND ("Markers"[Journal] OR "markers"[All Fields])) OR ("bone diseases, metabolic"[MeSH Terms] OR ("bone"[All Fields] AND "diseases"[All Fields] AND "metabolic"[All Fields]) OR "metabolic bone diseases"[All Fields] OR ("bone"[All Fields] AND "loss"[All Fields]) OR "bone loss"[All Fields]) OR ("fractures, bone"[MeSH Terms] OR ("fractures"[All Fields] AND "bone"[All Fields]) OR "bone fractures"[All Fields] OR "fracture"[All Fields]) OR ("fractures, bone"[MeSH Terms] OR ("fractures"[All Fields] AND "bone"[All Fields]) OR "bone fractures"[All Fields] OR ("bone"[All Fields] AND "fractures"[All Fields])) **Search string for intervention:** ("metformin"[mh] OR "thiazolidinediones"[mh] OR "glipizide"[mh] OR "glyburide"[mh] OR "Dipeptidyl-Peptidase IV Inhibitors"[mh] OR "Glucagon-Like Peptide 1"[mh] OR biguanide*[tiab] OR metformin[tiab] OR thiazolidinedione*[tiab] OR pioglitazone[tiab] OR rosiglitazone[tiab] OR sulfonylurea*[tiab] OR sulphonylurea*[tiab] OR glipizide[tiab] OR glyburide[tiab] OR glimepiride[tiab] OR glibenclamide[tiab] OR "insulin secretagogues"[tiab] OR sitagliptin*[tiab] OR saxagliptin*[tiab] OR dpp-4[tiab] OR dpp-iv[tiab] OR liraglutide[tiab] OR exenatide[tiab]) OR (linagliptin*[tiab] OR alogliptin*[tiab] OR albiglutide*[tiab] OR dulaglutide*[tiab] OR "sodium-glucose co-transporter 2 inhibitors"[tiab] OR "sodium-glucose cotransporter 2 inhibitor" [tiab] OR "SGLT-2" [tiab] OR "canagliflozin"[tiab] OR "dapagliflozin"[tiab]) **Search String for diabetes:** (insulin resistance) OR (Diabetes Mellitus[Mesh] OR Diabetes Mellitus, Experimental[Mesh] OR Glucose Metabolism Disorders[Mesh] OR Diabetes [tiab] OR Diabetic [tiab] or Diabetics[tiab] OR Hyperglycemia [tiab] OR Hyperglycaemia [tiab] OR High Blood Sugar [tiab] OR Streptozocin [tiab] OR STZ[tiab] OR Alloxan[tiab]) **Search String for animals:** [12]	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	First Pass: Screening based on title and abstract	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Three independent reviewer (MA, PV and SV) Discrepancies will be resolved by contacting (PG, AK and MS)	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Pre-clinical studies with control group Exclusion criteria: Review papers, opinion papers, non-diabetic studies, non-interventional studies	

24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: Experimental animals with diabetes Exclusion criteria: Human and in-vitro studies	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Any anti-diabetic medication in any dose, duration and frequency Exclusion criteria: other than anti-diabetic medication	
26.	Outcome measures	Inclusion criteria: Blood glucose level and bone biomarkers Exclusion criteria: N/A	
27.	Language restrictions	Inclusion criteria: English language papers Exclusion criteria: None	
28.	Publication date restrictions	Inclusion criteria: No restriction Exclusion criteria: N/A	
29.	Other	Inclusion criteria:N/A Exclusion criteria:N/A	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: first pass based on title/abstract 1. Non-diabetic studies 2. other interventions 3. Review or non original papers 4. Not English Selection phase: Second pass based on full text 1. Not an original paper 2. No data regarding bone 3. No control group	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	First author, title, year, journal	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Experimental setting Experimental groups Number of animals per group Type of animal model	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species Gender Diseased models (chemical induced such as streptozotocin, alloxane; Spontaneous autoimmune models; Genetically induced models)	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Name of the interventions Dose, duration, frequency and route of administration	
35.	Outcome measures	Blood glucose level and bone biomarkers (osteocalcin; RANKL; OPG; CTX; PINP; ALP; TRAP; calcium and sclerostin)	
36.	Other (*e.g.* drop-outs)		
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	(a) Two independent reviewers (MA and PV) will assess risk of bias of included studies. (b) Discrepancies will be resolved by contacting (SV, PG, AK and MS).	

38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	✕By use of SYRCLE's Risk of Bias tool[4] ☐By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	continuous outcomes : blood glucose level (mg/dl or mmol/L) and bone biomarkers (unit as per considerable markers)	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Extract data from table, text or figures For Incomplete or unavailable data respective authors will be contacted and if authors failed to respond then study will be excluded.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	(a) Two independent reviewers (MA and PV) will extract data. (b) Discrepancies will be resolved by contacting (SV, PG, AK and MS).	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	If data from more than three studies homogeneous in nature then meta-analysis will be performed.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Mean difference or standard mean difference and 95% confidence interval will be used.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effect model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Species Gender Diabetes duration Duration of drug treatment Type of intervention	
48.	Any sensitivity analyses you propose to perform	To be determined	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	If applicable, we will perform a Bonferroni correction for testing multiple subgroups. If one or more subgroup analyses cannot be performed due to insufficient data, the p-value will be adjusted accordingly. Also correction for multiple use of control groups will be performed by dividing the number of animals in the control group by the number of comparisons performed with this control group	

50.	The method for assessment of publication bias	Funnel plots	
Final approval by (names, affiliations):			Date:

References:

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