FLUORIDE
Quarterly reports

Fluoride-Induced Oxidative Stress in Non-Skeletal Tissues: A Systematic Review and Meta-Analysis

Unique digital address (Digital object identifier [DOI] equivalent):
https://www.fluorideresearch.online/epub/files/255.pdf

Linet Musungu ANGWA[1*], David MASINDE[2]

[1] Lecturer, Department of Clinical Medicine, Kabarak University, Kenya
[2] Lecturer, Department of Public Health, Maseno University, Kenya

***Corresponding author:**
Dr. Linet Angwa
Department of Clinical Medice
Kabarak University
Nakuru – Eldama Ravine road
20100, Nakuru, Kenya

E-mail: lynangwa@gmail.com

Acceptd: 2023 Oct 30
Epub as e255L 2023 Nov 24

ABSTRACT

Purpose: Several studies have investigated the oxidative stress parameters in non-skeletal tissues of animals exposed to fluoride, however, the findings from these studies are inconsistent. We conducted a systematic review and meta-analysis to evaluate the levels of oxidative stress biomarkers in experimental animals treated with fluoride compared with the control group.

Methods: The PubMed, Cochrane Library, EBSCO, and JSTOR databases were searched for studies reporting oxidative stress biomarkers in non-skeletal tissues of animals exposed to fluoride. A random effects model with the standardized mean difference (SMD) was used for meta-analyses. The heterogeneity of the studies was evaluated using Higgin's I2 statistics. The risk of bias was assessed using the SYRCLE's risk of bias tool and publication bias using Egger's test

Results: Compared to the control, the levels of ROS, LPO, and NO were significantly elevated and the levels of SOD, CAT, GSH-Px, and GSH significantly reduced in the studied tissues. The level of GST however showed no significant difference. The test for subgroup differences suggested that different animal species and tissues have varying susceptibilities and tolerance to fluoride. Furthermore, the extent of fluoride-induced oxidative stress damage can be modified by the intervention period. Meta-regression analysis indicated that the studies' effect size for LPO was influenced by animal species.

Conclusions: This meta-analysis's findings demonstrated the presence of oxidative stress and depletion of antioxidants in the non-skeletal tissues of experimental animals exposed to fluoride. More studies in humans are recommended to strengthen the current evidence.

Key-words: *Fluorosis; Oxidative stress; Non-skeletal tissue; Systematic review; Meta-analysis*

INTRODUCTION

It is well established that fluorosis is a worldwide health concern and is endemic in some areas where fluoride content is high in drinking water.[1,2] The global prevalence of dental and skeletal fluorosis is not clear. However, it is estimated that excessive fluoride concentration in drinking water has caused tens of millions of dental and skeletal fluorosis cases worldwide over a range of years.[3] After absorption from the gastrointestinal tract, 99% of the retained fluoride is deposited in mineralized tissues with 1% being found in soft tissues.[4,5] A steady state of distribution between the

extracellular and intracellular compartments is established in these tissues where intracellular fluoride concentrations change simultaneously and in proportion to changes in plasma fluoride levels.[4] The remaining proportion is excreted by the kidneys. Generally, the tissue-to-plasma concentration ratios fall between 1.0 and 4.0 except for the brain (<0.1) and the kidney (>4.0). The low value in the brain is attributed to the relative impermeability of the blood-brain barrier to fluoride. The kidney has the highest fluoride concentration compared to all other soft tissues due to its high concentration in the tubular fluid.[4,6] Excessive fluoride ingestion over a prolonged period can adversely influence several organs and tissues characterized by a vast array of symptoms and pathological changes. Apart from dental and skeletal fluorosis, Fluoride is now known to cause renal toxicity,[7] hepatotoxicity,[8] neurotoxicity,[9] cardiovascular system toxicity,[10,11] reproductive toxicity,[12,13] thyroid toxicity,[14] and Haematotoxicity.[15] Despite this, the molecular mechanisms of fluorosis are still unclear.

In recent decades, extensive research has reported a key role of oxidative stress in causing fluorosis in non-skeletal tissues.[16] This is further strengthened by evidence of consistent protection of cells from the lipid peroxidation (LPO) caused by fluoride exposure by antioxidant treatment.[17] Excessive accumulation of reactive oxygen species (ROS) or reactive nitrogen species (RNS), as a result of either increased ROS/RNS generation or impaired ROS/RNS clearance, leads to oxidative stress. To counteract the effects of ROS/RNS, living organisms possess a large number of enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px/GPx), glutathione S-transferase (GST) and catalase (CAT), and non-enzymatic antioxidants such as vitamins A, E, and C, GSH, and β-Carotene.[18–20] Enzymatic antioxidants act by converting oxidative products to hydrogen peroxide (H2O2) and then to water in the presence of cofactors like copper, manganese, and iron while non-enzymatic antioxidants act by intercepting and interrupting free radical chain reactions.[21] Fluoride is thought to decrease the levels of these antioxidants and increase oxidative stress.[22–24] However, previous studies addressing the oxidative stress status or detecting the biomarkers of oxidative stress in tissues of experimental animals with fluorosis yielded controversial results.[25–28]

In this study, we systematically reviewed the literature on oxidative stress induced by fluoride in the blood, liver, kidney, heart, and brain of experimental animals and conducted a meta-analysis on relevant studies. The primary objective was to quantitatively evaluate the levels of oxidative stress biomarkers (SOD, CAT, GSH-Px, GSH, GST, ROS, lipid peroxidation (LPO), and nitric oxide (NO)) in the aforementioned non-skeletal tissues of experimental animals exposed to fluoride compared to the controls. We hypothesized that there will be an increase in oxidative biomarkers and a decrease in antioxidative biomarkers in experimental animals with induced fluoride toxicity.

MATERIAL AND METHODS

Inclusion criteria

Studies were included in our analyses if they met the following criteria: 1) experimental studies measuring oxidative stress biomarkers in blood, brain, kidney, heart, and liver of experimental animals; 2) studies published in English; 3) studies that provided animal numbers, means, and standard deviations. Through our search strategy, we decided to focus on some enzymatic antioxidants (SOD, CAT, GSH-Px, and GST), non-enzymatic antioxidants (GSH), reactive oxygen species (ROS), free radicals (NO), and oxidative damage products (malondialdehyde (MDA)/ thiobarbituric acid reactive substances (TBARs): LPO), but not other oxidative stress biomarkers because of limited studies.

Exclusion criteria

Studies were excluded from our analyses if they met the following criteria: 1) conference abstracts, reviews, editorials, and letters; 2) human, *in vitro,* or other unrelated studies; 3) full-text not available in English; 4) studies with unavailable data/ unextractable data; 5) combined exposure with no fluoride only group; 6) studies not done in tissues of interest; 7) multigenerational studies; 8) studies on amelioration of fluoride toxicity; 9) studies that did not measure oxidative stress; 10) studies with incomplete or unclear results.

Literature search

We conducted searches in PubMed, Cochrane Library, EBSCO, and JSTOR to identify eligible articles on July 22nd, 2022, with the following keywords and Boolean operators: (“Fluoride OR Fluorosis”) AND (“Oxidative stress OR Oxidative damage”) AND (“Reactive oxygen species OR Reactive nitrogen species OR Lipid peroxidation OR Malondialdehyde OR Thiobarbituric acid reactive substance OR Nitric oxide OR Lipid hydroperoxide OR Superoxide dismutase OR Catalase OR Glutathione OR Vitamin C”) and articles were exported to Mendeley. An additional manual search of references and cited/related articles was done. Search terms were validated by ensuring the

search retrieved a selection of articles, representative of relevant works. Searches were restricted to the English language and Animals with no restriction on the date of publication. Abstracts were screened independently by two reviewers (LA and DM). Full-text screens were conducted to confirm eligibility. Differences between the two reviewers were resolved through discussion and consensus.

Data extraction and quality assessment

Two reviewers (LA and DM) extracted eligible studies independently through the review of titles, abstracts, and full texts. In case of disagreement, a final decision was made by consensus. Information was extracted in a systematic way as follows: 1) author; 2) year of publication; 3) animal species/strain; 4) sex; 5) age; 6) weight; 7) tissue/organ studied; 8) the number of animals in experimental and control groups; 9) study period; and 10) oxidative stress biomarkers (Table 1). In studies with multiple intervention groups, only one pair was selected and others were excluded from the meta-analysis: only the high-dose group was included in studies with multiple fluoride groups, the fluoride-only group was included in co-exposure studies, and in studies with different duration of treatment, the longest duration was selected for the study. In some studies, multiple datasets were extracted if reported and the mean of means and standard deviations (SD) were used for the meta-analysis. WebPlotDigitizer was used to facilitate graphical data extraction. The SD for studies that reported standard error (SE) of mean was obtained by multiplying by the square root of the sample size ($SD = SE \times \sqrt{n}$).

The quality of included studies was assessed independently by two reviewers (LA and DM) by using the SYRCLE's risk of bias tool and disagreements were resolved through consensus-oriented discussion. The SYRCLE's risk of bias tool contains 10 entries related to 6 types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. Signaling questions are used to assign a judgment of low, high or unclear risk of bias to each item mentioned in the tool.[29]

Statistical Analysis

All statistical analysis was performed using the R project software version 4.2.2 (R package meta). We used Hedge's g standardized mean difference (SMD) as a measure of effect size because the measures used are not the same in all studies and corresponding 95% confidence intervals (95% CIs) were presented. Pooled effect sizes (ES) were calculated according to DerSimonian and Laird for the random effects model because of the diversity of methods, species, and intervention protocols. The statistical heterogeneity was determined by the value of the I^2 index. A value of the I^2 index of around 25%, 50%, and 75% was considered as low-, moderate-, and high-heterogeneity, respectively.[87] For rigor, Publication bias was estimated by inspecting the funnel plot asymmetry and Egger's regression test. The leave-one-out sensitivity analysis was performed by excluding the studies identified as having a high risk of bias using the SYRCLE's risk of bias tool. For additional insight, subgroup analyses were performed based on the intervention period (<30, 30–90, >90 days); species of animals (mice, rats, others), and sample source (liver, kidney, brain, heart, blood) to determine the factors associated with differences among study results in the outcome indicators. Statistical significance was defined at $p < 0.05$.

Table 1. Characteristics of included studies

Author	Year	Species/ strain	Animal sex	Age	Weight	Sample Source	Animal Number: Control/ Intervention	Fluoride exposure period	Oxidative Stress Biomarkers
Afolabi, et al. [30]	2013	Rats	Male	9 - 10 weeks	Average 120g	Blood, Liver	8/8	30-90 days	MDA
Bhatnagar, et al. [24]	2006	Mice	Female	1 month	25 ± 5g	Brain, Liver,	10/15	30 days	SOD, CAT, MDA
Chouhan & Flora[28]	2008	Rats	Male	Adult	100–120g	Blood, Liver, Kidney, Brain	6/6	30-90 days	ROS, GSH
Chouhan, et al.[22]	2010	Rats	Male	Adult	100–120g	Blood, liver, kidney, brain	6/6	30-90 days	GSH, ROS
Deng, et al.[31]	2014	Avian Broiler Chickens	N/A	1 day old	N/A	Blood	5/5	30-90 days	SOD, MDA, GSH-PX, GSH, CAT*
Dubey, et al.[32]	2013	Rats	Male/female	Adult	150–200g	Liver	6/6	< 30 days	SOD, GSH, CAT, MDA
Flora, et al.[33]	2009	Mice	Male	Adult	25± 5 g	Brain	5/5	30-90 days	ROS, GPx, CAT, SOD, TBARS, GST
Flora, et al.[34]	2012	Mice	Male	Adult	Appx 30g	Blood, Brain, Liver	5/5	> 90 days	ROS, GSH, SOD GPx, CAT, TBARS
Guo, et al.[35]	2003	Rats	Male/ Female	4 weeks old	90–100 g	Liver	8/8	>90 days	MDA, SOD, GSH-Px
He & Chen[36]	2006	Rats	Male	N/A	80–120 g	Liver	5/5	< 30 days	ROS, MDA, GSH
Inkielewicz & Czarnowski[37]	2008	Rats	Male	Adult	189 ± 6.9 g	Blood, Brain, Kidney, Liver	6/6	30- 90 days	GSH, GPx, TBARS, CAT
Manivannan, et al.[38]	2013	Mice	Male	Adult (8-12 weeks old)	25–30 g	Liver	6/6	30 days	MDA, GST, GSH, CAT
Khan, et al. [39]	2018	Rats	Male/Female	N/A	150–200 g	Brain	6/6	< 30 days	GST, CAT, SOD, MDA
Kinawy[40]	2019	Rats	Male	Pups	N/A	Brain	8/8	30-90 days	MDA, GSH, NO
Liu, et al.[41]	1999	Rats	N/A	N/A	140–160 g	Liver, Kidney	14/14	> 90 days	MDA, SOD, GSH-Px,
Lopes, et al.[42]	2020	Mice	Male	21 days old	10 g ± 5 g	Brain	10/10	30-90 days	MDA, NO
Mittal & Flora[43]	2006	Mice	Male	Adult	Appx 30 g	Blood, Liver, Kidney	5/5	30-90 days	GSH, TBARS, ROS, GPx, SOD, CAT
Narayanaswamy & Piler[44]	2010	Rats	N/A	Pups	N/A	Brain	6/6	30-90 days	MDA, CAT, SOD, GSH-Px
Oyagbemi, et al.[45]	2017	Rats	Male	Adult	125–175 g	Kidney, heart	10/10	< 30 days	MDA, SOD, CAT, GSH, GPx, NO, GST
Panneerselvam, et al.[46]	2015	Rats	Male	3 months,	130–150 g	Heart	6/6	< 30 days	ROS, LPO, NO, GSH,

				2 weeks old					GPx, SOD, CAT, GST
Qin, et al. [47]	2015	Rats	Male/Female	N/A	90–120g	Kidney	10/10	>90 days	MDA
Quadri, et al.[48]	2018	Rats	Male	75 days old	N/A	Heart	6/6	30-90 days	TBARS, SOD, CAT
Ranjan, et al.[49]	2009	New Zealand white Rabbits	Male	4-6 weeks old	600–800 g	Liver, Kidney, Blood	6/6	90 days	LPO, SOD, CAT
Reddy, et al.[50]	2014	Rats	Male	4 months	150–200g	Brain, Blood	6/6	90 days	MDA, CAT, SOD, GPx
Shanthakumari, et al. [51]	2004	Rats	Male	N/A	120–160 g	Liver, Kidney	6/6	>90 days	TBARS, SOD, CAT, GSH, GPx,
Shivarajashankara, et al.[52]	2002	Rats	N/A	Pups	N/A	Brain	15/9	30-90 days	MDA, GSH, GST, GSH-Px,
Zhan, et al.[53]	2005	Pigs	Male (barrows)	50-day-old	Appx 17 kg	Liver, Kidney, Blood	8/8	30-90 days	MDA, NO, SOD, CAT, GSH-Px
Zhang, et al.[54]	2013	Rats	Male	8-week-old	195–210 g	Kidney, Liver	6/6	30-90 days	MDA. SOD
Banala & Karnati[26]	2015	Rats	N/A	Pups	N/A	Brain, Blood	5/5	30-90 days	SOD, LPO
Bartos, et al.[55]	2018	Rats	Female	Pups	N/A	Brain	5/5	>90 days	MDA, CAT, GPx
Bo, et al.[56]	2018	Bufo gargarizans	N/A	larvae	N/A	Liver	3/3	< 30 days	SOD, GPx
Bouaziz, et al.[57]	2007	Mice	Female	Adult	N/A	Kidney, liver, blood	6/6	<30 days	SOD, GSH-Px, TBARS
Campos-Pereira, et al.[58]	2017	Rats	Male	Adult	180–200g	Liver	10/10	30-90 days	CAT, MDA, SOD, GST,
Cao, et al.[59]	2013	Cyprinus carpio	N/A	Juveniles	15.8 ± 0.24 g	Liver	18/18	90 days	MDA, SOD, GSH
Chen, et al.[60]	2015	Cyprinus carpio	N/A	Juveniles	15.8 ± 0.24 g	Kidney	18/18	90 days	MDA, SOD, GSH
Dec, et al.[61]	2020	Rats	N/A	Pups	N/A	Brain	6/6	>90 days	CAT, SOD, GSH, GPx,
Gao, et al.[62]	2009	Rats	Male/Female	Young adult	90–120 g	Brain	8/8	>90 days	MDA
Inkielewicz-Stępniak & Knap[63]	2012	Rats	Male/Female	6 weeks old	Appx 220 g (M) & Appx 170 g (F)	Liver, Kidney	12/12	30-90 days	TBARS, NO, GSH
Kaur, et al.[64]	2009	Rats	Female	Adult	175–200g	Brain	8/8	30-90 days	MDA, SOD
Lu, et al.[65]	2017	Mice	N/A	4-week-old	N/A	Liver	8/8	30-90 days	GST, GSH-Px, CAT, SOD, ROS
Luo, et al.[66]	2017	Mice	N/A	4-week-old	N/A	Kidney	8/8	30-90 days	ROS, MDA, CAT, SOD, GSH, CAT, GSH-Px
Miranda, et al.[19]	2018	Mice	Male	21 days	30±10 g	Blood	10/10	30-90 days	TBARS, NO, SOD, CAT, GSH

Song, et al. [67]	2013	Rats	Male/Female	21 days	120±5 g	Kidney	12/12	30-90 days	CAT, SOD, ROS
Wang, et al.[68]	2019	Bufo gargarizans	N/A	Gosner stage (Gs) 26 Larvae	N/A	Liver	3/3	30-90 days	SOD, GPx
Zhan, et al.[69]	2006	Pigs	Male (barrows)		Appx 17 kgs	Liver	8/8	30-90 days	MDA
Zhong, et al.[70]	2021	Rats	Male	3-weeks-old	N/A	Blood	10/10	>90 days	MDA
Vani & Reddy[71]	2000	Mice	Female	Adult	30 ± 2 g	Brain	6/6	< 30 days	CAT, SOD, GST
Akinrinade, et al.[72]	2015	Rats	Male	Adult	180–250 g	Brain, Blood	5/5	< 30 days	SOD, MDA
Samir[73]	2017	Rats	Female	Adults	180–230 g	Liver	4/4	30-90 days	MDA, GSH, GST
Flora, et al.[74]	2011	Rats	Male	Adults	180–200 g	Blood	5/5	N/A	ROS, GSH
Chattopadhyay, et al.[75]	2011	Mice	Male	8-week-old	25–30 g	Liver, Kidney	5/5	90 days	MDA, GST, GSH
Mukhopadhyay, et al[76]	2015	Zebrafish	Female	Adult	0.7 ± 0.01 g	Liver	30/30	90 days	ROS, GSH, MDA, CAT, SOD, GST
Bartos, et al[77]	2019	Rats	Male	Pups	N/A	Brain	5/5	30-90 days	CAT, GPx, MDA
Baba, et al[78]	2016	Rats	N/A	N/A	175 ± 25 g	Kidney	6/6	< 30 days	CAT, SOD, GSH-Px, MDA
Mondal, et al[79]	2021	Zebrafish	Female	N/A	0.25–0.30 g	Brain	30/30	30-90 days	CAT, GSH, MDA
Zhou, et al. [80]	2015	Mice	Female	3-weeks old	N/A	Blood/Liver	36/36	30-90 days	ROS, SOD, GSH-Px, MDA, CAT
Inkielewicz & Czarnowski. [81]	2010	Rats	Male	6-weeks old	Appx 180 g	Blood, Brain, Kidney, Liver	6/6	< 30 days	TBARS
Khan, et al.[82]	2022	Rats	Female	3-month-old	140±20 g	Liver	5/5	90 days	NO, LPO, GSH
Sharma, et al. [83]	2022	Rats	Male	12–14 weeks	175–200 g	Blood, Brain	6/6	>90 days	GSH, SOD, CAT, GPx
Dong, et al.[84]	2020	Rats	Male	Pups	200–250 g	Liver	6/6	>90 days	MDA, CAT, GST
Tian, et al. [85]	2019	Rats	Male	Pups	200–250 g	Kidney	6/6	>90 days	GSH, GSH-Px, CAT, SOD, MDA
Morales-González, et al. [86]	2010	Rats	Male	N/A	240 ± 7 g	Blood	5/5	30-90 days	MDA, SOD, GSH-Px, CAT

SOD:superoxide dismutase; GPx/GSH-Px: glutathione peroxidase; CAT: catalase; GST: glutathione-s-transferase; GSH: glutathione ROS: reactive oxygen species; LPO: lipid peroxidation; MDA: malondialdehyde; TBARS: thiobarbituric acid reactive substances; NO: nitric oxide.

N.B: Total number of study animals was used in studies that did not report number of animals for each experiment.

RESULTS

Study Identification and Selection

We systematically identified a total of 619 articles using electronic databases (PubMed = 451, Cochrane Library = 2, EBSCO = 91, and JSTOR = 75) of which 614 were retrieved after deduplication. An additional 8 articles were identified through a manual search. Title and abstract screening excluded 531 articles. A full-text evaluation was conducted for the remaining 91 articles, and 29 articles were excluded for not fulfilling the inclusion criteria. Thus, 62 eligible studies were selected (Figure 1). The studies presented a high prevalence of rodents as laboratory animals, (52 out of 62). The characteristics of the animals and study design differed substantially among the studies.

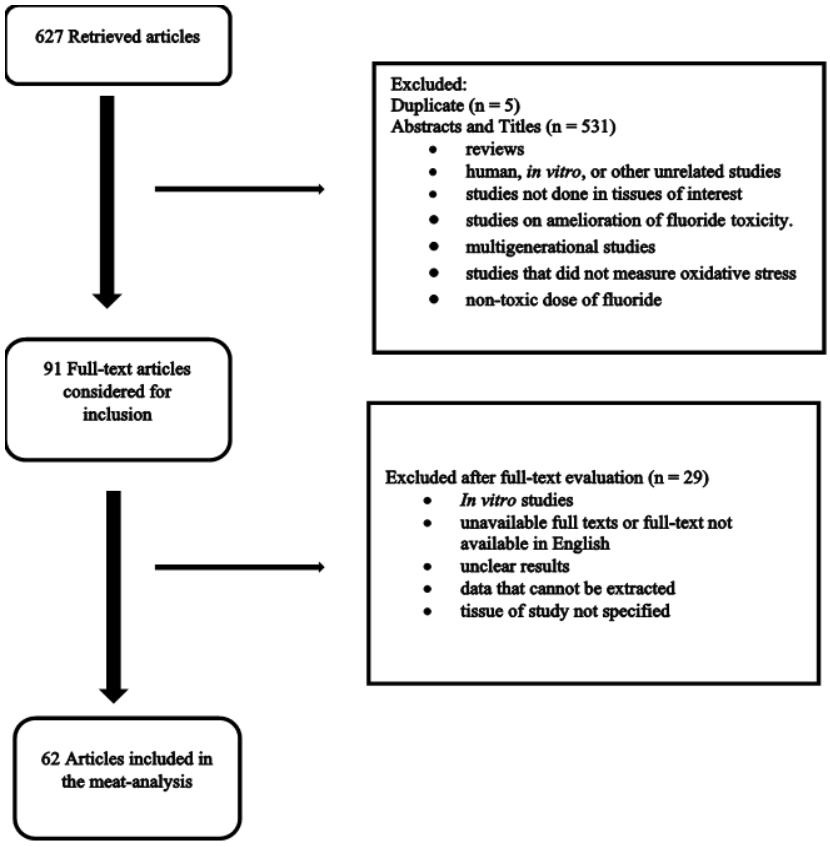


Figure 1. Flow chart of the literature search.

Risk of bias and quality of included studies

The risk of bias is categorized as high, low, or unclear. The majority of the studies presented a high number of unclear scores, indicating incomplete information related to the study design, resulting in difficulty accessing the actual risk of bias and not fully reproducible experimental protocols. The general result of the risk of bias assessment of this systematic review is presented in Figure 2. Concerning selection bias, 74.2% of the included studies reported randomization of the experimental units but the information provided was insufficient to assess whether the allocation sequence was adequately generated or adequately concealed. In 90.3% of the studies, the groups were similar at baseline with 9.7% presenting insufficient information. Additionally, 95.1%, and 100%, of the included studies registered unclear risk of bias regarding performance bias items "random housing" and "blinding" with 85.5% and 100% categorized as having unclear risk of bias regarding detection bias "random outcome assessment" and "blinding" respectively. Regarding the attrition bias, 75.8% had all the animals included in the study while 24.2% had insufficient information. All studies were free of reporting bias and 25.8% registered a low risk of bias from other sources.

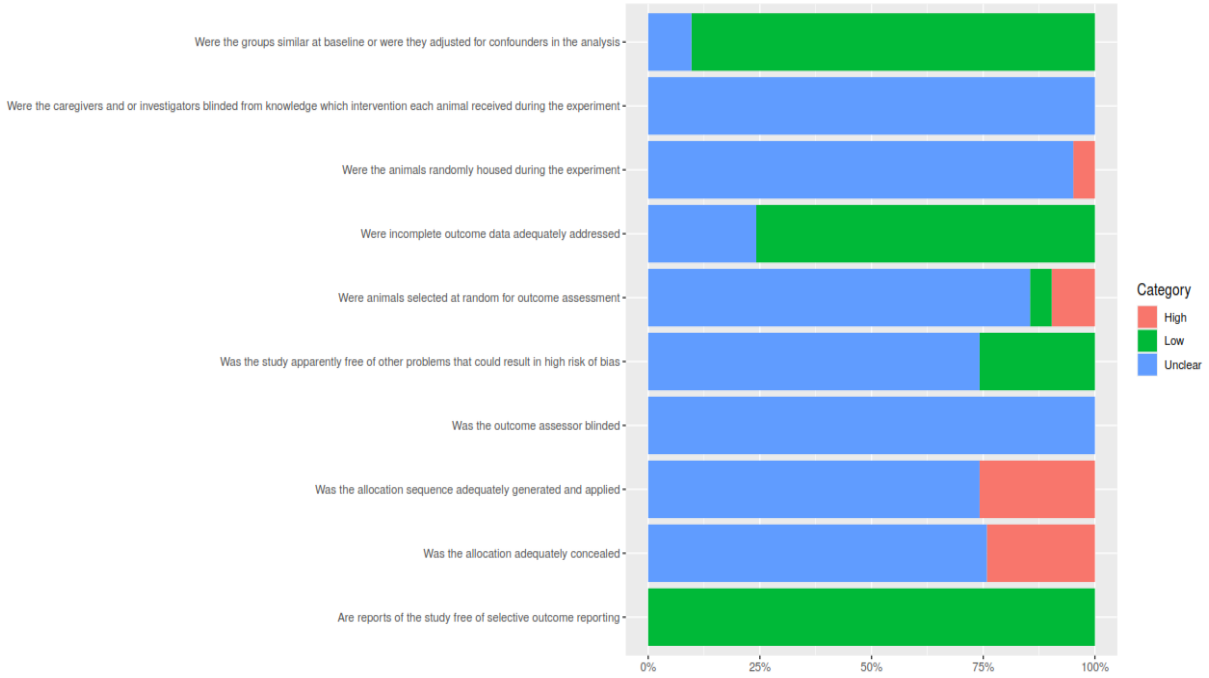


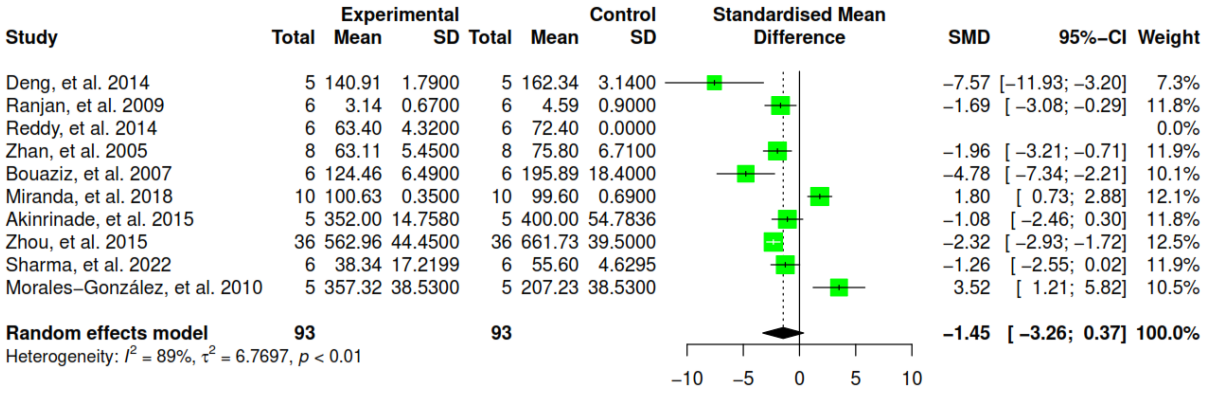
Figure 2. Risk of bias, average per item.

Meta-analysis of oxidative stress biomarkers

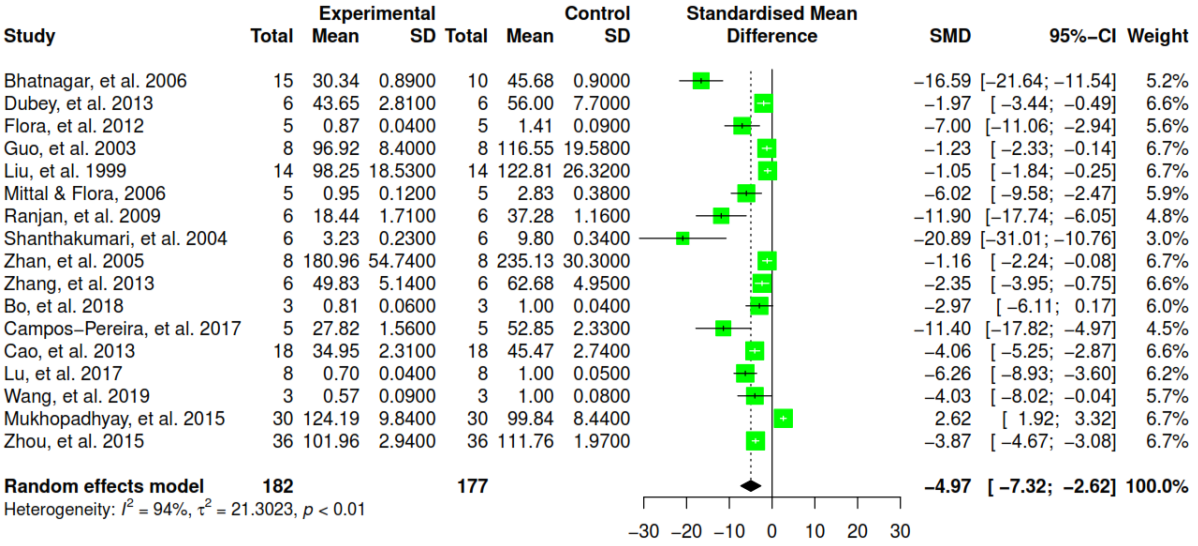
Meta-analysis of Superoxide Dismutase (SOD)

The overall effect size of SOD in the non-skeletal tissues was -4.2117 (95% CI: -5.4626; -2.9607, *Z*= -6.60, *p* < 0.0001) showing that the level of SOD was significantly lower in the treatment group compared to the controls. The statistical heterogeneity was notable (I^2 = 92%, p < 0.0001). More specifically, the effect sizes for SOD in blood, liver, kidney, heart, and brain were -1.4491(95% CI: -3.2640; 0.3657, *Z*= -1.56, *P*= 0.1176; I^2= 89%, *P*< 0.0001) -4.9682 (95% CI: -7.3196; -2.6169, *Z* =-4.14, *P* < 0.0001; I^2= 94%, *P*< 0.0001), -3.9651 (95% CI: -7.2393; -0.6909, *Z* -2.37, *P*= 0.0176; I^2= 90%, *P*< 0.0001), -5.0754 (95% CL: -8.3959; -1.7549, *Z*= -3, *P*= 0.0027; I^2= 63%, *P*= 0.0655), and -5.9072 (95% CI: -8.8593; -2.9551, *Z*= -3.92, *P* < 0.0001; I^2= 88%, *P*< 0.0001) respectively (Figure 3a-e). A visual inspection of the funnel plot showed asymmetry (Figure 3f). Egger's regression analysis displayed evidence of publication bias for this marker (Intercept -3.2373, t = -4.67, p-value < 0.0001). The exclusion of studies with a high risk of bias in any of the entries[24,26,35,40,41,49–51,56,58,64,80,83] yielded similar results and publication bias (SMD= -2.7346, 95%CI: -3.8546; -1.6146, Z = -4.79, *P*< 0.0001; I^2= 91%, *p* < 0.0001; Egger's regression intercept -3.5527, *t* = -3.50, p-value = 0.0014).

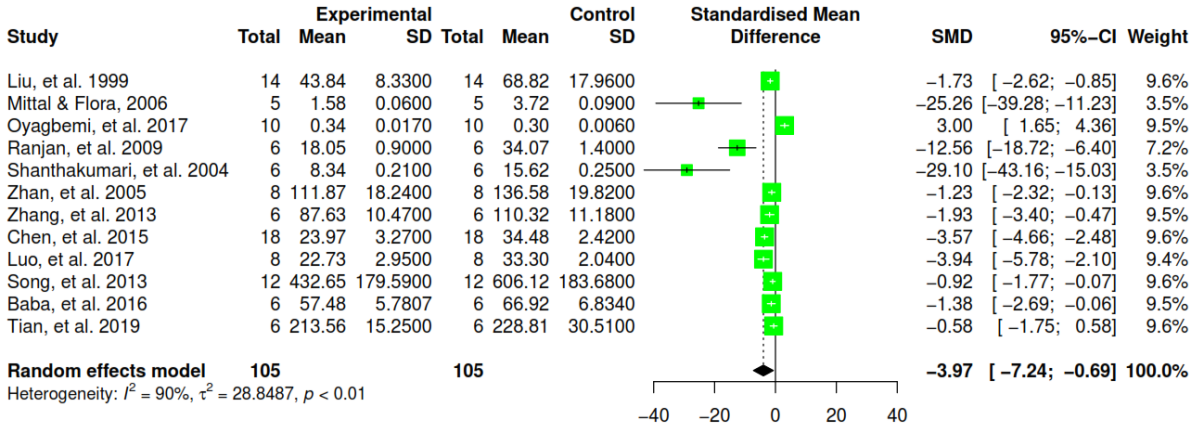
a



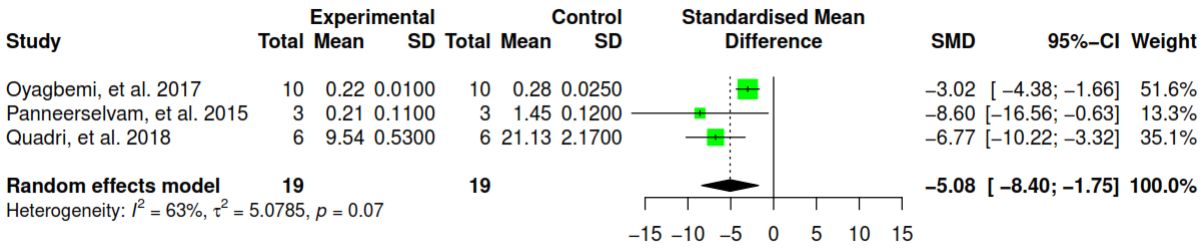
b


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%−CI Weight
Bhatnagar, et al. 2006 15 30.34 0.8900 10 45.68 0.9000 −16.59 [−21.64; −11.54] 5.2%
Dubey, et al. 2013 6 43.65 2.8100 6 56.00 7.7000 −1.97 [−3.44; −0.49] 6.6%
Flora, et al. 2012 5 0.87 0.0400 5 1.41 0.0900 −7.00 [−11.06; −2.94] 5.6%
Guo, et al. 2003 8 96.92 8.4000 8 116.55 19.5800 −1.23 [−2.33; −0.14] 6.7%
Liu, et al. 1999 14 98.25 18.5300 14 122.81 26.3200 −1.05 [−1.84; −0.25] 6.7%
Mittal & Flora, 2006 5 0.95 0.1200 5 2.83 0.3800 −6.02 [−9.58; −2.47] 5.9%
Ranjan, et al. 2009 6 18.44 1.7100 6 37.28 1.1600 −11.90 [−17.74; −6.05] 4.8%
Shanthakumari, et al. 2004 6 3.23 0.2300 6 9.80 0.3400 −20.89 [−31.01; −10.76] 3.0%
Zhan, et al. 2005 8 180.96 54.7400 8 235.13 30.3000 −1.16 [−2.24; −0.08] 6.7%
Zhang, et al. 2013 6 49.83 5.1400 6 62.68 4.9500 −2.35 [−3.95; −0.75] 6.6%
Bo, et al. 2018 3 0.81 0.0600 3 1.00 0.0400 −2.97 [−6.11; 0.17] 6.0%
Campos−Pereira, et al. 2017 5 27.82 1.5600 5 52.85 2.3300 −11.40 [−17.82; −4.97] 4.5%
Cao, et al. 2013 18 34.95 2.3100 18 45.47 2.7400 −4.06 [−5.25; −2.87] 6.6%
Lu, et al. 2017 8 0.70 0.0400 8 1.00 0.0500 −6.26 [−8.93; −3.60] 6.2%
Wang, et al. 2019 3 0.57 0.0900 3 1.00 0.0800 −4.03 [−8.02; −0.04] 5.7%
Mukhopadhyay, et al. 2015 30 124.19 9.8400 30 99.84 8.4400 2.62 [1.92; 3.32] 6.7%
Zhou, et al. 2015 36 101.96 2.9400 36 111.76 1.9700 −3.87 [−4.67; −3.08] 6.7%
Random effects model 182 177 −4.97 [−7.32; −2.62] 100.0%
Heterogeneity: I^2 = 94%, τ^2 = 21.3023, p < 0.01
−30 −20 −10 0 10 20 30

c


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%−CI Weight
Liu, et al. 1999 14 43.84 8.3300 14 68.82 17.9600 −1.73 [−2.62; −0.85] 9.6%
Mittal & Flora, 2006 5 1.58 0.0600 5 3.72 0.0900 −25.26 [−39.28; −11.23] 3.5%
Oyagbemi, et al. 2017 10 0.34 0.0170 10 0.30 0.0060 3.00 [1.65; 4.36] 9.5%
Ranjan, et al. 2009 6 18.05 0.9000 6 34.07 1.4000 −12.56 [−18.72; −6.40] 7.2%
Shanthakumari, et al. 2004 6 8.34 0.2100 6 15.62 0.2500 −29.10 [−43.16; −15.03] 3.5%
Zhan, et al. 2005 8 111.87 18.2400 8 136.58 19.8200 −1.23 [−2.32; −0.13] 9.6%
Zhang, et al. 2013 6 87.63 10.4700 6 110.32 11.1800 −1.93 [−3.40; −0.47] 9.5%
Chen, et al. 2015 18 23.97 3.2700 18 34.48 2.4200 −3.57 [−4.66; −2.48] 9.6%
Luo, et al. 2017 8 22.73 2.9500 8 33.30 2.0400 −3.94 [−5.78; −2.10] 9.4%
Song, et al. 2013 12 432.65 179.5900 12 606.12 183.6800 −0.92 [−1.77; −0.07] 9.6%
Baba, et al. 2016 6 57.48 5.7807 6 66.92 6.8340 −1.38 [−2.69; −0.06] 9.5%
Tian, et al. 2019 6 213.56 15.2500 6 228.81 30.5100 −0.58 [−1.75; 0.58] 9.6%
Random effects model 105 105 −3.97 [−7.24; −0.69] 100.0%
Heterogeneity: I^2 = 90%, τ^2 = 28.8487, p < 0.01
−40 −20 0 20 40

d


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%−CI Weight
Oyagbemi, et al. 2017 10 0.22 0.0100 10 0.28 0.0250 −3.02 [−4.38; −1.66] 51.6%
Panneerselvam, et al. 2015 3 0.21 0.1100 3 1.45 0.1200 −8.60 [−16.56; −0.63] 13.3%
Quadri, et al. 2018 6 9.54 0.5300 6 21.13 2.1700 −6.77 [−10.22; −3.32] 35.1%
Random effects model 19 19 −5.08 [−8.40; −1.75] 100.0%
Heterogeneity: I^2 = 63%, τ^2 = 5.0785, p = 0.07
−15 −10 −5 0 5 10 15

e

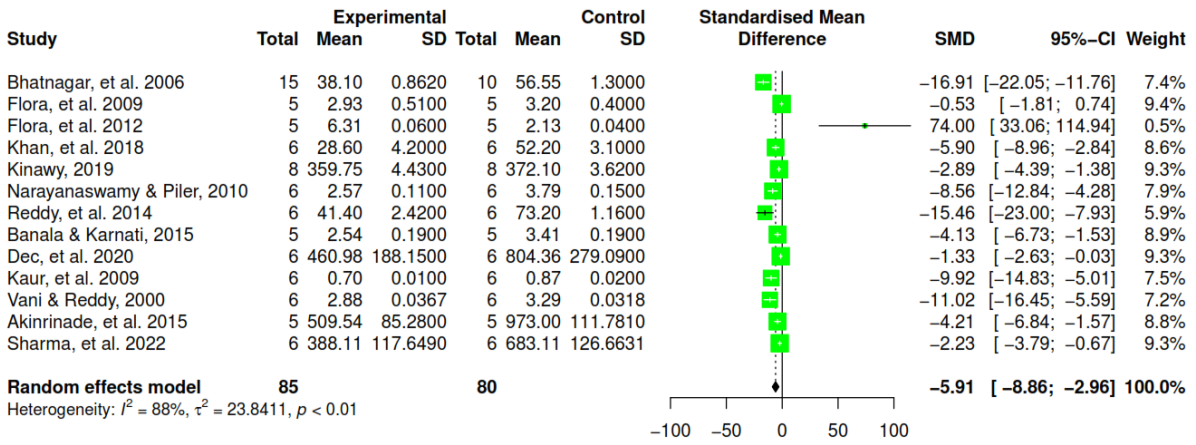


Figure 3. Forest plots for SOD meta-analysis: (a) Blood; (b) Liver; (c) Kidney; (d) Heart; (e) Brain.

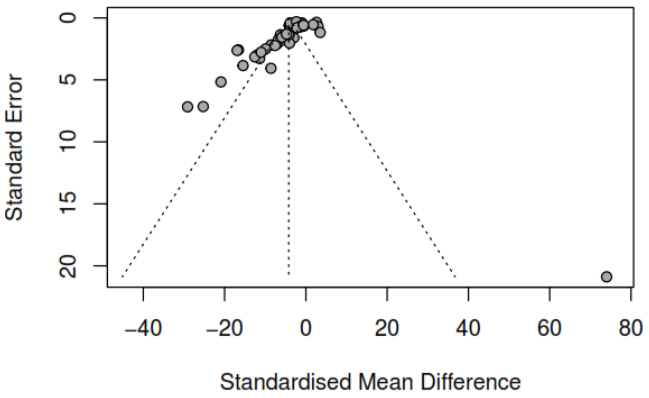
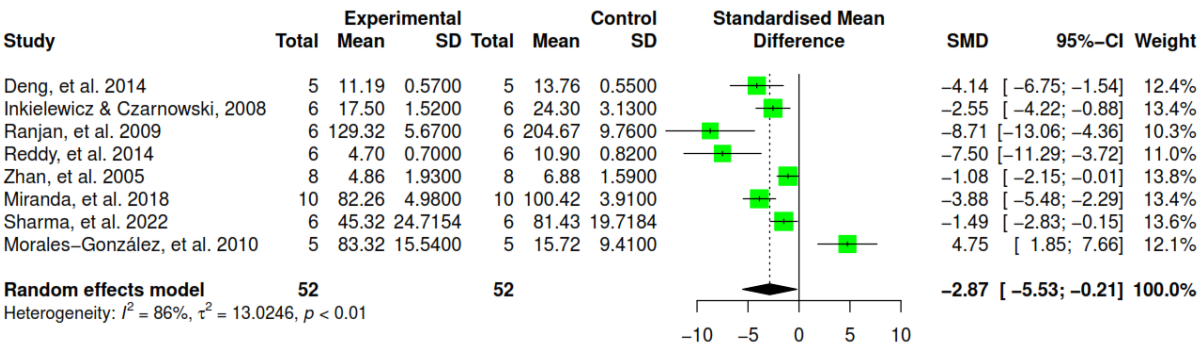


Figure 3f. SOD Funnel plot**.**

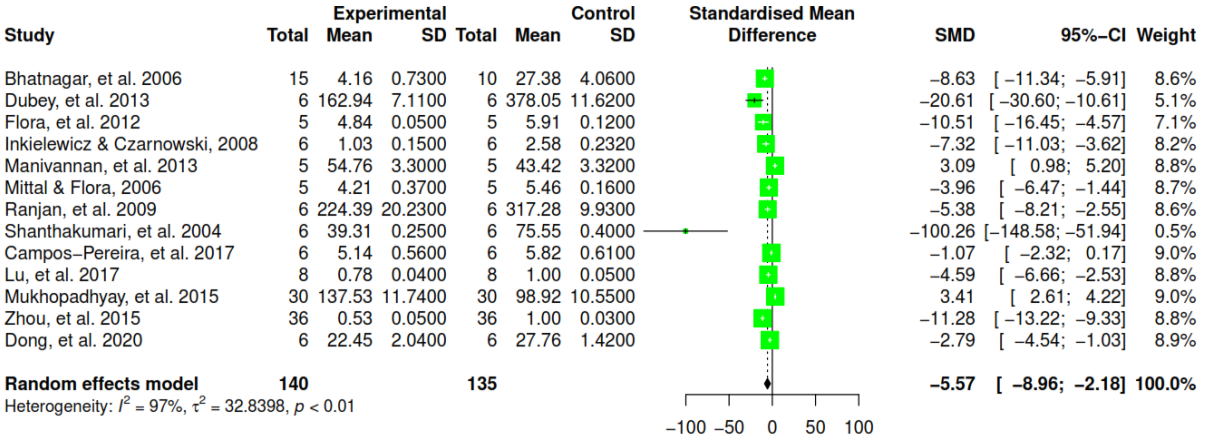
Meta- Meta-analysis of catalase (CAT)

The levels of CAT in non-skeletal tissues of experimental animals were remarkably lower than in the controls (SMD = -3.5470, 95% CI: -4.7155; -2.3786, Z = -5.95, p < 0.000). The statistical heterogeneity was high (I^2 = 92%, P < 0.0001). The effect sizes of CAT in blood (SMD = -2.8730, 95% CI: -5.5312; -0.2148, Z = -2.12, p = 0.034; I^2= 86%, p < 0.0001), liver (SMD = -5.5689, 95% CI: -8.9565; -2.1813, Z = -3.22, p = 0.0013; I^2 = 97%, p < 0.0001), heart (SMD = -4.0529, 95% CI: -5.3520; -2.7539, Z = -6.11, p < 0.0001; I^2 = 18%, p = 0.2972) and brain (SMD = -3.1932, 95% CI: -4.7950; -1.5915, Z = -3.91, p < 0.0001; I^2 = 85%, p < 0.0001) were also statistically significant (Figure 4a-d). The pooled effect size for kidney CAT (SMD = -2.1930, 95% CI: -4.4329; 0.0469, Z = -1.92, p = 0.0550; I^2= 90%, p < 0.0001) was however not significant (Figure 4e). The funnel plot was asymmetrical (Figure 4f) and an Egger's test was performed to detect publication bias and the results indicated the presence of publication bias (Egger's regression intercept -5.3276, t = -5.77, p-value < 0.0001). A sensitivity analysis with a random effect model was performed to calculate the pooled estimate of the effect after the exclusion of the studies with a high risk of bias[24,37,38,49–51,58,77,80,83,85,86], which showed no substantial variation of the results (SMD = -2.8297, 95% CI: -4.0840; -1.5754, Z = -4.42, p < 0.0001; I^2 = 92%, p < 0.0001; Egger's regression intercept -5.8654, t = -4.66, p-value = 0.0001).

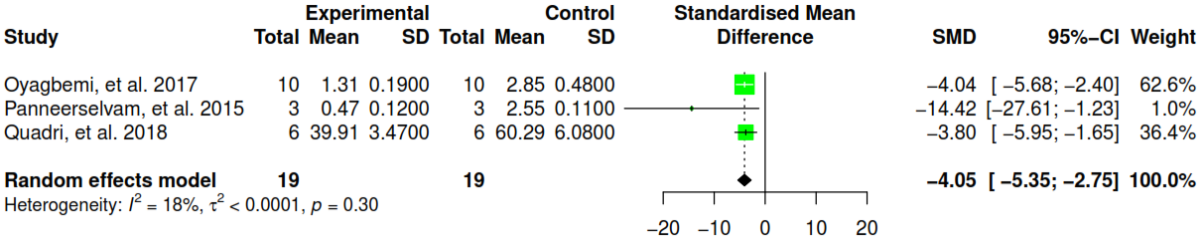
a


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Deng, et al. 2014 5 11.19 0.5700 5 13.76 0.5500 −4.14 [−6.75; −1.54] 12.4%
Inkielewicz & Czarnowski, 2008 6 17.50 1.5200 6 24.30 3.1300 −2.55 [−4.22; −0.88] 13.4%
Ranjan, et al. 2009 6 129.32 5.6700 6 204.67 9.7600 −8.71 [−13.06; −4.36] 10.3%
Reddy, et al. 2014 6 4.70 0.7000 6 10.90 0.8200 −7.50 [−11.29; −3.72] 11.0%
Zhan, et al. 2005 8 4.86 1.9300 8 6.88 1.5900 −1.08 [−2.15; −0.01] 13.8%
Miranda, et al. 2018 10 82.26 4.9800 10 100.42 3.9100 −3.88 [−5.48; −2.29] 13.4%
Sharma, et al. 2022 6 45.32 24.7154 6 81.43 19.7184 −1.49 [−2.83; −0.15] 13.6%
Morales−González, et al. 2010 5 83.32 15.5400 5 15.72 9.4100 4.75 [1.85; 7.66] 12.1%
Random effects model 52 52 −2.87 [−5.53; −0.21] 100.0%
Heterogeneity: I^2 = 86%, τ^2 = 13.0246, p < 0.01
−10 −5 0 5 10

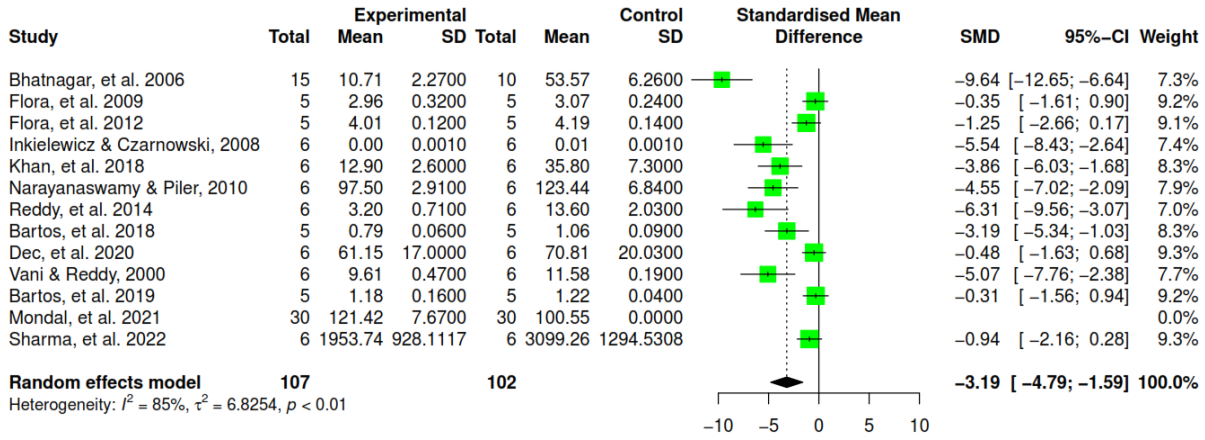
b


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Bhatnagar, et al. 2006 15 4.16 0.7300 10 27.38 4.0600 −8.63 [−11.34; −5.91] 8.6%
Dubey, et al. 2013 6 162.94 7.1100 6 378.05 11.6200 −20.61 [−30.60; −10.61] 5.1%
Flora, et al. 2012 5 4.84 0.0500 5 5.91 0.1200 −10.51 [−16.45; −4.57] 7.1%
Inkielewicz & Czarnowski, 2008 6 1.03 0.1500 6 2.58 0.2320 −7.32 [−11.03; −3.62] 8.2%
Manivannan, et al. 2013 5 54.76 3.3000 5 43.42 3.3200 3.09 [0.98; 5.20] 8.8%
Mittal & Flora, 2006 5 4.21 0.3700 5 5.46 0.1600 −3.96 [−6.47; −1.44] 8.7%
Ranjan, et al. 2009 6 224.39 20.2300 6 317.28 9.9300 −5.38 [−8.21; −2.55] 8.6%
Shanthakumari, et al. 2004 6 39.31 0.2500 6 75.55 0.4000 −100.26 [−148.58; −51.94] 0.5%
Campos−Pereira, et al. 2017 6 5.14 0.5600 6 5.82 0.6100 −1.07 [−2.32; 0.17] 9.0%
Lu, et al. 2017 8 0.78 0.0400 8 1.00 0.0500 −4.59 [−6.66; −2.53] 8.8%
Mukhopadhyay, et al. 2015 30 137.53 11.7400 30 98.92 10.5500 3.41 [2.61; 4.22] 9.0%
Zhou, et al. 2015 36 0.53 0.0500 36 1.00 0.0300 −11.28 [−13.22; −9.33] 8.8%
Dong, et al. 2020 6 22.45 2.0400 6 27.76 1.4200 −2.79 [−4.54; −1.03] 8.9%
Random effects model 140 135 −5.57 [−8.96; −2.18] 100.0%
Heterogeneity: I^2 = 97%, τ^2 = 32.8398, p < 0.01
−100 −50 0 50 100

c


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Oyagbemi, et al. 2017 10 1.31 0.1900 10 2.85 0.4800 −4.04 [−5.68; −2.40] 62.6%
Panneerselvam, et al. 2015 3 0.47 0.1200 3 2.55 0.1100 −14.42 [−27.61; −1.23] 1.0%
Quadri, et al. 2018 6 39.91 3.4700 6 60.29 6.0800 −3.80 [−5.95; −1.65] 36.4%
Random effects model 19 19 −4.05 [−5.35; −2.75] 100.0%
Heterogeneity: I^2 = 18%, τ^2 < 0.0001, p = 0.30
−20 −10 0 10 20

d


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Bhatnagar, et al. 2006 15 10.71 2.2700 10 53.57 6.2600 −9.64 [−12.65; −6.64] 7.3%
Flora, et al. 2009 5 2.96 0.3200 5 3.07 0.2400 −0.35 [−1.61; 0.90] 9.2%
Flora, et al. 2012 5 4.01 0.1200 5 4.19 0.1400 −1.25 [−2.66; 0.17] 9.1%
Inkielewicz & Czarnowski, 2008 6 0.00 0.0010 6 0.01 0.0010 −5.54 [−8.43; −2.64] 7.4%
Khan, et al. 2018 6 12.90 2.6000 6 35.80 7.3000 −3.86 [−6.03; −1.68] 8.3%
Narayanaswamy & Piler, 2010 6 97.50 2.9100 6 123.44 6.8400 −4.55 [−7.02; −2.09] 7.9%
Reddy, et al. 2014 6 3.20 0.7100 6 13.60 2.0300 −6.31 [−9.56; −3.07] 7.0%
Bartos, et al. 2018 5 0.79 0.0600 5 1.06 0.0900 −3.19 [−5.34; −1.03] 8.3%
Dec, et al. 2020 6 61.15 17.0000 6 70.81 20.0300 −0.48 [−1.63; 0.68] 9.3%
Vani & Reddy, 2000 6 9.61 0.4700 6 11.58 0.1900 −5.07 [−7.76; −2.38] 7.7%
Bartos, et al. 2019 5 1.18 0.1600 5 1.22 0.0400 −0.31 [−1.56; 0.94] 9.2%
Mondal, et al. 2021 30 121.42 7.6700 30 100.55 0.0000 0.0%
Sharma, et al. 2022 6 1953.74 928.1117 6 3099.26 1294.5308 −0.94 [−2.16; 0.28] 9.3%
Random effects model 107 102 −3.19 [−4.79; −1.59] 100.0%
Heterogeneity: I^2 = 85%, τ^2 = 6.8254, p < 0.01
−10 −5 0 5 10

e

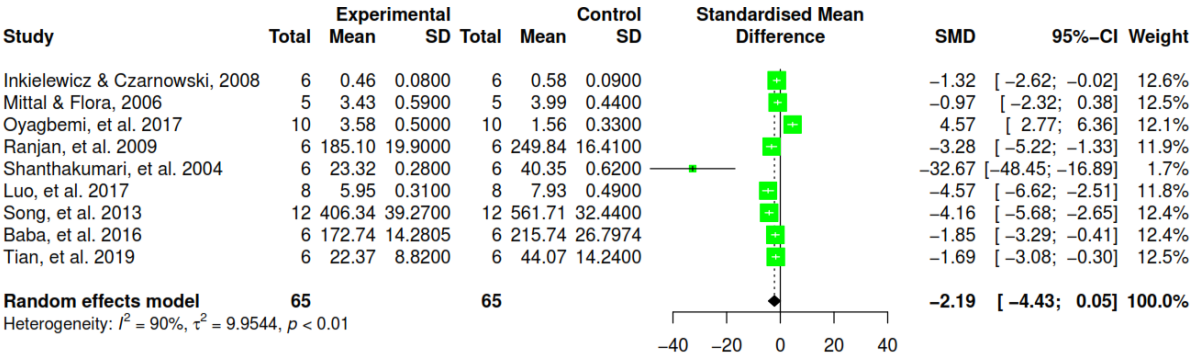


Figure 4. Forest plots for CAT meta-analysis: (a) Blood; (b) Liver; (c) Heart; (d) Brain; (e) Kidney

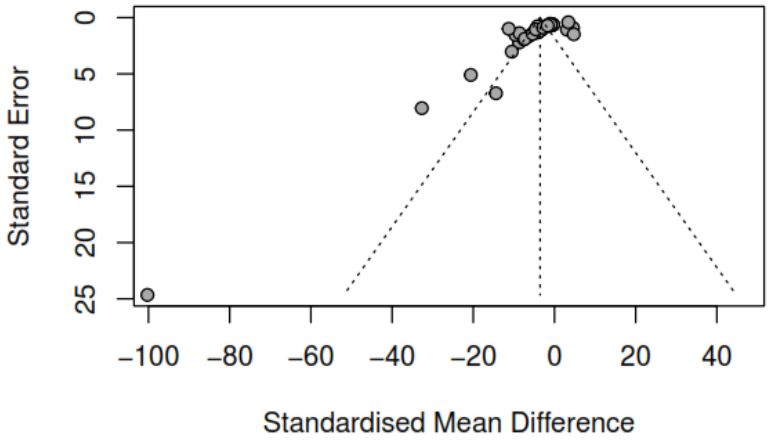


Figure 4f. CAT Funnel plot.

Meta-analysis of glutathione peroxidase (GSH-Px)

The overall effect size for GSH-Px in non-skeletal tissues was -2.5319 (95% CI: -3.9412; -1.1226, Z = -3.52, p = 0.0004) indicating that the levels of GSH-Px were significantly lower in the treatment groups compared to the controls. High heterogeneity of the studies was observed (I^2 = 86%, p < 0.0001). Statistically significant results were also seen in the meta-analysis of the articles reporting GSH-Px levels in the blood (SMD = -3.3938, 95% CI: -6.5339; -0.2538, Z = -2.12, p = 0.0341; I^2 = 88%, p < 0.0001), liver (SMD = -2.6349, 95% CI: -3.9062; -1.3636, Z = 4.06, p < 0.0001; I^2 = 76%, p < 0.0001), kidney (SMD = -1.7080, 95% CI: -2.6243; -0.7917, Z = -3.65, p = 0.0003; I^2 = 75%, p = 0.0003), and heart (SMD = -5.6537, 95% CI: -10.7131; -0.5942, Z = -2.19, p = 0.0285; I^2 = 89%, p = 0.0001). No significant change in GSH-Px was seen in the brain (SMD = -0.0574, 95% CI: -5.3120; 5.1972, Z = -0.02, p = 0.9829; I^2 = 91%, p < 0.0001) (Figure 5a-e). The Egger's test for asymmetry of the funnel plot showed evidence of publication bias (Egger's regression intercept -1.2193, t = -1.42, p = 0.1634). The funnel plot is presented in Figure 5f. The effect size was -2.9586 (95% CI: -4.6038; -1.3135, Z = -3.52, p = 0.0004; I^2= 85%, p < 0.0001), and Egger's regression (intercept -3.6063, t = -3.56, p = 0.0022) after excluding articles with a high risk of bias.[35,37,41,50–52,56,77,80,83,85,86]

Meta-analysis of Glutathione (GSH)

The level of GSH was significantly lower in non-skeletal tissues of animals treated with fluoride compared to the controls (SMD = -3.2790, 95% CI: -3.9433; -2.6147, Z = -9.67, p < 0.0001). The heterogeneity was found to be high (I^2 = 76%, p < 0.0001). The level of GSH in blood (SMD = -4.6776, 95% CI: -6.3518; -3.0034, Z = -5.48, p < 0.0001; I^2 = 73%, p = 0.0003), liver (SMD = -2.61, 95% CI: -3.7148; -1.5052, Z = -4.63, p < 0.0001; I^2 = 80%, p < 0.0001), kidney (SMD = -3.4747, 95% CI: -4.7208; -2.2285, Z = -5.46, p < 0.0001; I^2 = 79%, p < 0.0001), heart (SMD = -3.9344, 95% CI: -7.6808; -0.1881, Z = -2.06, p = 0.0396; I^2 = 80%, p = 0.0253), and brain (SMD = -1.6222, 95% CI: -2.7999; -

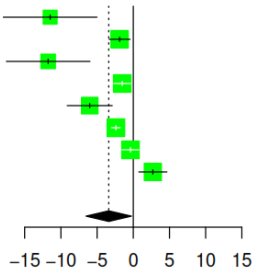
0.4445, Z = -2.70, p = 0.0069; I^2 = 71%, p = 0.0156) of animals treated with fluoride compared to the control was found to be significantly lower (Figure 6a-e). The Egger's test for asymmetry of the funnel plot showed evidence of publication bias (Egger's regression intercept -3.0807, t = -4.52, p < 0.0001).

The funnel plot is presented in Figure 6f. A sensitivity analysis was performed by the exclusion of studies with a high risk of bias[36–38,40,51,52,63,73,79,83,85] which showed similar results (SMD = -3.6340, 95% CI: -4.6631; -2.6050, Z = -6.92, p < 0.0001; I^2 = 82%, p < 0.0001; Egger's regression intercept -2.9614, t = -2.89, p = 0.0101).

a

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Deng, et al. 2014	5	302.12	6.5500	5	442.57	14.1700	−11.49	[−17.96; −5.01]	8.8%
Inkielewicz & Czarnowski, 2008	6	1.35	0.3400	6	2.10	0.3900	−1.89	[−3.34; −0.44]	13.8%
Reddy, et al. 2014	6	48.90	0.5000	6	58.10	0.8900	−11.76	[−17.54; −5.98]	9.6%
Zhan, et al. 2005	8	613.03	153.3900	8	802.72	63.3800	−1.53	[−2.68; −0.38]	13.9%
Bouaziz, et al. 2007	6	12.61	0.8700	6	24.73	2.4800	−6.02	[−9.13; −2.91]	12.4%
Zhou, et al. 2015	36	370.37	19.7500	36	424.69	24.6900	−2.40	[−3.02; −1.79]	14.1%
Sharma, et al. 2022	6	9.51	7.2505	6	11.69	1.1513	−0.39	[−1.53; 0.76]	13.9%
Morales–González, et al. 2010	5	89.25	10.1500	5	61.42	8.3600	2.70	[0.76; 4.64]	13.4%
Random effects model	**78**			**78**			**−3.39**	**[−6.53; −0.25]**	**100.0%**

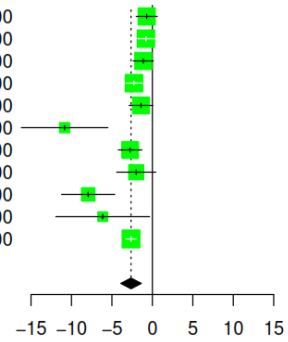
Heterogeneity: I^2 = 88%, τ^2 = 18.1057, p < 0.01



b

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Flora, et al. 2012	5	0.66	0.0400	5	0.74	0.1400	−0.70	[−2.00; 0.60]	10.9%
Guo, et al. 2003	8	4.58	3.6500	8	8.44	5.3300	−0.80	[−1.83; 0.23]	11.3%
Inkielewicz & Czarnowski, 2008	6	35.70	3.0400	6	42.00	6.6500	−1.12	[−2.38; 0.13]	10.9%
Liu, et al. 1999	14	246.57	39.4600	14	386.40	74.4800	−2.28	[−3.26; −1.30]	11.4%
Mittal & Flora, 2006	5	1.96	0.1600	5	2.21	0.1600	−1.41	[−2.88; 0.05]	10.5%
Shanthakumari, et al. 2004	6	5.04	0.2900	6	9.49	0.4500	−10.85	[−16.19; −5.50]	3.9%
Zhan, et al. 2005	8	8.69	1.9300	8	15.18	2.5000	−2.75	[−4.21; −1.28]	10.5%
Bo, et al. 2018	3	0.71	0.1200	3	1.01	0.1200	−1.99	[−4.41; 0.42]	8.5%
Lu, et al. 2017	8	0.62	0.0400	8	1.00	0.0500	−7.93	[−11.22; −4.64]	6.7%
Wang, et al. 2019	3	0.44	0.0500	3	1.00	0.0900	−6.14	[−11.94; −0.34]	3.5%
Zhou, et al. 2015	36	92.16	2.9400	36	100.00	2.9400	−2.64	[−3.28; −2.00]	11.9%
Random effects model	**102**			**102**			**−2.63**	**[−3.91; −1.36]**	**100.0%**

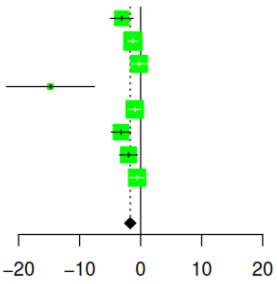
Heterogeneity: I^2 = 76%, τ^2 = 3.4373, p < 0.01



c

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Inkielewicz & Czarnowski, 2008	6	12.00	0.6000	6	17.50	2.2400	−3.10	[−4.97; −1.22]	10.8%
Liu, et al. 1999	14	58.76	24.9300	14	92.46	26.4700	−1.27	[−2.10; −0.45]	17.1%
Mittal & Flora, 2006	5	2.85	0.4000	5	2.94	0.1400	−0.27	[−1.52; 0.98]	14.5%
Shanthakumari, et al. 2004	6	3.22	0.3100	6	7.46	0.2100	−14.78	[−21.99; −7.57]	1.5%
Zhan, et al. 2005	8	9.75	3.6800	8	13.04	2.6800	−0.97	[−2.02; 0.09]	15.7%
Luo, et al. 2017	8	48.35	4.4000	8	66.15	5.9400	−3.22	[−4.83; −1.61]	12.3%
Baba, et al. 2016	6	9.07	0.6858	6	10.89	0.9797	−1.99	[−3.47; −0.50]	13.1%
Tian, et al 2019	6	76.27	24.1500	6	91.53	24.1500	−0.58	[−1.75; 0.58]	15.0%
Random effects model	**59**			**59**			**−1.71**	**[−2.62; −0.79]**	**100.0%**

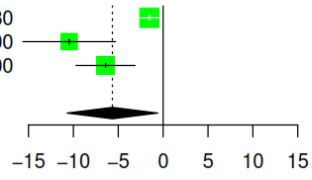
Heterogeneity: I^2 = 75%, τ^2 = 1.1029, p < 0.01



d

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Oyagbemi, et al. 2017	10	32.29	1.1960	10	36.19	3.2680	−1.52	[−2.54; −0.50]	38.6%
Panneerselvam, et al. 2015	6	0.81	0.1200	6	3.93	0.3700	−10.47	[−15.64; −5.30]	27.8%
Quadri, et al. 2018	6	7.24	0.5500	6	13.20	1.0800	−6.42	[−9.71; −3.12]	33.6%
Random effects model	**22**			**22**			**−5.65**	**[−10.71; −0.59]**	**100.0%**

Heterogeneity: I^2 = 89%, τ^2 = 17.0033, p < 0.01



e

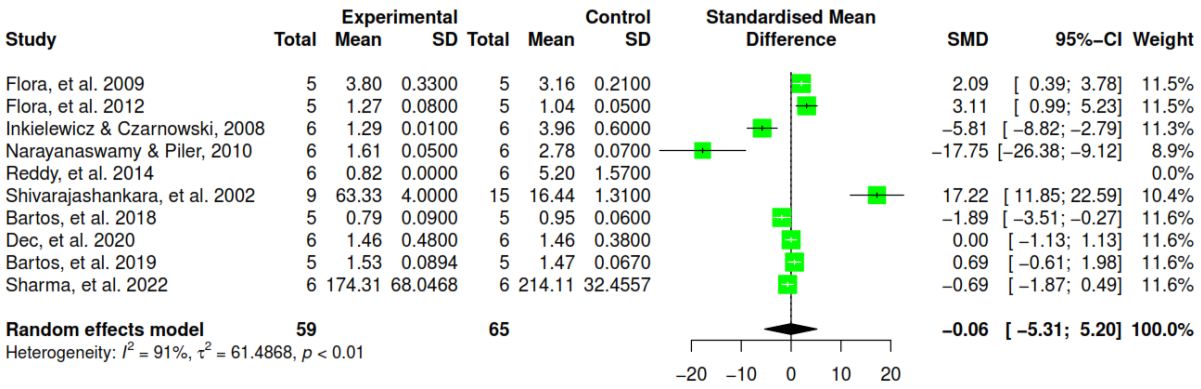


Figure 5. Forest plots for GSH-Px meta-analysis: (a) Blood; (b) Liver; (c) Kidney; (d) Heart; (e) Brain.

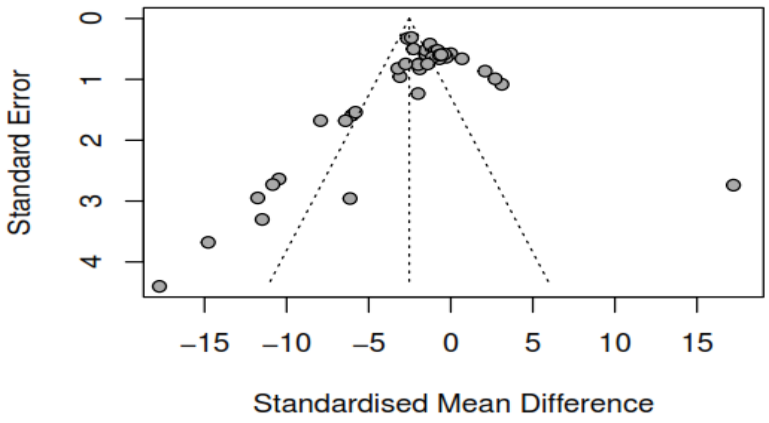
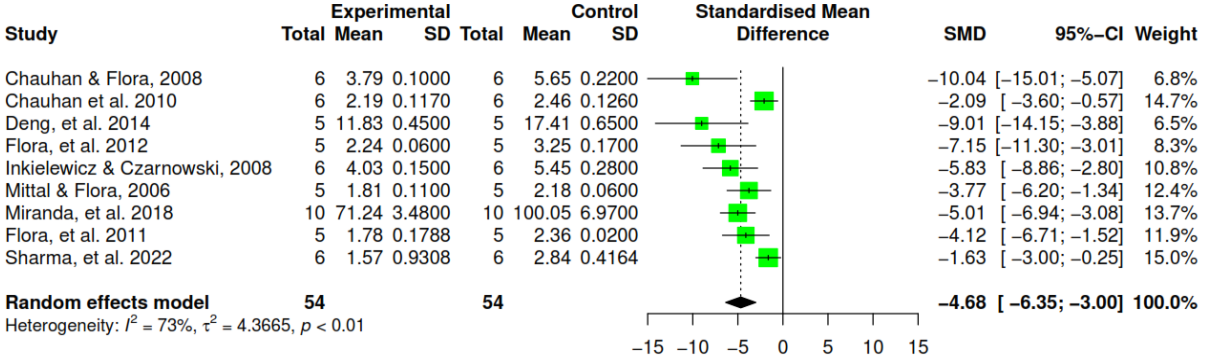
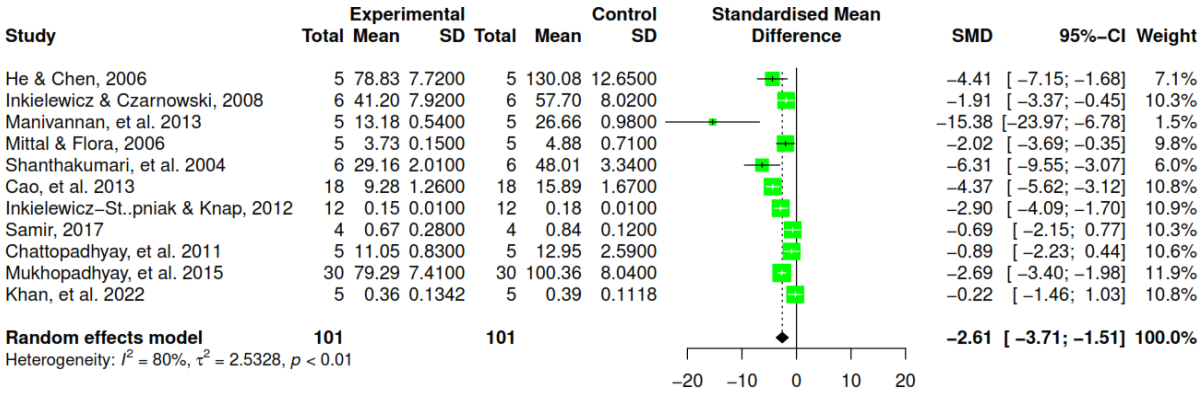


Figure 5f. GSH-Px Funnel plot.

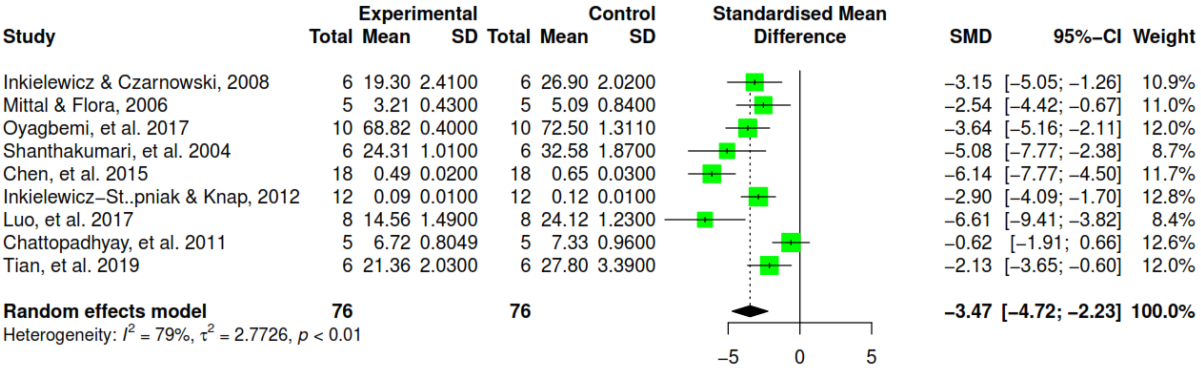
a



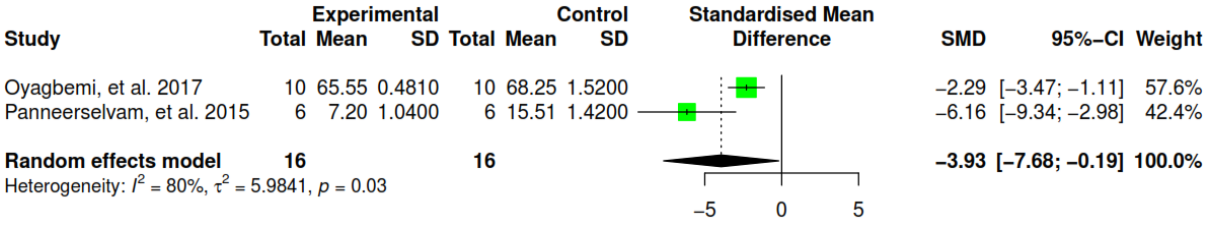
b



c



d



e

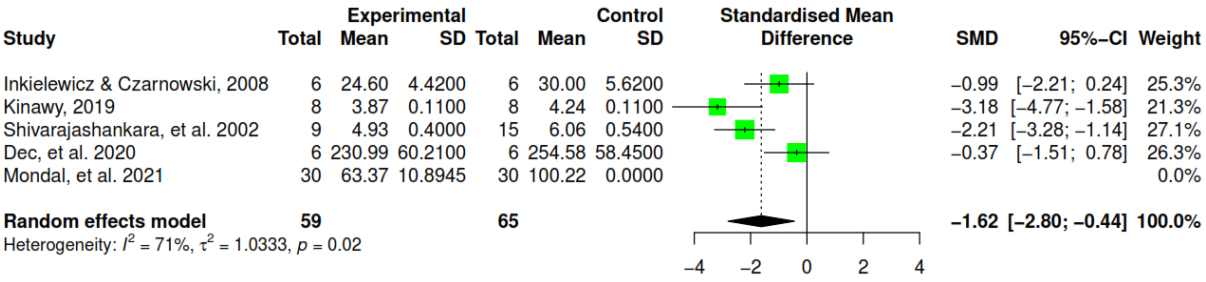


Figure 6. Forest plots for GSH meta-analysis: (a) Blood; (b) Liver; (c) Kidney; (d) Heart; (e) Brain.

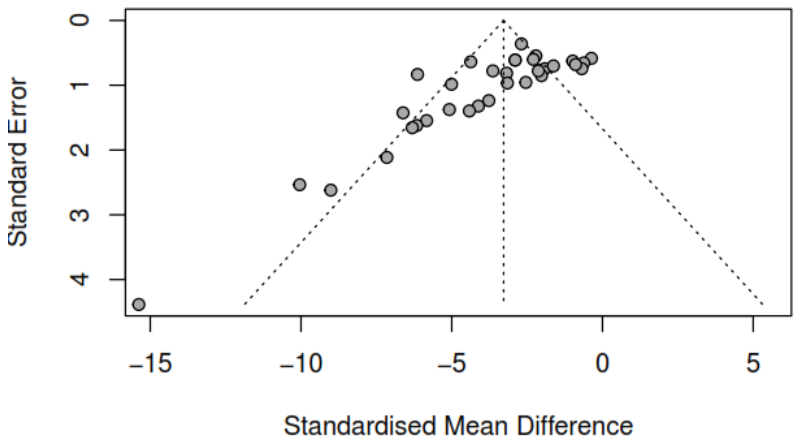


Figure 6f. GSH Funnel plot.

Meta-analysis of glutathione S-transferase (GST)

The difference in the levels of GST between the treatment groups and the controls was non-significant (SMD = -1.5579, 95% CI: -4.1415; 1.0257, Z = -1.18, p = 0.2373). Significant heterogeneity was found (I^2 = 94%, p < 0.0001). Similarly, results from the analysis of the level of GST in individual tissues was found to be non-significant: liver (SMD = -2.8939, 95% CI: -7.0783; 1.2906, Z = -1.36, p = 0.1753; I^2 = 95%, p < 0.0001), kidney (SMD = -1.7370, 95% CI: -4.3792; 0.9052, Z = -1.29, p = 0.1976; I^2 = 88%, p = 0.0047) and brain (SMD = 0.6964, 95% CI: -3.8534; 5.2463, Z = 0.30, p = 0.7642; I^2 = 89%, p < 0.0001) (Figure 7a-c). No studies measured GST in blood and heart. A visual inspection of the funnel plot showed asymmetry (Figure 7d) with evidence of publication bias on Egger's regression test (Egger's regression intercept -5.2524, t = -2.25, p = 0.0439). The significance of the results did not change after a sensitivity analysis was done with the exclusion of the studies with a high risk of bias[38,52,58,73] (SMD = -1.2318, 95% CI: -4.4643; 2.0008, Z = -0.75, p = 0.4552; I^2 = 95.2%, p < 0.0001). However, Egger's regression test showed no evidence of publication bias (Egger's regression intercept -5.4283, t = -1.52, p = 0.1665).

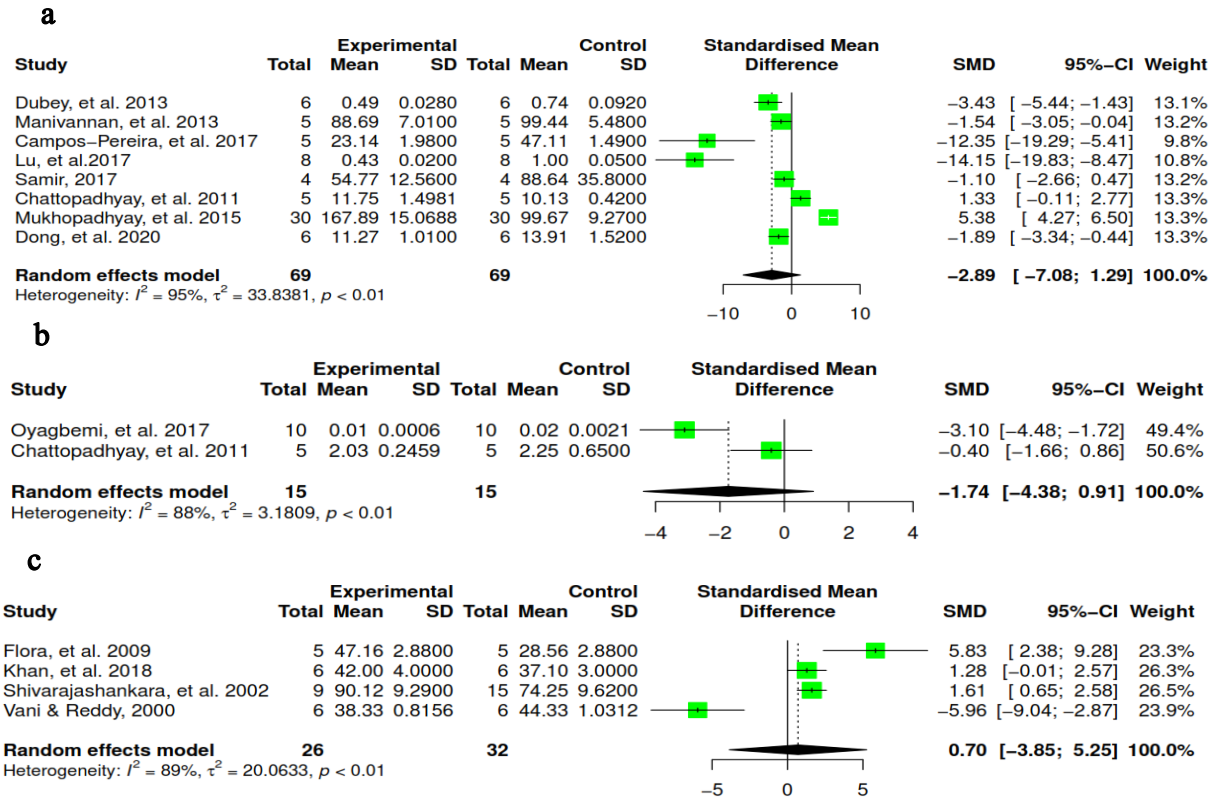


Figure 7. Forest plots for GST meta-analysis: (a) Liver; (b) Kidney; (c) Brain.

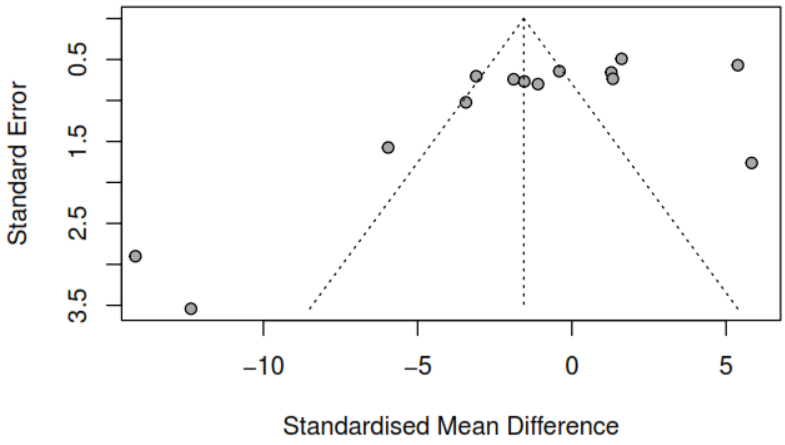


Figure 7d. GST Funnel plot.

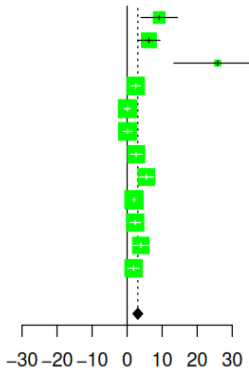
Meta-analysis of Lipid peroxidation (LPO)

The LPO levels in non-skeletal tissues of experimental animals were significantly higher compared to the controls (SMD = 3.4165, 95% CI: 2.8569; 3.9761, Z = 11.97, p < 0.0001). High statistical heterogeneity was found (I^2= 85%, p < 0.0001). Meta-analysis of levels of LPO in individual tissues was also significant, blood (SMD = 3.0534, 95% CI: 1.6614; 4.4454, Z = 4.30, p < 0.0001; I^2 = 84%, p < 0.0001), liver (SMD = 3.4668, 95% CI: 2.4776; 4.4560, Z = 6.87, p < 0.0001; I^2=88%, p < 0.0001), kidney (SMD = 2.9527, 95% CI: 2.0353; 3.8700, Z = 6.31, p < 0.0001; I^2 = 81%, p < 0.0001), heart (SMD = 4.8766, 95% CI: 1.3816; 8.3715, Z = 2.73, p = 0.0062; I^2=87%, p = 0.0006), and brain (SMD = 4.0750, 95% CI: 2.5517; 5.5984, Z = 5.24, p < 0.0001; I^2 = 84%, p < 0.0001) (Figure 8a-e). The funnel plot showed evidence of publication bias (Figure 8f) which was confirmed by Egger's regression analysis (Egger's regression intercept 3.9938, t = 10.22, p < 0.0001). The pooled estimate of the effect did not vary substantially with the exclusion of the studies with a high risk of bias[24,26,35–38,40,41,49–52,58,63,64,73,77,79,80,83,85,86](SMD = 3.5875, 95% CI: 2.8567; 4.3183, Z = 9.62, p < 0.0001; I^2 = 89%, p < 0.0001; Egger's regression intercept 5.2682, t = 7.77, p < 0.0001).

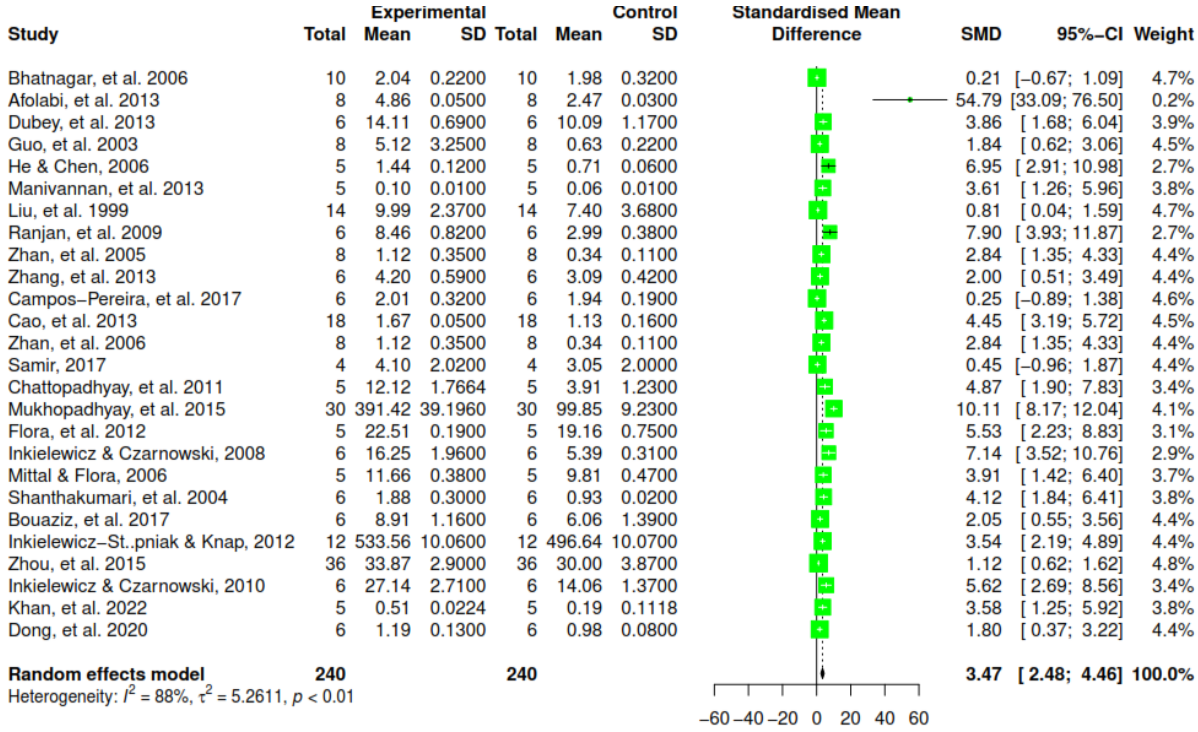
a

Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	SMD	95%–CI	Weight
Deng, et al. 2014	5	8.90	0.4000	5	5.96	0.0900	9.15	[3.94; 14.37]	4.3%
Ranjan, et al. 2009	6	6.92	0.6700	6	3.39	0.3200	6.20	[3.01; 9.40]	7.0%
Reddy, et al. 2014	6	291.90	1.9400	6	232.30	2.3000	25.85	[13.34; 38.35]	1.1%
Zhan, et al. 2005	8	9.36	1.0900	8	5.97	1.5000	2.44	[1.07; 3.82]	9.9%
Zhong, et al. 2021	10	9.58	1.2700	10	9.42	2.1200	0.09	[−0.79; 0.96]	10.5%
Akinrinade, et al. 2015	5	3.03	0.1565	5	2.96	0.7155	0.12	[−1.12; 1.36]	10.1%
Inkielewicz & Czarnowski, 2008	6	11.27	0.9800	6	9.06	0.5400	2.58	[0.90; 4.26]	9.5%
Miranda, et al. 2018	10	154.78	12.8600	10	100.38	3.9600	5.48	[3.40; 7.55]	8.8%
Zhou, et al. 2015	36	17.04	2.5500	36	13.06	1.2700	1.95	[1.39; 2.52]	10.8%
Inkielewicz & Czarnowski, 2010	6	24.41	4.1200	6	16.82	1.2800	2.30	[0.71; 3.88]	9.6%
Sharma, et al. 2022	6	11.97	3.2578	6	2.21	0.3429	3.89	[1.70; 6.08]	8.6%
Morales–González, et al. 2010	5	375.36	56.5900	5	265.40	51.4700	1.84	[0.23; 3.44]	9.6%
Random effects model	**109**			**109**			**3.05**	**[1.66; 4.45]**	**100.0%**

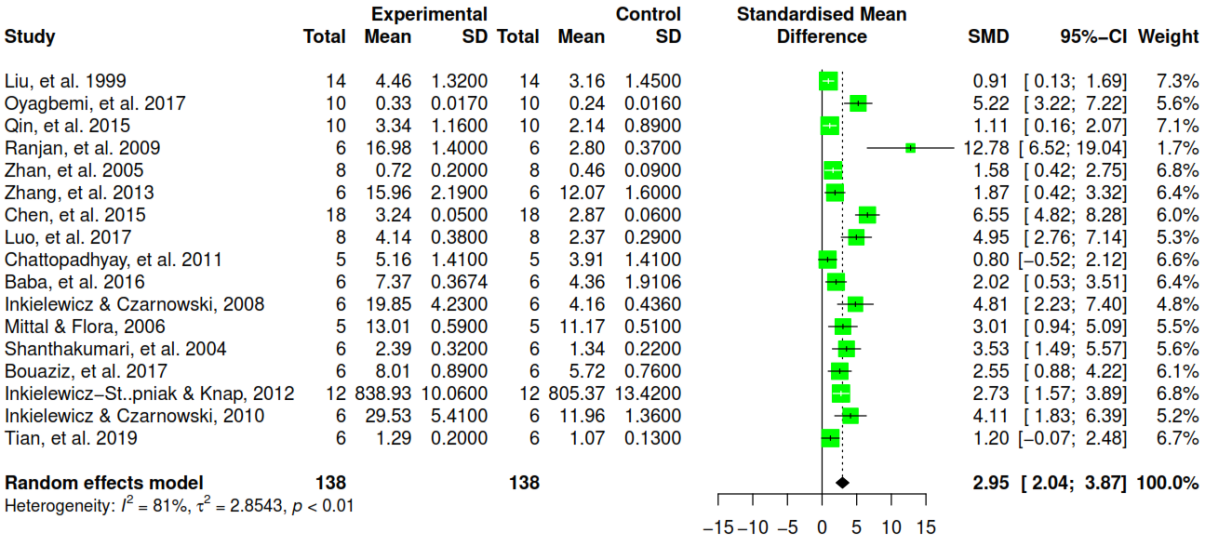
Heterogeneity: I^2 = 84%, τ^2 = 4.5850, p < 0.01



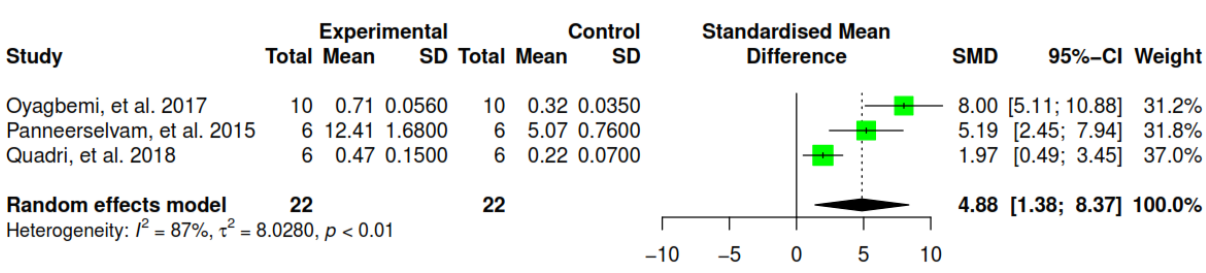
b


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Bhatnagar, et al. 2006 10 2.04 0.2200 10 1.98 0.3200 0.21 [–0.67; 1.09] 4.7%
Afolabi, et al. 2013 8 4.86 0.0500 8 2.47 0.0300 54.79 [33.09; 76.50] 0.2%
Dubey, et al. 2013 6 14.11 0.6900 6 10.09 1.1700 3.86 [1.68; 6.04] 3.9%
Guo, et al. 2003 8 5.12 3.2500 8 0.63 0.2200 1.84 [0.62; 3.06] 4.5%
He & Chen, 2006 5 1.44 0.1200 5 0.71 0.0600 6.95 [2.91; 10.98] 2.7%
Manivannan, et al. 2013 5 0.10 0.0100 5 0.06 0.0100 3.61 [1.26; 5.96] 3.8%
Liu, et al. 1999 14 9.99 2.3700 14 7.40 3.6800 0.81 [0.04; 1.59] 4.7%
Ranjan, et al. 2009 6 8.46 0.8200 6 2.99 0.3800 7.90 [3.93; 11.87] 2.7%
Zhan, et al. 2005 8 1.12 0.3500 8 0.34 0.1100 2.84 [1.35; 4.33] 4.4%
Zhang, et al. 2013 6 4.20 0.5900 6 3.09 0.4200 2.00 [0.51; 3.49] 4.4%
Campos–Pereira, et al. 2017 6 2.01 0.3200 6 1.94 0.1900 0.25 [–0.89; 1.38] 4.6%
Cao, et al. 2013 18 1.67 0.0500 18 1.13 0.1600 4.45 [3.19; 5.72] 4.5%
Zhan, et al. 2006 8 1.12 0.3500 8 0.34 0.1100 2.84 [1.35; 4.33] 4.4%
Samir, 2017 4 4.10 2.0200 4 3.05 2.0000 0.45 [–0.96; 1.87] 4.4%
Chattopadhyay, et al. 2011 5 12.12 1.7664 5 3.91 1.2300 4.87 [1.90; 7.83] 3.4%
Mukhopadhyay, et al. 2015 30 391.42 39.1960 30 99.85 9.2300 10.11 [8.17; 12.04] 4.1%
Flora, et al. 2012 5 22.51 0.1900 5 19.16 0.7500 5.53 [2.23; 8.83] 3.1%
Inkielewicz & Czarnowski, 2008 6 16.25 1.9600 6 5.39 0.3100 7.14 [3.52; 10.76] 2.9%
Mittal & Flora, 2006 5 11.66 0.3800 5 9.81 0.4700 3.91 [1.42; 6.40] 3.7%
Shanthakumari, et al. 2004 6 1.88 0.3000 6 0.93 0.0200 4.12 [1.84; 6.41] 3.8%
Bouaziz, et al. 2017 6 8.91 1.1600 6 6.06 1.3900 2.05 [0.55; 3.56] 4.4%
Inkielewicz–St..pniak & Knap, 2012 12 533.56 10.0600 12 496.64 10.0700 3.54 [2.19; 4.89] 4.4%
Zhou, et al. 2015 36 33.87 2.9000 36 30.00 3.8700 1.12 [0.62; 1.62] 4.8%
Inkielewicz & Czarnowski, 2010 6 27.14 2.7100 6 14.06 1.3700 5.62 [2.69; 8.56] 3.4%
Khan, et al. 2022 5 0.51 0.0224 5 0.19 0.1118 3.58 [1.25; 5.92] 3.8%
Dong, et al. 2020 6 1.19 0.1300 6 0.98 0.0800 1.80 [0.37; 3.22] 4.4%
Random effects model 240 240 3.47 [2.48; 4.46] 100.0%
Heterogeneity: I^2 = 88%, τ^2 = 5.2611, p < 0.01
–60 –40 –20 0 20 40 60

c


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Liu, et al. 1999 14 4.46 1.3200 14 3.16 1.4500 0.91 [0.13; 1.69] 7.3%
Oyagbemi, et al. 2017 10 0.33 0.0170 10 0.24 0.0160 5.22 [3.22; 7.22] 5.6%
Qin, et al. 2015 10 3.34 1.1600 10 2.14 0.8900 1.11 [0.16; 2.07] 7.1%
Ranjan, et al. 2009 6 16.98 1.4000 6 2.80 0.3700 12.78 [6.52; 19.04] 1.7%
Zhan, et al. 2005 8 0.72 0.2000 8 0.46 0.0900 1.58 [0.42; 2.75] 6.8%
Zhang, et al. 2013 6 15.96 2.1900 6 12.07 1.6000 1.87 [0.42; 3.32] 6.4%
Chen, et al. 2015 18 3.24 0.0500 18 2.87 0.0600 6.55 [4.82; 8.28] 6.0%
Luo, et al. 2017 8 4.14 0.3800 8 2.37 0.2900 4.95 [2.76; 7.14] 5.3%
Chattopadhyay, et al. 2011 5 5.16 1.4100 5 3.91 1.4100 0.80 [–0.52; 2.12] 6.6%
Baba, et al. 2016 6 7.37 0.3674 6 4.36 1.9106 2.02 [0.53; 3.51] 6.4%
Inkielewicz & Czarnowski, 2008 6 19.85 4.2300 6 4.16 0.4360 4.81 [2.23; 7.40] 4.8%
Mittal & Flora, 2006 5 13.01 0.5900 5 11.17 0.5100 3.01 [0.94; 5.09] 5.5%
Shanthakumari, et al. 2004 6 2.39 0.3200 6 1.34 0.2200 3.53 [1.49; 5.57] 5.6%
Bouaziz, et al. 2017 6 8.01 0.8900 6 5.72 0.7600 2.55 [0.88; 4.22] 6.1%
Inkielewicz–St..pniak & Knap, 2012 12 838.93 10.0600 12 805.37 13.4200 2.73 [1.57; 3.89] 6.8%
Inkielewicz & Czarnowski, 2010 6 29.53 5.4100 6 11.96 1.3600 4.11 [1.83; 6.39] 5.2%
Tian, et al. 2019 6 1.29 0.2000 6 1.07 0.1300 1.20 [–0.07; 2.48] 6.7%
Random effects model 138 138 2.95 [2.04; 3.87] 100.0%
Heterogeneity: I^2 = 81%, τ^2 = 2.8543, p < 0.01
–15 –10 –5 0 5 10 15

d


Experimental
Control
Standardised Mean Difference
Study Total Mean SD Total Mean SD SMD 95%–CI Weight
Oyagbemi, et al. 2017 10 0.71 0.0560 10 0.32 0.0350 8.00 [5.11; 10.88] 31.2%
Panneerselvam, et al. 2015 6 12.41 1.6800 6 5.07 0.7600 5.19 [2.45; 7.94] 31.8%
Quadri, et al. 2018 6 0.47 0.1500 6 0.22 0.0700 1.97 [0.49; 3.45] 37.0%
Random effects model 22 22 4.88 [1.38; 8.37] 100.0%
Heterogeneity: I^2 = 87%, τ^2 = 8.0280, p < 0.01
–10 –5 0 5 10

e

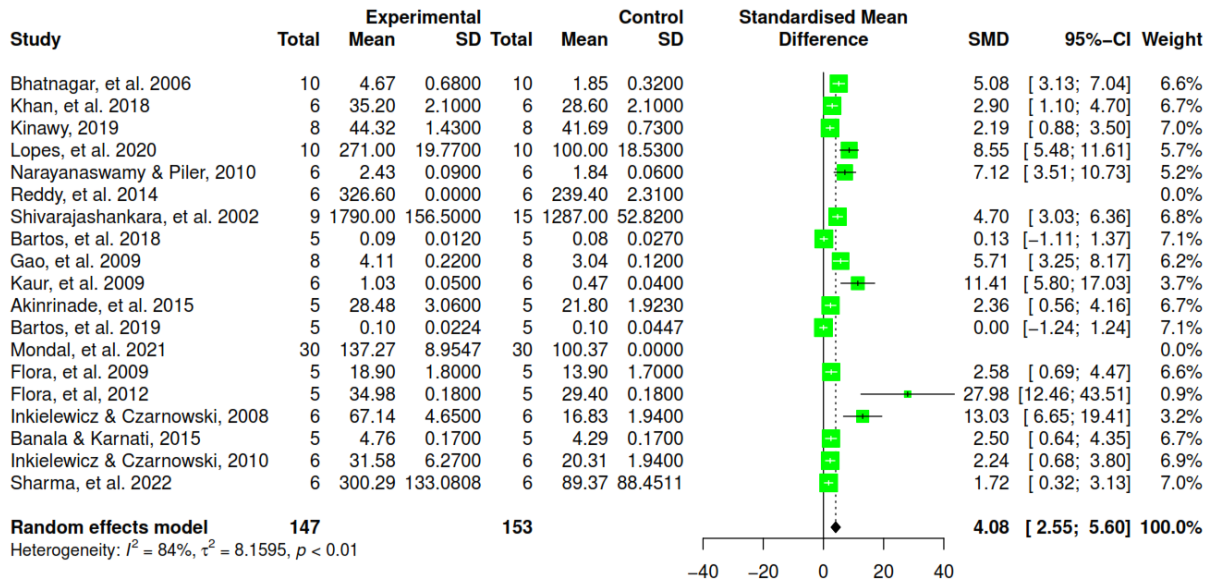


Figure 8. Forest plots for LPO meta-analysis: (a) Blood; (b) Liver; (c) Kidney; (d) Heart; (e) Brain.

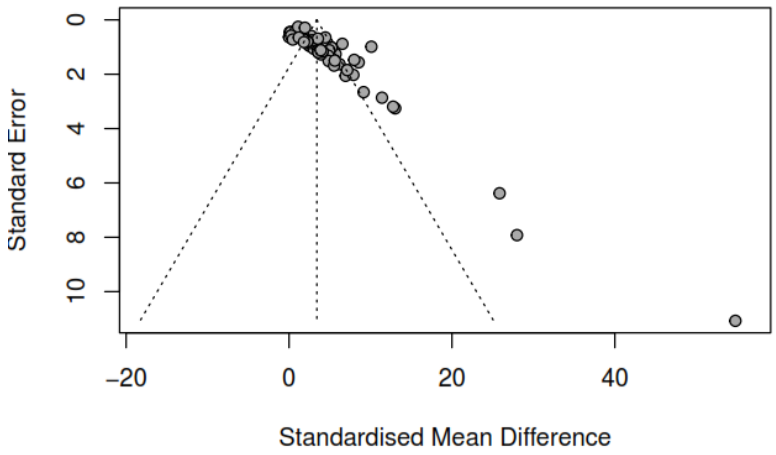


Figure 8f. LPO Funnel plot.

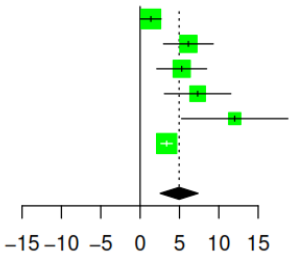
Meta-analysis of Lipid peroxidation (ROS)

The results on ROS indicate a statistically significant increase in the levels of ROS in the non-skeletal tissues of experimental animals as compared to the controls (SMD = 4.5185 (95% CI: 3.1926; 5.8444, Z = 6.68, p < 0.0001). The heterogeneity of the included studies was high (I^2 = 86%, p < 0.0001). Only one study included in the meta-analysis reported the levels of ROS in the heart. A meta-analysis of the studies on ROS in the blood (SMD = 4.9560, 95% CI: 2.5505; 7.3616, Z = 4.04, p < 0.0001; I^2 = 78%, p = 0.0004), liver (SMD = 5.0346, 95% CI: 1.4152; 8.6540, Z = 2.73, p = 0.0064; I^2 = 92%, p < 0.0001), kidney (SMD = 6.2111, 95% CI: 3.8443; 8.5780, Z = 5.14, p < 0.0001; I^2 = 60%, p = 0.057), and brain (SMD = 2.3064, 95% CI: 00.4871; 4.1256, Z = 2.48, p = 0.0130; I^2 = 79%, p = 0.0028) was statistically significant (Figure 9a-d). The funnel plot was asymmetrical (Figure 9e) and the publication bias evaluated through Egger's test showed statistically significant evidence of publication bias (Egger's regression intercept 2.9315, t = 3.27, p = 0.004). The results were unchanged after the exclusion of two studies with a high risk of bias[36,80] (SMD = 4.6235, 95% CI: 3.1309; 6.1162, Z = 6.07, p < 0.0001; I^2 = 87%, p < 0.0001; Egger's regression intercept 4.0824, t = 4.14, p = 0.0007).

a

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Chauhan & Flora, 2008	6	1.18	0.6300	6	0.52	0.0400	1.36	[0.06; 2.67]	21.9%
Chauhan et al. 2010	6	0.03	0.0012	6	0.02	0.0016	6.13	[2.97; 9.30]	16.7%
Flora, et al. 2012	5	14.15	0.5400	5	10.49	0.7000	5.29	[2.11; 8.46]	16.6%
Mittal & Flora, 2006	5	1.04	0.0800	5	0.50	0.0500	7.31	[3.08; 11.53]	13.6%
Flora, et al, 2011	5	11.09	0.5590	5	2.73	0.6900	12.02	[5.26; 18.78]	8.2%
Zhou, et al. 2015	36	1.29	0.0800	36	1.00	0.0900	3.37	[2.64; 4.10]	22.9%
Random effects model	**63**			**63**			**4.96**	**[2.55; 7.36]**	**100.0%**

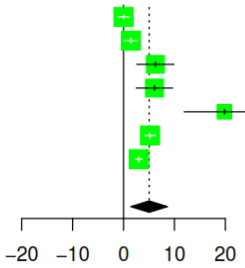
Heterogeneity: I^2 = 78%, τ^2 = 6.4269, p < 0.01



b

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Chauhan & Flora, 2008	6	2.91	0.1400	6	2.91	0.1500	0.00	[−1.13; 1.13]	15.8%
Chauhan et al. 2010	6	0.05	0.0030	6	0.05	0.0020	1.45	[0.12; 2.78]	15.7%
Flora, et al. 2012	5	61.42	1.6900	5	45.28	2.8300	6.25	[2.58; 9.92]	13.8%
He & Chen, 2006	5	143.45	11.7600	5	75.57	8.0500	6.08	[2.50; 9.66]	13.9%
Lu, et al. 2017	8	11.86	0.6000	8	2.79	0.1200	19.82	[11.91; 27.72]	9.1%
Mukhopadhyay, et al. 2015	30	178.59	17.6322	30	98.69	12.2100	5.20	[4.11; 6.29]	15.8%
Zhou, et al. 2015	36	1.38	0.1300	36	1.00	0.1200	3.00	[2.32; 3.69]	16.0%
Random effects model	**96**			**96**			**5.03**	**[1.42; 8.65]**	**100.0%**

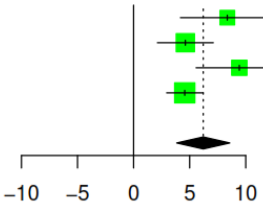
Heterogeneity: I^2 = 92%, τ^2 = 21.2528, p < 0.01



c

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Chauhan & Flora, 2008	6	2.02	0.1400	6	0.88	0.1100	8.36	[4.17; 12.54]	18.0%
Chauhan et al. 2010	6	0.07	0.0010	6	0.07	0.0010	4.61	[2.12; 7.11]	28.1%
Luo, et al. 2017	8	10.43	1.0200	8	2.51	0.4700	9.43	[5.57; 13.28]	19.6%
Song, et al. 2013	12	84.08	7.8000	12	52.06	5.5700	4.56	[2.94; 6.18]	34.3%
Random effects model	**32**			**32**			**6.21**	**[3.84; 8.58]**	**100.0%**

Heterogeneity: I^2 = 60%, τ^2 = 3.5676, p = 0.06



d

Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%–CI	Weight
Chauhan & Flora, 2008	6	3.35	0.1200	6	2.87	0.1500	3.26	[1.32; 5.20]	24.7%
Chauhan et al. 2010	6	0.08	0.0100	6	0.08	0.0050	0.12	[−1.02; 1.25]	30.2%
Flora, et al. 2009	5	0.54	0.0300	5	0.47	0.0200	2.48	[0.63; 4.33]	25.3%
Flora, et al. 2012	5	47.26	1.9900	5	37.92	1.9900	4.24	[1.59; 6.89]	19.8%
Random effects model	**22**			**22**			**2.31**	**[0.49; 4.13]**	**100.0%**

Heterogeneity: I^2 = 79%, τ^2 = 2.5172, p < 0.01

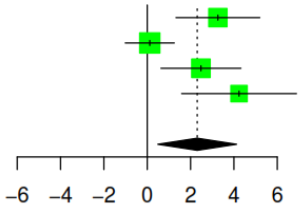


Figure 9. Forest plots for ROS meta-analysis: (a) Blood; (b) Liver; (c) Kidney; (d) Brain.

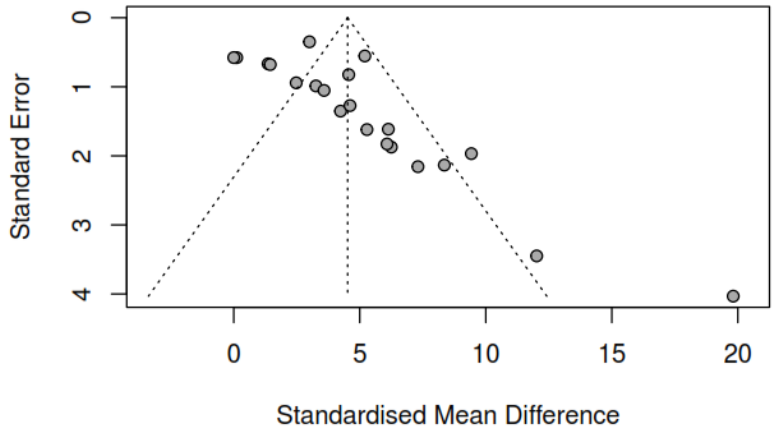
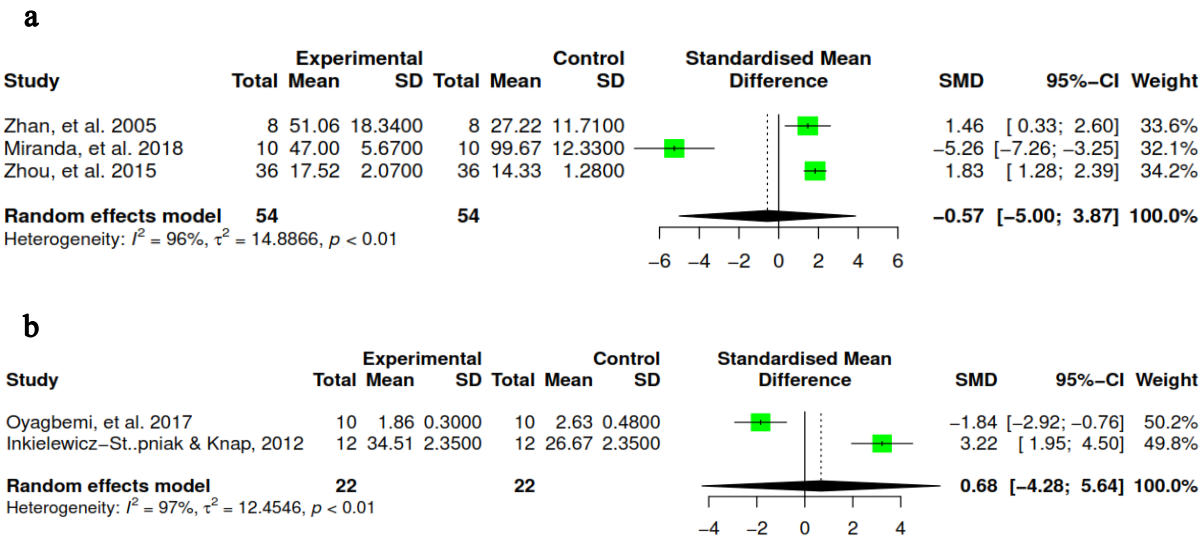


Figure 9e. ROS Funnel plot.

Meta-analysis of Nitric Oxide (NO)

NO level was found to be significantly higher in non-skeletal tissues of experimental animals treated with fluoride compared to the controls (SMD = 3.1115, 95% CI: 0.0142; 6.2087, Z = 1.97, p = 0.049). High heterogeneity was found among the studies measuring NO (I^2 = 95%, p < 0.0001). The NO level in the blood (SMD = -0.5690, 95% CI: -5.0046; 3.8666, Z = -0.25, p = 0.8015; I^2 = 96%, p < 0.0001), kidney (SMD = 0.6776, 95% CI: -4.2842; 5.6394, Z = 0.27, p = 0.7890; I^2 = 97%, p < 0.0001), and heart (SMD= 4.8567, 95% CI: -13.3212; 23.0345, Z = 0.52, p = 0.6005; I^2 = 96%, p < 0.0001) of experimental animals treated with fluoride was non-significant compared to the controls. The NO level was however, significantly higher in the brain (SMD = 8.6347, 95% CI: 4.9441; 12.3253, Z = 4.59, p < 0.0001; I^2 = 53%, p = 0.1426) and liver (SMD = 2.4717, 95% CI: 1.8812; 3.0622, Z = 8.20, p < 0.0001; I^2 = 0%, p = 0.5301) of experimental animals treated with fluoride compared to the controls (10a-e). The funnel plot was asymmetrical (Figure 10b). However, aEgger's regression test showed no evidence of publication bias (Egger's regression intercept 1.1559, t = 0.5, p = 0.6311). Sensitivity analysis done by removing three studies[40,63,80] was non-significant (SMD = 1.6737, 95% CI: -2.9603; 6.3078, Z = 0.71, p = 0.4790) but the heterogeneity remained unchanged (I^2=95%, p < 0.0001). As bias examination using a funnel plot is not recommended for the analysis with less than 10 studies,[88] we did not examine the studies included in the sensitivity analysis for publication bias.



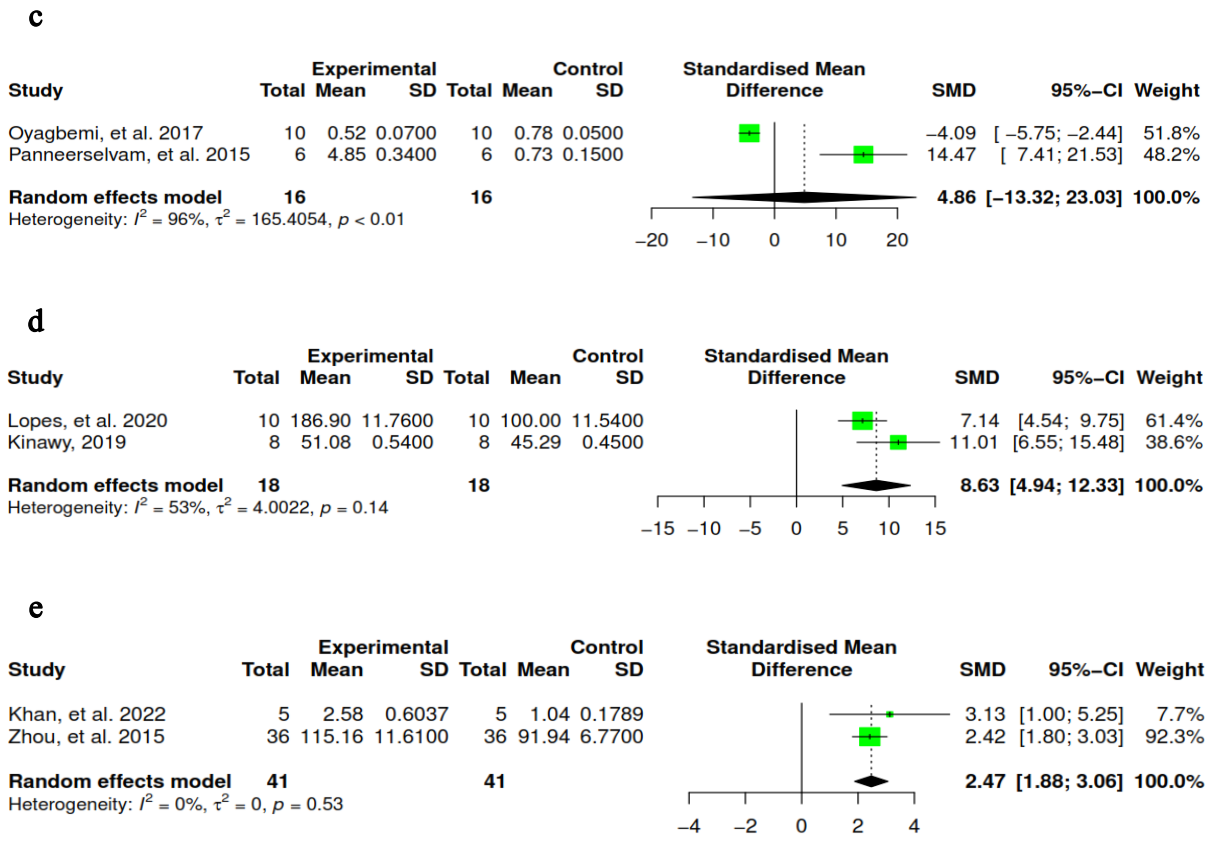


Figure 10. Forest plots for NO meta-analysis: (a) Blood; (b) Kidney; (c) Heart; (d) Brain; (e) Liver.

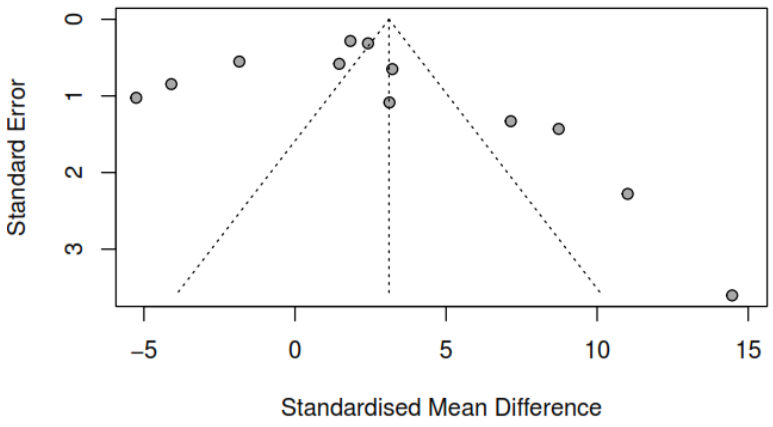


Figure 10f. NO Funnel plot

Subgroup Analysis

A Subgroup analysis assessing the intervention period (<30, 30–90, >90 days), species of animals (mice, rats, others), and sample source (liver, kidney, brain, other tissue) was conducted. The test for subgroup differences suggests that there is a statistically significant subgroup effect (intervention period for SOD (p = 0.0003), CAT (p = 0.03) and LPO (p = 0.007) (Figure 11); animal species for LPO (p = 0.04; Figure 12); and sample source for SOD (p = 0.04), GSH (p = 0.04), ROS (p =0.048), and NO (p = 0.02) (Figure 13)). No statistical difference was detected for other indicators.

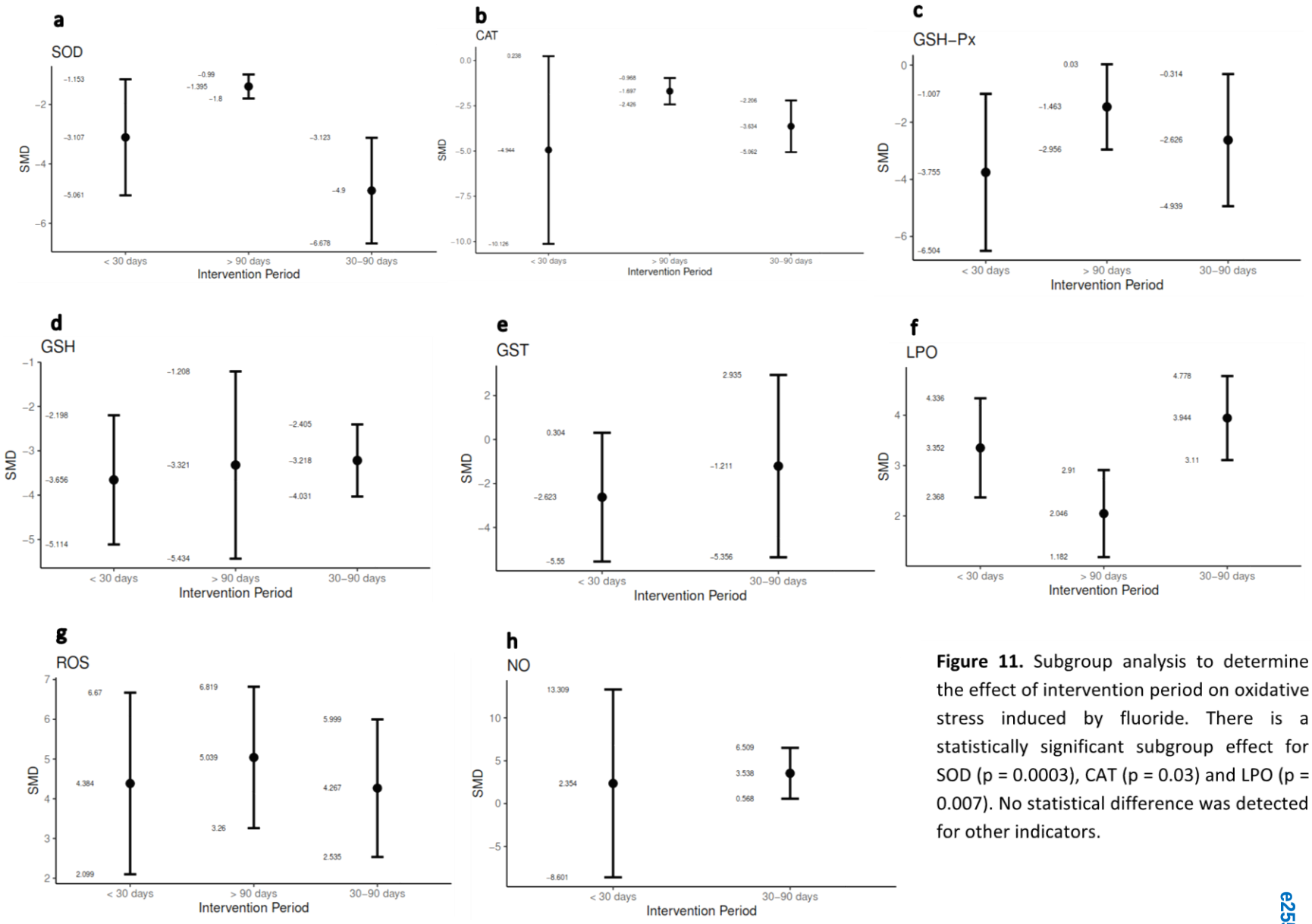


Figure 11. Subgroup analysis to determine the effect of intervention period on oxidative stress induced by fluoride. There is a statistically significant subgroup effect for SOD ($p = 0.0003$), CAT ($p = 0.03$) and LPO ($p = 0.007$). No statistical difference was detected for other indicators.

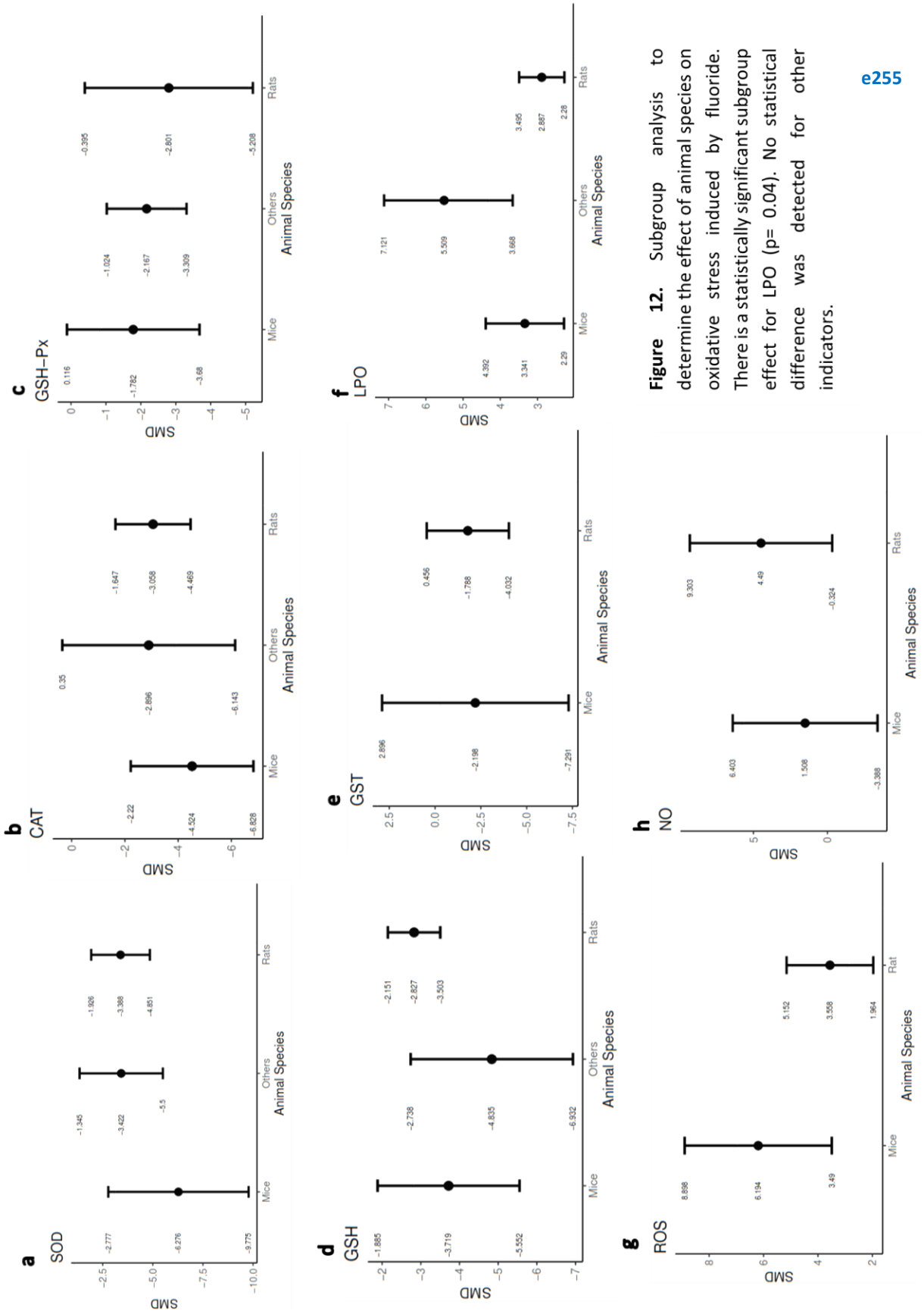


Figure 12. Subgroup analysis to determine the effect of animal species on oxidative stress induced by fluoride. There is a statistically significant subgroup effect for LPO (p= 0.04). No statistical difference was detected for other indicators.

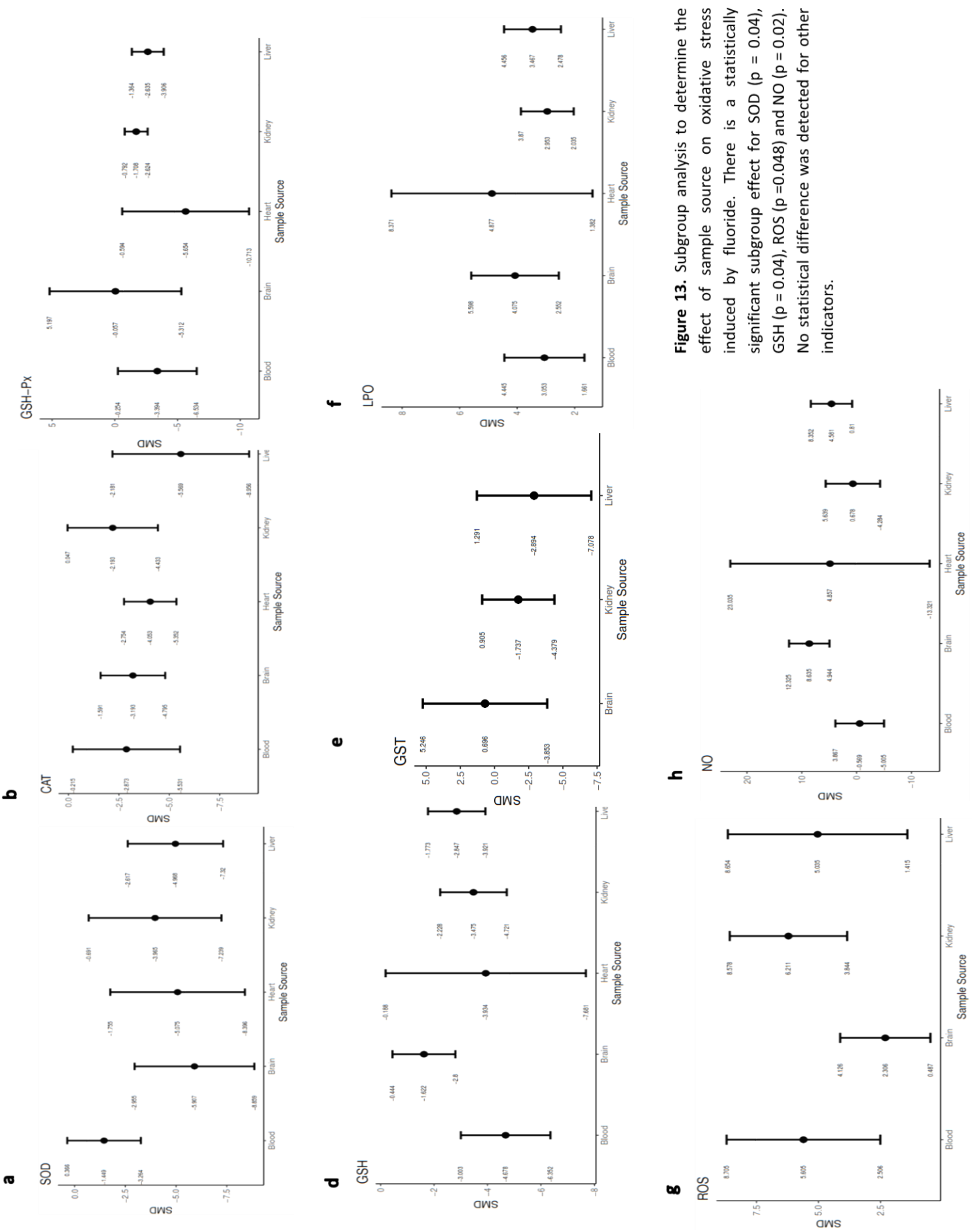


Figure 13. Subgroup analysis to determine the effect of sample source on oxidative stress induced by fluoride. There is a statistically significant subgroup effect for SOD (p = 0.04), GSH (p = 0.04), ROS (p =0.048) and NO (p = 0.02). No statistical difference was detected for other indicators.

Meta-regression

A meta-regression analysis was performed with each indicator of oxidative stress as the outcome and with the intervention period (<30, 30–90, >90 days); animal species (rats, mice, others); and source of samples (kidney, liver, brain, heart, blood) as factors. There was a significant influence of animal species (p = 0.02) for LPO. All the other moderators had no influence on the studies' effect size (Table 2). The source of heterogeneity was found to be from all factors (intervention: LPO (4.1%); animal species: SOD (2.7%), GSH (3.7%), LPO (11%), and ROS (8.2%); sample source: SOD (6%), GSH-Px (3.5%), GSH (3.3%), ROS (3.8%) and NO (2.2%).

Table 2. Results of the meta-regression analysis

	Coefficient	SE	Z value	*P* value	95% CI
SOD					
▪ **Intervention period (Reference < 30 days)**					
✓ **30-90 days**	-1.4080	1.6379	-0.8596	0.3900	-4.6182 – 1.8023
✓ **> 90 days**	-0.0020	2.0681	-0.0010	0.9992	-4.0554 – 4.0515
▪ **Animal species (Reference mice)**					
✓ **Rats**	2.3968	1.5563	1.5400	0.1236	-0.6536 – 5.4472
✓ **Others**	2.3896	1.8342	1.3028	0.1926	-1.2054 – 5.9846
▪ **Sample source (Reference blood)**					
✓ **Kidney**	-1.8243	1.9534	-0.9339	0.3503	-5.6529 – 2.0042
✓ **Liver**	-3.2729	1.8067	-1.8115	0.0701	-6.8140 – 0.2682
✓ **Heart**	-4.0347	3.0881	-1.3065	0.1914	-10.0873 – 2.0178
✓ **Brain**	-4.2570	1.9466	-2.1869	0.0288	-8.0724 – -0.4417
CAT					
▪ **Intervention period (Reference < 30 days)**					
✓ **30-90 days**	0.1844	1.8160	0.1015	0.9191	-3.3750 – 3.7437
✓ **> 90 days**	0.6915	2.1564	0.3207	0.7484	-3.5349 – 4.9180
▪ **Animal species (Reference mice)**					
✓ **Rats**	1.2902	1.3501	0.9556	0.3393	-1.3560 – 3.9364
✓ **Others**	1.6203	1.9266	0.8410	0.4003	-2.1557 – 5.3963
▪ **Sample source (Reference blood)**					
✓ **Kidney**	0.5064	1.9800	0.2558	0.7981	-3.3743 – 4.3871
✓ **Liver**	-2.0595	1.8681	-1.1025	0.2703	-5.7209 – 1.6019
✓ **Heart**	-2.2404	2.9927	-0.7486	0.4541	-8.1060 – 3.6251
✓ **Brain**	-0.4309	1.8362	-0.2347	0.8145	-4.0299 – 3.1680
GSH-Px					
▪ **Intervention period (Reference < 30 days)**					
✓ **30-90 days**	1.5329	2.2885	0.6698	0.5030	-2.9524 – 6.0182
✓ **> 90 days**	2.0769	2.4460	0.8491	0.3958	-2.7172 – 6.8709
▪ **Animal species (Reference mice)**					
✓ **Rats**	-0.7676	1.7390	-0.4414	0.6589	-4.1759 – 2.6407
✓ **Others**	-1.7164	2.4167	-0.7102	0.4776	-6.4532 – 3.0203
▪ **Sample source (Reference blood)**					
✓ **Kidney**	0.7536	2.2008	0.3424	0.7320	-3.5598 – 5.0670
✓ **Liver**	0.1932	2.0550	0.0940	0.9251	-3.8344 – 4.2209
✓ **Heart**	-2.2892	3.0477	-0.7511	0.4526	-8.2625 – 3.6842
✓ **Brain**	3.5809	2.1682	1.6515	0.0986	-0.6687 – 7.8305
GSH					
▪ **Intervention period (Reference < 30 days)**					
✓ **30-90 days**	0.6217	1.1237	0.5533	0.5801	-1.5807 – 2.8241
✓ **> 90 days**	0.7466	1.3525	0.5520	0.5810	-1.9043 – 3.3974
▪ **Animal species (Reference mice)**					
✓ **Rats**	0.4242	0.8028	0.5285	0.5972	-1.1492 – 1.9976

✓ Others	-1.3756	1.1805	-1.1652	0.2439	-3.6893 – 0.9382
▪ Sample source (Reference blood)					
✓ Kidney	1.0041	0.9583	1.0477	0.2948	-0.8743 – 2.8824
✓ Liver	1.5602	0.9473	1.6471	0.0995	-0.2964 – 3.4168
✓ Heart	0.7720	1.5684	0.4922	0.6226	-2.3020 – 3.8459
✓ Brain	2.8213	1.1399	2.4750	0.0133	0.5871 – 5.0556
GST					
▪ Intervention period (Reference < 30 days)					
✓ 30-90 days	1.6799	3.2129	0.5229	0.6011	-4.6172 - 7.977
▪ Animal species (Reference mice)					
✓ Rats	-0.1534	2.7282	-0.0562	0.9552	-5.5007 - 5.1938
▪ Sample source (Reference brain)					
✓ Kidney	-2.4449	4.4802	-0.5457	0.5853	-11.2258 - 6.3361
✓ Liver	-3.4545	3.2290	-1.0698	0.2847	-9.7832 - 2.8742
LPO					
▪ Intervention period (Reference < 30 days)					
✓ 30-90 days	0.3420	0.7189	0.4757	0.6343	-1.0670 - 1.7510
✓ > 90 days	-1.1967	0.8694	-1.3764	0.1687	-2.9007 - 0.5074
▪ Animal species (Reference mice)					
✓ Rats	-0.4183	0.6657	-0.6283	0.5298	-1.7230 - 0.8865
✓ Others	1.8370	0.9271	1.9815	0.0475	0.0200 - 3.6540
▪ Sample source (Reference blood)					
✓ Kidney	-0.0259	0.9489	-0.0273	0.9782	-1.8857 - 1.8339
✓ Liver	0.3575	0.8908	0.4013	0.6882	-1.3886 - 2.1035
✓ Heart	1.6936	1.6510	1.0257	0.3050	-1.5424 - 4.9295
✓ Brain	0.7313	0.9755	0.7496	0.4535	-1.1807 - 2.6432
ROS					
▪ Intervention period (Reference < 30 days)					
✓ 30-90 days	-0.5565	2.3193	-0.2399	0.8104	-5.1021 - 3.9892
✓ > 90 days	0.5144	2.8494	0.1805	0.8567	-5.0703 - 6.0992
▪ Animal species (Reference mice)					
✓ Rats	-2.2948	1.4507	-1.5819	0.1137	-5.1381 - 0.5485
▪ Sample source (Reference blood)					
✓ Kidney	0.8882	2.2144	0.4011	0.6883	-3.4520 - 5.2284
✓ Liver	-1.1578	1.9302	-0.5998	0.5486	-4.9409 - 2.6254
✓ Brain	-3.1068	2.1300	-1.4586	0.1447	-7.2816 - 1.0679
NO					
▪ Intervention period (Reference < 30 days)					
✓ 30-90 days	2.0093	3.8155	0.5266	0.5985	-5.4689 - 9.4874
▪ Animal species (Reference mice)					
✓ Rats	2.9135	3.7208	0.7830	0.4336	-4.3790 - 10.2061
▪ Sample source (Reference blood)					
✓ Kidney	1.2903	4.8220	0.2676	0.7890	-8.1606 - 10.7411
✓ Liver	5.2942	4.3416	1.2194	0.2227	-3.2153 - 13.8036
✓ Heart	4.1357	5.0938	0.8119	0.4168	-5.8479 - 14.1193
✓ Brain	9.5767	4.9762	1.9245	0.0543	-0.1765 - 19.3299

DISCUSSION

In this study, we have undertaken the first meta-analysis to investigate the alterations of oxidative stress biomarkers in non-skeletal tissues of experimental animals exposed to fluoride compared with the controls. We included 62 studies measuring 8 oxidative stress biomarkers. Overall, in comparison to the controls, animals treated with fluoride showed a significant increase in the levels of ROS, LPO, and NO and a significant decrease in the antioxidant levels of SOD, CAT, GSH-Px, and GSH. The results on the levels of GST were, however, not significant. All biomarkers showed high levels of heterogeneity. Significant publication bias was found in all biomarkers except for NO. The sensitivity analysis showed significant differences in the oxidative stress biomarkers between animals treated with fluoride and the controls were not

influenced by any single study, suggesting the robustness of the outcome of the meta-analysis. However, the effect size for studies measuring NO was not significant and there was no publication bias for studies measuring GST after a sensitivity analysis.

We demonstrated an increase in oxidative stress and a decrease in antioxidants in blood, liver, kidney, heart, and brain in line with the available evidence suggesting fluoride-induced oxidative stress as a mechanism involved in fluoride toxicity.[16,89] The liver, being a site of active metabolism and detoxification of foreign substances[90] is susceptible to fluoride toxicity. Evidence suggests that oxidative stress contributes to the development of many liver diseases.[91–93] The cellular structures that are primarily affected by ROS/RNS include the hepatocytic proteins, lipids, and DNA. This results in structural and functional abnormalities in the liver.[91] Our study found that the levels of ROS, LPO, and NO, were elevated in the liver of experimental animals compared to the controls. A significant decrease in SOD, CAT, GSH-Px, and GSH was also observed.

Studies done on the renal system have focused largely on the kidney. Several studies have shown that excess fluoride causes direct adverse effects on the kidneys.[28,60,94–98] The kidney is exposed to higher concentrations of fluoride than all other soft tissues except for the pineal gland, a major site of fluoride accumulation in humans.[99] About half of the daily intake of fluoride is cleared by the kidneys in healthy, young, or middle-aged adults.[100] This exposure to a high concentration of fluoride makes the kidney a target organ for the adverse effects of fluoride. Available data suggests that oxidative stress is a major factor in the deterioration of renal function.[101,102] We found a significant increase in the level of ROS and LPO and a decrease in SOD, GSH-Px, and GSH in the kidney. However, there was no significant change in the level of NO and CAT.

Oxidative stress is an important cause of brain damage.[103] Fluoride is a known neurotoxin associated with cretinism, low Intelligence (IQ), headache, attention deficit hyperactivity disorder, delirium, insomnia, increased pain sensation, tremors, seizures, paralysis, decreased learning ability, decreased long-term memory, anxiety, and depression.[104] Fluoride crosses the blood-brain barrier and accumulates in the brain causing metabolic, structural, and functional damage to the nervous system.[9,105–107] Fluoride is also capable of crossing the placental barrier and accumulating in the brain tissue before birth.[108,109] Our meta-analysis found an increase in the level of ROS, LPO, and NO, and a decrease in the level of SOD, CAT, and GSH in the brain of animals treated with fluoride as compared to the controls. There was no significant change in the level of GSH-Px.

Fluoride has been shown to concentrate in the cardiovascular system[110] leading to vascular calcification, coronary artery disease,[110] atherosclerosis,[111,112] hypertension,[113] and myocardial damage.[10,114] In addition to classic cardiovascular risk factors, oxidative stress is considered to be one of the potential aetiologies in various CVDs.[115] Oxidative stress has been reported to contribute to atherosclerosis and vascular stiffness.[115–117] One of the known molecular mechanisms through which fluoride induces cardiovascular damage is through oxidative stress.[10,45,111,114] However, fluoride can indirectly increase the risk of cardiovascular disease by causing or exacerbating diabetes[118–120] and thyroid dysfunction.[121,122] An increase in the level of ROS and LPO, and a decrease in SOD, CAT, GSH-Px, and GSH was observed in the heart and blood. No significant difference was observed in the level of NO in these tissues.

NO is one of the reactive nitrogen species (RNS) produced by the catalytic action of nitric oxide synthase (NOS) during the generation of L-citrulline from L-arginine and oxygen.[123] Fluoride can either induce[124] or suppress the synthesis of NO.[125] RNS initiates lipid peroxidation, reacts with thiols including glutathione (GSH), creating S-nitrosothiols which can inactivate proteins, leading to increased impaired cellular respiration, oxidative stress, or necrotic cell death. Further, excess NO combines with superoxide, producing peroxynitrite (ONOO-) that is responsible for much of the cytotoxicity.[123] Evidence suggests that oxidative stress also inhibits NO production by impairing endothelial NOS expression and activity. Excessive or deficient NO increases ROS/RNS production while lowering antioxidant levels.[126] Of the 12 studies included in the meta-analysis, 2 recorded lower levels of NO in blood, heart, and kidney.[19,45] The results of this meta-analysis should be interpreted with caution since only 2 or 3 studies were included in the analysis of each organ.

SOD forms the first line of defense against superoxide radicals by conversion to hydrogen peroxide (H_2O_2). H_2O_2 is either detoxified to H_2O and O_2 by GSH-Px or diffuses into the cytosol and is detoxified by catalase (CAT) in peroxisomes. GSH protects against oxygen radicals and toxic compounds and acts as a coenzyme for enzymes.[127] The role of GSH-Px is dependent upon the availability of GSH.[128] A decrease in these antioxidants suggests an impaired ability of the antioxidant defense mechanism to inactivate ROS and scavenge free radicals. GST through their Se-independent glutathione peroxidase activity can reduce lipid hydroperoxides and detoxify lipid peroxidation end products such as 4-hydroxynonenal.[129] No significant change was found in the level of GST in non-skeletal

tissues of experimental animals compared with the control.

We observed a statistically significant subgroup effect suggesting that different animal species and tissues have varying susceptibility and tolerance to fluoride, and the intervention period can determine the level of oxidative damage in experimental animals. However, since there was substantial heterogeneity between the studies within each of these groups, the validity of the treatment effect estimate for each subgroup is uncertain. The absolute SMD for SOD and LPO was higher in the 30-90 days subgroup while that of CAT was higher in the < 30 days subgroup. The severity of fluorosis is dependent on the dose and duration of fluoride exposure. In an in vivo study on the effect of sodium fluoride on sperm motility, sodium fluoride decreased sperm motility in a dose and time-dependent manner. The sperm abnormality was significantly increased at 10 and 100mg/ml of NaF at the 30-minute time interval. [130] In another study, the alterations found in the liver of rats at 60 days were less evident than those observed at 20 days, in both groups treated with 15mg/l and 50mg/l.[131] Mukhopadhyay, et al.[76] however, found that the effect of fluoride was different for the measured parameters e.g., Cytochrome P450 1 A (Cyp1A) mRNA expression increased in a dose-dependent manner up to 30 mg NaF for 30 days treatment group but decreased in the 90 days treatment group while the downregulation of Kelch-like ECH- associated protein 1 (keap 1) was most prominent after 15 mg NaF treatment for 90 days. The differences observed in these studies are likely to be a result of different study designs, dosage, animal species, and duration of treatment. The significant animal species subgroup effect seen in LPO confirms the variation in genetic susceptibility found in different strains of animals.[132–134] This difference is likely to be greater in animals from different species. There was also a significant sample source subgroup effect in SOD, GSH, ROS, and NO with the absolute SMD for SOD being lower in samples from the brain and GSH in samples from blood. The SMD for ROS was higher in samples from the kidney and NO in samples from the brain. The available evidence suggests that the fluoride levels in the brain are generally low due to the relative impermeability of the blood-brain barrier. Conversely, the kidneys are exposed to high levels of fluoride and tend to have the highest fluoride concentration compared to all non-skeletal tissues.[4,135,136] These results should, however, be interpreted with caution since the number of studies contributing data to different subgroups was unequal thus the analysis may not be able to detect subgroup differences.

LIMITATIONS

Our study had some limitations. First, the heterogeneity between the included studies was high. The dissimilarity seen in the studies analysed could be as a result of differences in ages of experimental animals, animal species, kind of tissue examined, dose and mode of fluoride exposure, time of exposure, and methods for biochemical assay. Second, due to the limited number of studies, some comparisons had to contain only two or three studies per item. Thirdly, a subgroup analysis based on fluoride dose could not be done since it was not possible to generate different dose ranges. Further meta-analysis with more studies included would be necessary to verify the results of this study.

CONCLUSIONS

Our meta-analysis findings demonstrated the presence of oxidative stress evidenced by elevated ROS, LPO, and NO and depletion of antioxidants SOD, CAT, GSH-Px, and GSH in non-skeletal tissues of experimental animals exposed to fluoride. However, there was no significant change in the level of GST. Subgroup analysis suggested that different animal species and tissues have varying susceptibilities and tolerance to fluoride. The meta-regression revealed that the extent of fluoride-induced oxidative stress damage can be modified by the intervention period. These findings strengthen the evidence that fluoride toxicity is accompanied by increased oxidative stress response not only in skeletal tissues but also in non-skeletal tissues. Our results also suggested that different animal species and tissues have varying susceptibilities and tolerance to fluoride. Manipulation of oxidative stress marker concentrations should be investigated for potential therapeutic strategies for the disease. Further, studies in humans are recommended to strengthen the current evidence. This will contribute to providing evidence-based guidance for mitigating the toxic effects of fluoride.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

[1] Hewavithana PB, Jayawardhane WM, Gamage R, Goonaratna C. Skeletal fluorosis in Vavuniya District: an observational study. Ceylon Med. J. 2018 Sep 30;63(3):139-42. DOI: 10.4038/cmj.v63i3.8723

[2] Wang C, Gao Y, Wang W, Zhao L, Zhang W, Han H, Shi Y, Yu G, Sun D. A national cross-sectional study on effects of fluoride-safe water supply on the prevalence of fluorosis in China. BMJ Open 2: e001564. DOI: 10.1136/bmjopen-2012-001564

[3] World Health Organization. Preventing Disease Through Healthy Environments. Inadequate or Excess Fluoride: A Major Public Health Concern. World Health Organization; 2010.

[4] Buzalaf MA, editor. Fluoride and the oral environment. Karger Medical and Scientific Publishers; 2011. DOI: 10.1159/isbn.978-3-8055-9659-6

[5] Kanduti D, Sterbenk P, Artnik B. Fluoride: a review of use and effects on health. Materia socio-medica. 2016 Apr;28(2):133. DOI: 10.5455/msm.2016.28.133-137

[6] Whitford GM, Pashley DH, Reynolds KE. Fluoride tissue distribution: short-term kinetics. American Journal of Physiology-Renal Physiology. 1979 Feb 1;236(2):F141-8. DOI: 10.1152/ajprenal.1979.236.2.F141

[7] Dharmaratne RW. Exploring the role of excess fluoride in chronic kidney disease: a review. Human & experimental toxicology. 2019 Mar;38(3):269-79. DOI: 10.1177/0960327118814161

[8] Raina R, Baba NA, Verma PK, Sultana M, Singh M. Hepatotoxicity induced by subchronic exposure of fluoride and chlorpyrifos in Wistar rats: Mitigating effect of ascorbic acid. Biological trace element research. 2015 Aug;166(2):157-62. DOI: 10.1007/s12011-015-0263-1

[9] Grandjean P. Developmental fluoride neurotoxicity: an updated review. Environmental Health. 2019 Dec;18(1):1-7. DOI: 10.1186/s12940-019-0551-x

[10] Basha MP, Sujitha NS. Chronic fluoride toxicity and myocardial damage: antioxidant offered protection in second generation rats. Toxicology international. 2011 Jul;18(2):99. DOI: 10.4103/0971-6580.84260

[11] Varol E, Varol S. Effect of fluoride toxicity on cardiovascular systems: role of oxidative stress. Archives of toxicology. 2012 Oct;86:1627. DOI: 10.1007/s00204-012-0862-y

[12] Kumar N, Sood S, Arora B, Singh M. Effect of duration of fluoride exposure on the reproductive system in male rabbits. Journal of human reproductive sciences. 2010 Sep;3(3):148-52. DOI: 10.4103/0974-1208.74159

[13] Li M, Cao J, Zhao Y, Wu P, Li X, Khodaei F, Han Y, Wang J. Fluoride impairs ovary development by affecting oogenesis and inducing oxidative stress and apoptosis in female zebrafish (Danio rerio). Chemosphere. 2020 Oct 1;256:127105. DOI: 10.1016/j.chemosphere.2020.127105

[14] Selim AO, Abd El-Haleem MR, Ibrahim IH. Effect of sodium fluoride on the thyroid gland of growing male albino rats: histological and biochemical study. Egyptian Journal of Histology. 2012 Sep 1;35(3):470-82. DOI: 10.1097/01.EHX.0000418503.12452.9a

[15] Abbas M, Siddiqi MH, Khan K, Zahra K. Haematological evaluation of sodium fluoride toxicity in oryctolagus cunniculus. Toxicology Reports. 2017 Jan 1;4:450-4. DOI: 10.1016/j.toxrep.2017.07.002

[16] Barbier O, Arreola-Mendoza L, Del Razo LM. Molecular mechanisms of fluoride toxicity. Chemico-biological interactions. 2010 Nov 5;188(2):319-33. DOI: 10.1016/j.cbi.2010.07.011

[17] Angwa LM, Jiang Y, Pei J, Sun D. Antioxidant phytochemicals for the prevention of fluoride-induced oxidative stress and apoptosis: A review. Biological Trace Element Research. 2022 Mar;200(3):1418-41. DOI: 10.1007/s12011-021-02729-8

[18] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World allergy organization journal. 2012 Dec;5:9-19. DOI: 10.1097/WOX.0b013e3182439613

[19] Miranda GH, Gomes BA, Bittencourt LO, Aragão WA, Nogueira LS, Dionizio AS, Buzalaf MA, Monteiro MC, Lima RR. Chronic exposure to sodium fluoride triggers oxidative biochemistry misbalance in mice: effects on peripheral blood circulation. Oxidative medicine and cellular longevity. 2018 Aug 27;2018. DOI: 10.1155/2018/8379123

[20] Chlubek D, Poland S. Fluoride and oxidative stress. Fluoride. 2003 Nov 1;36(4):217-28.

[21] Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC advances. 2015;5(35):27986-8006. DOI: 10.1039/C4RA13315C

[22] Chouhan S, Lomash V, Flora SJ. Fluoride-induced changes in haem biosynthesis pathway, neurological variables and tissue histopathology of rats. Journal of Applied Toxicology: An International Journal. 2010 Jan;30(1):63-73. DOI: 10.1002/jat.1474

[23] Mohammed AT, Mohamed AA, Ali H. Pulmonary apoptotic and oxidative damaging effects of Triclosan alone or in combination with Fluoride in Sprague Dawley rats. Acta histochemica. 2017 May 1;119(4):357-63. DOI: 10.1016/j.acthis.2017.03.004

[24] Bhatnagar M, Rao P, Saxena A, Bhatnagar R, Meena P, Barbar S, Chouhan A, Vimal S. Biochemical changes in brain and other tissues of young adult female mice from fluoride in their drinking water. Fluoride. 2006 Dec;39(4):280-4.

[25] Chlubek D, Grucka-Mamczar E, Birkner E, Polaniak R, Stawiarska-Pięta B, Duliban H. Activity of pancreatic antioxidative enzymes and malondialdehyde concentrations in rats with hyperglycemia caused by fluoride intoxication. Journal of Trace Elements in Medicine and Biology. 2003 Jan 1;17(1):57-60. DOI: 10.1016/S0946-672X(03)80047-0

[26] Banala RR, Karnati PR. Vitamin A deficiency: An oxidative stress marker in sodium fluoride (NaF) induced oxidative damage in developing rat brain. International Journal of Developmental Neuroscience. 2015 Dec 1;47:298-303. DOI: 10.1016/j.ijdevneu.2015.08.010

[27] Reddy GB, Khandare AL, Reddy PY, Rao GS, Balakrishna N, Srivalli I. Antioxidant defense system and lipid peroxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits. Toxicological Sciences. 2003 Apr 1;72(2):363-8. DOI: 10.1093/toxsci/kfg030

[28] Chouhan S, Flora SJ. Effects of fluoride on the tissue oxidative stress and apoptosis in rats: biochemical assays supported by IR spectroscopy data. Toxicology. 2008 Dec 5;254(1-2):61-7. DOI: 10.1016/j.tox.2008.09.008

[29] Hooijmans CR, Rovers MM, De Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC medical research methodology. 2014 Dec;14:1-9. DOI: 10.1186/1471-2288-14-43

[30] Afolabi OK, Oyewo EB, Adekunle AS, Adedosu OT, Adedeji AL. Oxidative indices correlate with dyslipidemia and pro-inflammatory cytokine levels in fluoride-exposed rats. Archives of Industrial Hygiene and Toxicology. 2013 Dec 15;64(4):521-9. DOI: 10.2478/10004-1254-64-2013-2351

[31] Deng Y, Cui H, Peng X, Fang J, Zuo Z, Deng J, Luo Q. Effects of high dietary fluoride on serum biochemistry and oxidative stress parameters in broiler chickens. Health. 2014 Jul 28;2014. DOI:10.4236/health.2014.614216

[32] Dubey N, Khan AM, Raina R. Sub-acute deltamethrin and fluoride toxicity induced hepatic oxidative stress and biochemical alterations in rats. Bulletin of environmental contamination and toxicology. 2013 Sep;91:334-8. DOI: 10.1007/s00128-013-1052-1

[33] Flora SJ, Mittal M, Mishra D. Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic amines and DNA damage in mouse brain. Journal of the neurological sciences. 2009 Oct 15;285(1-2):198-205. DOI: 10.1016/j.jns.2009.07.001

[34] Flora SJ, Mittal M, Pachauri V, Dwivedi N. A possible mechanism for combined arsenic and fluoride induced cellular and DNA damage in mice. Metallomics. 2012 Jan;4(1):78-90. DOI: 10.1039/c1mt00118c

[35] Guo XY, Sun GF, Sun YC. Oxidative stress from fluoride-induced hepatotoxicity in rats. Fluoride. 2003 Feb 1;36(1):25-9.

[36] He LF, Chen JG. DNA damage, apoptosis and cell cycle changes induced by fluoride in rat oral mucosal cells and hepatocytes. World journal of gastroenterology: WJG. 2006 Feb 2;12(7):1144-8. DOI: 10.3748/wjg.v12.i7.1144

[37] Inkielewicz I, Czarnowski W, Gdańsk P. Oxidative stress parameters in rats exposed to fluoride and aspirin. Fluoride. 2008 Jan 1;41(1):76-82.

[38] Manivannan J, Sinha S, Ghosh M, Mukherjee A. Evaluation of multi-endpoint assay to detect genotoxicity and oxidative stress in mice exposed to sodium fluoride. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2013 Feb 18;751(1):59-65. DOI: 10.1016/j.mrgentox.2012.11.006

[39] Khan AM, Raina R, Dubey N, Verma PK. Effect of deltamethrin and fluoride co-exposure on the brain antioxidant status and cholinesterase activity in Wistar rats. Drug and chemical toxicology. 2018 Apr 3;41(2):123-7. DOI: 10.1080/01480545.2017.1321009

[40] Kinawy AA. Synergistic oxidative impact of aluminum chloride and sodium fluoride exposure during early stages of brain development in the rat. Environmental Science and Pollution Research. 2019 Apr 1;26(11):10951-60. DOI: 10.1007/s11356-019-04491-w

[41] Liu KT, Wang G, Ma L, Jang P, Xiao BY, Zhang C. Adverse effects of combined arsenic and fluoride on liver and kidney in rats. Fluoride. 1999 Nov 1;32(4):243-7.

[42] Lopes GO, Martins Ferreira MK, Davis L, Bittencourt LO, Bragança Aragão WA, Dionizio A, Rabelo Buzalaf MA, Crespo-Lopez ME, Maia CS, Lima RR. Effects of fluoride long-term exposure over the cerebellum: global proteomic profile, oxidative biochemistry, cell density, and motor behavior evaluation. International journal of molecular sciences. 2020 Oct 2;21(19):7297. DOI: 10.3390/ijms21197297

[43] Mittal M, Flora SJ. Effects of individual and combined exposure to sodium arsenite and sodium fluoride on tissue oxidative stress, arsenic and fluoride levels in male mice. Chemico-biological interactions. 2006 Aug 25;162(2):128-39. DOI: 10.1016/j.cbi.2006.05.018

[44] Narayanaswamy M, Piler MB. Effect of maternal exposure of fluoride on biometals and oxidative stress parameters in developing CNS of rat. Biological trace element research. 2010 Jan;133:71-82. DOI: 10.1007/s12011-009-8413-y

[45] Oyagbemi AA, Omobowale TO, Asenuga ER, Adejumobi AO, Ajibade TO, Ige TM, Ogunpolu BS, Adedapo AA, Yakubu MA. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Environmental toxicology. 2017 Apr;32(4):1089-101. DOI: 10.1002/tox.22306

[46] Panneerselvam L, Govindarajan V, Ameeramja J, Nair HR, Perumal E. Single oral acute fluoride exposure causes changes in cardiac expression of oxidant and antioxidant enzymes, apoptotic and necrotic markers in male rats. Biochimie. 2015 Dec 1;119:27-35. DOI: 10.1016/j.biochi.2015.10.002

[47] Qin SL, Deng J, Lou DD, Yu WF, Pei J, Guan ZZ. The decreased expression of mitofusin-1 and increased fission-1 together with alterations in mitochondrial morphology in the kidney of rats with chronic fluorosis may involve elevated oxidative stress. Journal of Trace Elements in Medicine and Biology. 2015 Jan 1;29:263-8. DOI: 10.1016/j.jtemb.2014.06.001

[48] Quadri JA, Sarwar S, Kar P, Singh S, Mallick SR, Arava S, Nag TC, Roy TS, Shariff A. Fluoride induced tissue hypercalcemia, IL-17 mediated inflammation and apoptosis lead to cardiomyopathy: ultrastructural and biochemical findings. Toxicology. 2018 Aug 1;406:44-57. DOI: 10.1016/j.tox.2018.05.012

[49] Ranjan R, Swarup D, Patra RC. Oxidative stress indices in erythrocytes, liver, and kidneys of fluoride-exposed rabbits. Fluoride. 2009 Apr 1;42(2):88-93.

[50] Reddy YP, Tiwari SK, Shaik AP, Alsaeed A, Sultana A, Reddy PK. Effect of sodium fluoride on neuroimmunological parameters, oxidative stress and antioxidative defenses. Toxicology mechanisms and methods. 2014 Jan 1;24(1):31-6. DOI: 10.3109/15376516.2013.843224

[51] Shanthakumari D, Srinivasalu S, Subramanian S. Effect of fluoride intoxication on lipidperoxidation and antioxidant status in experimental rats. Toxicology. 2004 Nov 15;204(2-3):219-28. DOI: 10.1016/j.tox.2004.06.058

[52] Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH. Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. Fluoride. 2002 Aug 1;35(3):197-203.

[53] Zhan XA, Xu ZR, Li JX, Wang M. Effects of fluorosis on lipid peroxidation and antioxidant systems in young pigs. Fluoride. 2005 May 1;38(2):157-61.

[54] Zhang J, Song J, Zhang J, Chen X, Zhou M, Cheng G, Xie X. Combined effects of fluoride and cadmium on liver and kidney function in male rats. Biological trace element research. 2013 Dec;155:396-402. DOI: 10.1007/s12011-013-9807-4

[55] Bartos M, Gumilar F, Gallegos CE, Bras C, Dominguez S, Mónaco N, del Carmen Esandi M, Bouzat C, Cancela LM, Minetti A. Alterations in the memory of rat offspring exposed to low levels of fluoride during gestation and lactation: Involvement of the α7 nicotinic receptor and oxidative stress. Reproductive Toxicology. 2018 Oct 1;81:108-14. DOI: 10.1016/j.reprotox.2018.07.078

[56] Bo X, Mu D, Wu M, Xiao H, Wang H. The morphological changes and molecular biomarker responses in the liver of fluoride-exposed Bufo gargarizans larvae. Ecotoxicology and environmental safety. 2018 Apr 30;151:199-205. DOI: 10.1016/j.ecoenv.2018.01.027

[57] Bouaziz H, Croute F, Boudawara T, Soleilhavoup JP, Zeghal N. Oxidative stress induced by fluoride in adult mice and their suckling pups. Experimental and Toxicologic Pathology. 2007 Apr 26;58(5):339-49. DOI: 10.1016/j.etp.2006.11.004

[58] Campos-Pereira FD, Lopes-Aguiar L, Renosto FL, Nogueira GA, Costa EF, Pulz RB, Silva-Zacarin EC, Oliveira CA, Pigoso AA, Severi-Aguiar GD. Genotoxic effect and rat hepatocyte death occurred after oxidative stress induction and antioxidant gene downregulation caused by long term fluoride exposure. Chemico-biological interactions. 2017 Feb 25;264:25-33. DOI: 10.1016/j.cbi.2017.01.005

[59] Cao J, Chen J, Wang J, Jia R, Xue W, Luo Y, Gan X. Effects of fluoride on liver apoptosis and Bcl-2, Bax protein expression in freshwater teleost, Cyprinus carpio. Chemosphere. 2013 May 1;91(8):1203-12. DOI: 10.1016/j.chemosphere.2013.01.037

[60] Chen J, Cao J, Wang J, Jia R, Xue W, Xie L. Fluoride-induced apoptosis and expressions of caspase proteins in the kidney of carp (Cyprinus carpio). Environmental toxicology. 2015 Jul;30(7):769-81. DOI: 10.1002/tox.21956

[61] Dec K, Łukomska A, Skonieczna-Żydecka K, Jakubczyk K, Tarnowski M, Lubkowska A, Dec K, Łukomska A, Skonieczna-Żydecka K, Jakubczyk K, Tarnowski M, Lubkowska A, Baranowska-Bosiacka I, Styburski D, Skórka-Majewicz M, Maciejewska D, Gutowska I. Chronic exposure to fluoride affects GSH level and NOX4 expression in rat model of this element of neurotoxicity. Biomolecules. 2020 Mar 9;10(3):422. DOI: 10.3390/biom10030422

[62] Gao Q, Liu YJ, Guan ZZ. Decreased learning and memory ability in rats with fluorosis: increased oxidative stress and reduced cholinesterase activity in the brain. Fluoride. 2009 Oct 1;42(4):277-85.

[63] Inkielewicz-Stępniak I, Knap N. Effect of exposure to fluoride and acetaminophen on oxidative/nitrosative status of liver and kidney in male and female rats. Pharmacological reports. 2012 Jul 1;64(4):902-11. DOI: 10.1016/S1734-1140(12)70885-X

[64] Kaur T, Bijarnia RK, Nehru B. Effect of concurrent chronic exposure of fluoride and aluminum on rat brain. Drug and chemical toxicology. 2009 Jul 1;32(3):215-21. DOI: 10.1080/01480540902862251

[65] Lu Y, Luo Q, Cui H, Deng H, Kuang P, Liu H, Fang J, Zuo Z, Deng J, Li Y, Wang X. Sodium fluoride causes oxidative stress and apoptosis in the mouse liver. Aging (Albany NY). 2017 Jun;9(6):1623-39. DOI: 10.18632/aging.101257

[66] Luo Q, Cui H, Deng H, Kuang P, Liu H, Lu Y, Fang J, Zuo Z, Deng J, Li Y, Wang X. Histopathological findings of renal tissue induced by oxidative stress due to different concentrations of fluoride. Oncotarget. 2017 Aug 8;8(31):50430-46. DOI: 10.18632/oncotarget.17365

[67] Song Y, Wang JC, Xu H, Du ZW, Zhang GZ, Selim HA, Li GS, Wang Q, Gao ZL. Fluorosis caused cellular apoptosis and oxidative stress of rat kidneys. Chemical Research in Chinese Universities. 2013 Apr;29:263-9. DOI: 10.1007/s40242-013-2430-2

[68] Wang X, Zheng R, Yao Q, Liang Z, Wu M, Wang H. Effects of fluoride on the histology, lipid metabolism, and bile acid secretion in liver of Bufo gargarizans larvae. Environmental Pollution. 2019 Nov 1;254:113052. DOI: 10.1016/j.envpol.2019.113052

[69] Zhan XA, Wang M, Xu ZR, Li WF, Li JX. Evaluation of caspase-dependent apoptosis during fluoride-induced liver lesion in pigs. Archives of toxicology. 2006 Feb;80:74-80. DOI: 10.1007/s00204-005-0019-3

[70] Zhong N, Yao Y, Ma Y, Meng X, Sowanou A, Pei J. Effects of fluoride on oxidative stress markers of lipid, gene, and protein in rats. Biological Trace Element Research. 2021 Jun;199:2238-46. DOI: 10.1007/s12011-020-02336-z

[71] Vani ML, Reddy KP. Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluoride. 2000 Feb 1;33(1):17-26.

[72] Akinrinade ID, Memudu AE, Ogundele OM, Ajetunmobi OI. Interplay of glia activation and oxidative stress formation in fluoride and aluminium exposure. Pathophysiology. 2015 Mar 1;22(1):39-48. DOI: 10.1016/j.pathophys.2014.12.001

[73] Anfal D, Samir D. Study of fluoride-induced haematological alterations and liver oxidative stress in rats. World journal of pharmacy and pharmaceutical sciences. 2017 Mar 6;6(5):211-21.

[74] Flora SJ, Pachauri V, Mittal M, Kumar D. Interactive effect of arsenic and fluoride on cardio-respiratory disorders in male rats: possible role of reactive oxygen species. Biometals. 2011 Aug;24:615-28. DOI: 10.1007/s10534-011-9412-y

[75] Chattopadhyay A, Podder S, Agarwal S, Bhattacharya S. Fluoride-induced histopathology and synthesis of stress protein in liver and kidney of mice. Archives of toxicology. 2011 Apr;85:327-35. DOI: 10.1007/s00204-010-0588-7

[76] Mukhopadhyay D, Srivastava R, Chattopadhyay A. Sodium fluoride generates ROS and alters transcription of genes for xenobiotic metabolizing enzymes in adult zebrafish (Danio rerio) liver: expression pattern of Nrf2/Keap1 (INrf2). Toxicology mechanisms and methods. 2015 Jun 13;25(5):364-73. DOI: 10.3109/15376516.2015.1025348

[77] Bartos M, Gumilar F, Gallegos CE, Bras C, Dominguez S, Cancela LM, Minetti A. Effects of perinatal fluoride exposure on short-and long-term memory, brain antioxidant status, and glutamate metabolism of young rat pups. International Journal of Toxicology. 2019 Sep;38(5):405-14. DOI: 10.1177/1091581819857558

[78] Baba N, Raina R, Verma P, Sultana M. Free radical-induced nephrotoxicity following repeated oral exposureto chlorpyrifos alone and in conjunction with fluoride in rats. Turkish journal of medical sciences. 2016;46(2):512-7. DOI: 10.3906/sag-1403-109

[79] Mondal P, Shaw P, Bhowmik AD, Bandyopadhyay A, Sudarshan M, Chakraborty A, Chattopadhyay A. Combined effect of arsenic and fluoride at environmentally relevant concentrations in zebrafish (Danio rerio) brain: Alterations in stress marker and apoptotic gene expression. Chemosphere. 2021 Apr 1;269:128678. DOI: 10.1016/j.chemosphere.2020.128678

[80] Zhou BH, Zhao J, Liu J, Zhang JL, Li J, Wang HW. Fluoride-induced oxidative stress is involved in the morphological damage and dysfunction of liver in female mice. Chemosphere. 2015 Nov 1;139:504-11. DOI: 10.1016/j.chemosphere.2015.08.030

[81] Inkielewicz-Stepniak I, Czarnowski W. Oxidative stress parameters in rats exposed to fluoride and caffeine. Food and Chemical Toxicology. 2010 Jun 1;48(6):1607-11. DOI: 10.1016/j.fct.2010.03.033

[82] Khan H, Verma Y, Rana SV. Significance of inflammation and apoptosis in hepatocellular death in rat, co-treated with arsenic and fluoride. Biological trace element research. 2022 Jul;200(7):3227-35. DOI: 10.1007/s12011-021-02929-2

[83] Sharma P, Verma PK, Sood S, Singh R, Gupta A, Rastogi A. Distribution of fluoride in plasma, brain, and bones and associated oxidative damage after induced chronic fluorosis in Wistar rats. Biological trace element research. 2022 Apr;200(4):1710-21. DOI: 10.1007/s12011-021-02782-3

[84] Dong N, Feng J, Xie J, Tian X, Li M, Liu P, Zhao Y, Wei C, Gao Y, Li B, Qiu Y. Co-exposure to arsenic-fluoride results in endoplasmic reticulum stress-induced apoptosis through the PERK signaling pathway in the liver of offspring rats. Biological trace element research. 2020 Sep;197:192-201. DOI: 10.1007/s12011-019-01975-1

[85] Tian X, Feng J, Dong N, Lyu Y, Wei C, Li B, Ma Y, Xie J, Qiu Y, Song G, Ren X. Subchronic exposure to arsenite and fluoride from gestation to puberty induces oxidative stress and disrupts ultrastructure in the kidneys of rat offspring. Science of the Total Environment. 2019 Oct 10;686:1229-37. DOI: 10.1016/j.scitotenv.2019.04.409

[86] Morales-González JA, Gutiérrez-Salinas J, García-Ortiz L, Chima-Galán MD, Madrigal-Santillán E, Esquivel-Soto J, Esquivel-Chirino C, González-Rubio MG. Effect of sodium fluoride ingestion on malondialdehyde concentration and the activity of antioxidant enzymes in rat erythrocytes. International Journal of Molecular Sciences. 2010 Jun 11;11(6):2443-52. DOI: 10.3390/ijms11062443

[87] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Bmj. 2003 Sep 4;327(7414):557-60. DOI: 10.1136/bmj.327.7414.557

[88] Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, Carpenter J, Rücker G, Harbord RM, Schmid CH, Tetzlaff J. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. Bmj. 2011 Jul 22;343. DOI: 10.1136/bmj.d4002

[89] Shivarajashankara YM, Shivashankara AR. Neurotoxic effects of fluoride in endemic skeletal fluorosis and in experimental chronic fluoride toxicity. Journal of clinical and diagnostic research. 2012;6(4):740-4. DOI: 10.7860/JCDR/2012/.2179

[90] Kalra A, Yetiskul E, Wehrle CJ, Tuma F. Physiology, Liver. StatPearls [Internet]. 2021 May 9 [cited 2023 Sept 28]; Available from: https://www.ncbi.nlm.nih.gov /books/NBK535438/

[91] Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World journal of gastroenterology: WJG. 2014 Jul 7;20(25):8082- 91. DOI: 10.3748/wjg.v20.i25.8082

[92] Arauz J, Ramos-Tovar E, Muriel P. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. Annals of Hepatology. 2016 Feb 15;15(2):160-73. DOI: 10.5604/16652681.1193701

[93] Conde de la Rosa L, Goicoechea L, Torres S, Garcia-Ruiz C, Fernandez-Checa JC. Role of oxidative stress in liver disorders. Livers. 2022 Oct 14;2(4):283-314. DOI: 10.3390/livers2040023

[94] Iano FG, Ferreira MC, Quaggio GB, Fernandes MS, Oliveira RC, Ximenes VF, Buzalaf MA. Effects of chronic fluoride intake on the antioxidant systems of the liver and kidney in rats. Journal of Fluorine Chemistry. 2014 Dec 1;168:212-7. DOI: 10.1016/j.jfluchem.2014.09.029

[95] Gao J, Tian X, Yan X, Wang Y, Wei J, Wang X, Yan X, Song G. Selenium exerts protective effects against fluoride-induced apoptosis and oxidative stress and altered the expression of Bcl-2/caspase family. Biological Trace Element Research. 2021 Feb;199:682-92. DOI: 10.1007/s12011-020-02185-w

[96] Cao J, Chen J, Xie L, Wang J, Feng C, Song J. Protective properties of sesamin against fluoride-induced oxidative stress and apoptosis in kidney of carp (Cyprinus carpio) via JNK signaling pathway. Aquatic Toxicology. 2015 Oct 1;167:180-90. DOI: 10.1016/j.aquatox.2015.08.004

[97] Błaszczyk I, Grucka-Mamczar E, Kasperczyk S, Birkner E. Influence of fluoride on rat kidney antioxidant system: effects of methionine and vitamin E. Biological trace element research. 2008 Jan;121:51-9. DOI: 10.1007/s12011-007-8030-6

[98] Yu RA, Xia T, Wang AG, Chen XM. Effects of selenium and zinc on renal oxidative stress and apoptosis induced by fluoride in rats. Biomedical and environmental sciences. 2006 Dec 20;19(6):439-44.

[99] Panda L, Kar BB, Patra BB. Fluoride and its health impacts-a critical review. IOSR J Electron Commun Eng. 2015;10:79-91.

[100] Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride [Internet]. National Academies Press; 1997 [cited 2023 Sept 26]. Available from: https://pubmed. ncbi.nlm.nih.gov/23115811/ DOI:10.17226/5776

[101] Kao MP, Ang DS, Pall AA, Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. Journal of human hypertension. 2010 Jan;24(1):1-8. DOI: 10.1038/jhh.2009.70

[102] Daenen K, Andries A, Mekahli D, Van Schepdael A, Jouret F, Bammens B. Oxidative stress in chronic kidney disease. Pediatric nephrology. 2019 Jun 1;34:975-91. DOI: 10.1007/s00467-018-4005-4

[103] Cobley JN, Fiorello ML, Bailey DM. 13 reasons why the brain is susceptible to oxidative stress. Redox biology. 2018 May 1;15:490-503. DOI: 10.1016/j.redox.2018.01.008

[104] Pain G. Mechanisms of Fluoride Neurotoxicity A quick guide to the literature. Appl Sci. 2017;10:1-24.

[105] Ghosh D, Ghosh S. Fluoride and brain: A review. Int. J. Pharm. Sci. Res. 2020;11:2011-7.

[106] Shen L, Feng C, Xia S, Wei Y, Zhang H, Zhao D, Yao F, Liu X, Zhao Y, Zhang H. Progressive research in the molecular mechanisms of chronic fluorosis. In: Environmental Chemistry and Recent Pollution Control Approaches 2019 Feb 22 (p. 41). IntechOpen.

[107] Dec K, Łukomska A, Maciejewska D, Jakubczyk K, Baranowska-Bosiacka I, Chlubek D, Wąsik A, Gutowska I. The influence of fluorine on the disturbances of homeostasis in the central nervous system. Biological trace element research. 2017 Jun;177:224-34. DOI: 10.1007/s12011-016-0871-4

[108] Valdez-Jiménez L, Fregozo CS, Beltrán MM, Coronado OG, Vega MP. Effects of the fluoride on the central nervous system. Neurología (English Edition). 2011 Jan 1;26(5):297-300. DOI: 10.1016/S2173-5808(11)70062-1

[109] Ferreira MK, Aragão WA, Bittencourt LO, Puty B, Dionizio A, de Souza MP, Buzalaf MA, de Oliveira EH, Crespo-Lopez ME, Lima RR. Fluoride exposure during pregnancy and lactation triggers oxidative stress and molecular changes in hippocampus of offspring rats. Ecotoxicology and environmental safety. 2021 Jan 15;208:111437. DOI: 10.1016/j.ecoenv.2020.111437

[110] Li Y, Berenji GR, Shaba WF, Tafti B, Yevdayev E, Dadparvar S. Association of vascular fluoride uptake with vascular calcification and coronary artery disease. Nuclear medicine communications. 2012 Jan 1;33(1):14-20. DOI: 10.1097/MNM.0b013e32834c187e

[111] Liu H, Gao Y, Sun L, Li M, Li B, Sun D. Assessment of relationship on excess fluoride intake from drinking water and carotid atherosclerosis development in adults in fluoride endemic areas, China. International journal of hygiene and environmental health. 2014 Mar 1;217(2-3):413-20. DOI: 10.1016/j.ijheh.2013.08.001

[112] Korotenko OY, Panev NI, Zakharenkov VV, Filimonov SN, Semenova EA, Panev RN. Chronic fluoride intoxication as a risk factor for the development of atherosclerosis. Gigiena i Sanitariia. 2015 Sep 1;94(5):91-4.

[113] Li M, Zhao Y, Tian X, Liu P, Xie J, Dong N, Feng J, Gao Y, Fan Y, Qiu Y, Tian F. Fluoride exposure and blood pressure: a systematic review and meta-analysis. Biological Trace Element Research. 2021 Mar;199:925-34. DOI: 10.1007/s12011-020-02232-6

[114] Sharma P, Verma PK, Sood S, Singh M, Verma D. Impact of chronic sodium fluoride toxicity on antioxidant capacity, biochemical parameters, and histomorphology in cardiac, hepatic, and renal tissues of wistar rats. Biological Trace Element Research. 2023 Jan;201(1):229-41. DOI: 10.1007/s12011-022-03113-w

[115] Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: still a therapeutic target?. Nutrients. 2019 Sep 4;11(9):2090. DOI: 10.3390/nu11092090

[116] Delles C, Zimmerli LU, McGrane DJ, Koh-Tan CH, Pathi VL, McKay AJ, Steedman T, Dargie HJ, Hamilton CA, Dominiczak AF. Vascular stiffness is related to superoxide generation in the vessel wall. Journal of hypertension. 2008 May 1;26(5):946-55. DOI: 10.1097/HJH.0b013e3282f7677c

[117] Noma K, Goto C, Nishioka K, Jitsuiki D, Umemura T, Ueda K, Kimura M, Nakagawa K, Oshima T, Chayama K, Yoshizumi M. Roles of rho-associated kinase and oxidative stress in the pathogenesis of aortic stiffness. Journal of the American College of Cardiology. 2007 Feb 13;49(6):698-705. DOI: 10.1016/j.jacc.2006.06.082

[118] Basha MP, Saumya SM. Influence of fluoride on streptozotocin induced diabetic nephrotoxicity in mice: Protective role of Asian ginseng (Panax ginseng) & banaba (Lagerstroemia speciosa) on mitochondrial oxidative stress. The Indian Journal of Medical Research. 2013 Feb;137(2):370-9.

[119] García-Montalvo EA, Reyes-Pérez H, Del Razo LM. Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress. Toxicology. 2009 Sep 19;263(2-3):75-83. DOI: 10.1016/j.tox.2009.06.008

[120] Chiba FY, Colombo NH, Shirakashi DJ, da Silva VC, Moimaz SA, Garbin CA, Antoniali C, Sumida DH. NaF treatment increases TNF-α and resistin concentrations and reduces insulin signal in rats. Journal of Fluorine Chemistry. 2012 Apr 1;136:3-7. DOI: 10.1016/j.jfluchem.2011.12.006

[121] Malin AJ, Riddell J, McCague H, Till C. Fluoride exposure and thyroid function among adults living in Canada: Effect modification by iodine status. Environment International. 2018 Dec 1;121:667-74. DOI: 10.1016/j.envint.2018.09.026

[122] Kheradpisheh Z, Mirzaei M, Mahvi AH, Mokhtari M, Azizi R, Fallahzadeh H, Ehrampoush MH. Impact of drinking water fluoride on human thyroid hormones: a case-control study. Scientific reports. 2018 Feb 8;8(1):2674. DOI: 10.1038/s41598-018-20696-4

[123] Fredstrom S. Nitric oxide, oxidative stress, and dietary antioxidants. Nutrition. 2002;6(18):537-9.

[124] Bergandi L, Aina V, Malavasi G, Morterra C, Ghigo D. The toxic effect of fluoride on MG-63 osteoblast cells is also dependent on the production of nitric oxide. Chemico-biological interactions. 2011 Apr 25;190(2-3):179-86. DOI: 10.1016/j.cbi.2011.02.003

[125] Huang Y, Sun M, Li F, Li H, Jiang Z. Preliminary study of mechanisms of fluoride-induced suppression of nitric oxide synthesis in human umbilical vein endothelial cells. Biological trace element research. 2018 Oct;185:311-5. DOI: 10.1007/s12011-018-1252-y

[126] Levine AB, Punihaole D, Levine TB. Characterization of the role of nitric oxide and its clinical applications. Cardiology. 2012 Jun 19;122(1):55-68. DOI: 10.1159/000338150

[127] Dalvi SM, Patil VW, Ramraje NN. The roles of glutathione, glutathione peroxidase, glutathione reductase and the carbonyl protein in pulmonary and extra pulmonary tuberculosis. Journal of clinical and diagnostic research: JCDR. 2012 Nov;6(9):1462-5. DOI: 10.7860/JCDR/2012/4410.2533

[128] Marín-García J. Oxidative stress and cell death in cardiovascular disease: a post-genomic appraisal. Post-Genomic Cardiology, JM Garcia, Ed. 2014:471-98. DOI: 10.1016/B978-0-12-404599-6.00014-7

[129] Sharma R, Yang Y, Sharma A, Awasthi S, Awasthi YC. Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. Antioxidants and Redox Signaling. 2004 Apr 1;6(2):289-300. DOI: 10.1089/152308604322899350

[130] Chaithra B, Sarjan HN, Shivabasavaiah. Dose and time-dependent effects of sodium fluoride on sperm motility: An in vitro study. Toxicology and Industrial Health. 2018 Dec;34(12):813-8. DOI: 10.1177/0748233718795926

[131] da Silva Pereira HA, Dionizio AS, Araujo TT, da Silva Fernandes M, Iano FG, Buzalaf MA. Proposed mechanism for understanding the dose-and time-dependency of the effects of fluoride in the liver. Toxicology and applied pharmacology. 2018 Nov 1;358:68-75. DOI: 10.1016/j.taap.2018.09.010

[132] Kobayashi CA, Leite AL, Peres-Buzalaf C, Carvalho JG, Whitford GM, Everett ET, Siqueira WL, Buzalaf MA. Bone response to fluoride exposure is influenced by genetics. PloS one. 2014 Dec 11;9(12):e114343. DOI: 10.1371/journal.pone.0114343

[133] Everett ET, McHenry MA, Reynolds N, Eggertsson H, Sullivan J, Kantmann C, Martinez-Mier EA, Warrick JM, Stookey GK. Dental fluorosis: variability among different inbred mouse strains. Journal of dental research. 2002 Nov;81(11):794-8. DOI: 10.1177/0810794

[134] Katsura I. In search of new mutants in cell-signaling systems of the nematode Caenorhabditis elegans. Genetica. 1993 Jun;88:137-46. DOI: 10.1007/BF02424470

[135] Dote T, Kono K, Usuda K, Nishiura H, Tagawa T, Miyata K, Shimahara M, Hashiguchi N, Senda J, Tanaka Y. Toxicokinetics of intravenous fluoride in rats with renal damage caused by high-dose fluoride exposure. International archives of occupational and environmental health. 2000 Jul;73:S90-2. DOI: 10.1007/PL00014633

[136] Fluoride Action Network. Kidney: A potential target for fluoride toxicity [Internet]. 2012 [cited 2021 Dec 29]. Available from: https://fluoridealert.org/ studies/kidney06/