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Original article

Systematic review and meta-analysis of anti-hyperglycaemic effects of Pu-erh tea

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Summary Pu-erh tea was presumed to have anti-hyperglycaemic effects with limited evidence. This study uses meta-analysis to investigate anti-hyperglycaemic effect of Pu-erh tea. Five English databases and three Chinese ones were systematically searched up to July 31, 2018. Those databases were searched to identify studies containing keywords of 'Pu-erh', 'Pu'er', 'blood sugar', 'blood glucose' and 'hyperglycaemia'. RevMan 5 and Stata were then utilized to conduct meta-analysis. Systematic reviews collected two mice studies with sixteen records for meta-analysis. Meta-analysis results showed that Pu-erh tea has significant anti-hyperglycaemic effect on mice. Pooled weight mean difference of blood sugar on mice studies were 71 and 116 mg dL^{-1} at 21st day and 28th day respectively. Meta-regression disclosed over a longer intervention period showed that Pu-erh tea can reduce fasting blood glucose. Secondly, a higher dose of Pu-erh is shown to lower fasting blood glucose more significantly.

Keywords Blood sugar, meta-analysis, Pu-erh tea, systematic review.

Introduction

Tea drinking has long been part of Chinese culture. Tea was believed to have a multitude of benefits on health. Green tea, black tea or oolong tea is shown to be good for rats with high fructose diet. These teas can ameliorate plasma triglyceride and total cholesterol concentration. Extracts of Pu-erh tea can significantly lower plasma concentrations of glucose, insulin, triglycerides and free fatty acids (Yang *et al.*, 2001; Wu *et al.*, 2004; Hsu, 2009; Yamashita *et al.*, 2012). In recent years, many people drink Pu-erh tea because of its potential health benefits. Recent studies have confirmed that catechins, caffeine, polyphenols, amino acids and polysaccharides in extracts of Pu-erh tea have beneficial effect on the *in vivo* glucose homeostasis for people with type 2 diabetes (Du *et al.*, 2012). The carbohydrates derived from Pu-erh tea polysaccharides (PTPS) can inhibit alpha-glucosidase (Deng *et al.*, 2015). Some studies have shown that Pu-erh tea can even take up the roles as a new natural neuroprotective agent via a biological compound that binds with transcription factors so as to inhibit metabotropic glutamate Receptor 5 (mGluR5) to protect neurons from glutamate (Li *et al.*, 2017). To provide diabetes patients with better choices on controlling their blood sugar, researchers need to understand the effect of Pu-erh tea on regulating diabetes mellitus. This study has investigated the effects of Pu-erh tea on lowering blood glucose via both a systematic literature review and a meta-analysis method to analyse all relevant researches. This study also attempted to make comprehensive conclusions to the effectiveness of Pu-reh tea on lowering blood sugar.

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Pu-erh tea has long been considered as a health beverage. 'Compendium of Materia Medica' of 1596 A.D. in China has recorded below passages: 'Pu-erh tea bitter moment, the solution for oily cattle and sheep poisoning, phlegm, including Tong vent, Pu-erh tea paste black paint, sober first, the green is better, digestion Sputum, stomach and fluid.'

Some studies have investigated the effect of Pu-erh tea on physical health using scientific methods. Seven-week-old SD rats were used as study samples, The study showed that Pu-erh tea can prevent hyperlipidaemia of SD rats on high-fat diet, while the cooked tea has an even more significant effect (Xu *et al*., 2015). Pu-erh tea protects the vascular endothelium, with possible mechanism related to its antioxidant, PGI2 (Prostaglandin I2) synthesis and the inhibition of TXA2 (Thromboxane A2) synthesis. Li explored the protective mechanism of Pu-erh tea on nervous system (Li *et al*., 2017). Pu-erh tea contains biological compounds that bind with transcription factors and subsequently inhibit mGluR5 (mluR5) expression. Inhibition of mGluR5 can lead to nerve cells protection. As such, Pu-erh tea can be regarded as a new natural neuroprotective agent. The effective components of Pu-erh tea include tea polysaccharide, tea-browning, tea polyphenols, Gallic acid and trace elements (Hsu, 2009).

Method

Systemic literature review and meta-analysis were conducted to investigate the effect of Pu-erh tea on reducing blood glucose and other related factors.

Information sources and searches

Eight bibliographic databases were searched to include PubMed, EBSCO, SCOPUS, Cochrane Library, Web of science, Airiti Library, CKND (China Known Network Database) and Google Scholar. Chinese literatures on Airiti Library and China Knowledge Network database were also searched. The study used Google Scholar to search other studies and restricted search criteria on publications in English or Chinese only.

Three independent reviewers (Lee CT, Yen YY and Lin HC) have examined and confirmed the adequacy of literatures searched from these bibliographies database, using keywords of (Pu-erh OR Pu'er OR Pu-erh*) AND (blood sugar* OR blood glucose OR hyperglycemia).

Study selection

Firstly, the authors of this study have unanimously determined that only articles meeting inclusion criteria as defined by PICO principle (population, interventions, compare and outcome) will be selected and included in the studies. Secondly, based upon search criteria of research topic and abstract, obviously irrelevant studies were initially filtered out. Investigators then examined full-text articles and abstracts determined to be compliant with inclusion criteria. Thirdly, searched trials were considered fully eligible only when specific research has allocated mice to a Pu-erh tea intervention versus a control group. Additionally, the study has further established excluding criteria that screen out review articles, duplicate articles, non-full text, articles without data of blood glucose changes and non-animal trial studies from being included in meta-analysis. Outcome from systemic review and meta-analysis of this study has supported that Pu-erh tea intervention can lead to decreases in blood sugar levels.

Data analysis

Data extraction

Eligible articles of this study were primarily interventional researches with statistical data recording before and after blood glucose changes in both experimental group and control group. As some studies showed research results only graphically and due to the way the results were presented by related literatures, our study has obtained the results in two ways as shown below:

Firstly, for studies with average blood glucose and standard deviation values of before and after the intervention, we calculated the pooled standard deviation and the difference in average blood glucose value, as shown by below formula:

$$d_E = \bar{X}_{E\_\mathrm{pos}} - \bar{X}_E$$

$$S_{E\_\mathrm{dif}} = \sqrt{\frac{(N_{E\_\mathrm{pos}}-1)(SD_{E\_\mathrm{pos}})^2 + (N_{E\_\mathrm{pre}}-1)(SD_{E\_\mathrm{pre}})^2}{(N_{E\_\mathrm{pos}}-1)(N_{E\_\mathrm{pre}}-1)}}$$

Where d_E is the average difference in the blood glucose value between before and after Pu-erh tea intervention in the trial group; and S_{E_dif} is the standard deviation of the average value of blood glucose before and after the experiment.

Secondly, for those studies where blood glucose data were only graphically represented, the author used Screen Ruler Pro program tools (http://www.wonderwebware.com/screen-ruler-pro/) to measure average values of blood glucose and associated standard deviation.

The effect size

In the past, results of consolidation analysis were mainly expressed by a significant level combination method. Some scholars argued that the effect size (ES) in those studies should be analysed to understand the

size and significance of impact differences (Glass, 1976). Meta-analysis takes two additional steps than primary study, namely clarifying research methods first and then quantifying results of studies. By following these two additional steps, standard common unit or 'ES' between different studies can be determined accordingly. After calculating respective ES in each study, we subsequently determined the 'overall average ES' to validate the intervention effect. Because most studies used different measurement tools or scales, the effect of averaging differences needed to be standardised. In case if all studies used the same measurement tools or scales, then the mean difference can be used as an effective surrogate of consolidation analysis value, rendering standardisation processes unnecessary.

Risk bias and quality assessment

Two authors (Chang Lee SN and Lan SJ) assessed studies on the presence of a high, low or unclear risk of bias. The Cochrane's risk of bias table was used to determine the methodological quality of each trial (Green, 2017). A total of seven potential risks of bias were evaluated: random sequence generation, allocation concealment, blinding of participants, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. Studies involving three or more high risks of bias were considered as poor methodological quality (Figs 1 and 2).

Analysis

This study used Stata version 13 (Stata Corp LP, College Station, TX, USA) and Review Manager(RevMan) version 5.3 (The Cochrane Collaboration 2014, NordicCochrane Centre Copenhagen, Denmark) in analysing those studies by a random effect meta-analysis for high heterogeneity. SMD and 95% confidence intervals (CIs) were used as ES measurement. Researchers then used Forest Plot to describe the weighted mean difference and 95% Confidence Interval (95% CI) and simultaneously compiled the statistic report of Begg's test. Meta-regression was consequently used to evaluate the effect of intervention period (time) and dose.

Results

Search results

A total of 132 studies were identified in the first stage of the literature search. Among the forty-one articles written in English, thirteen studies were from PubMed dataset, six from EBSCO database, thirteen from SCOPUS dataset, one from Cochrane Library and eight from Web of Science. As for the remaining eligible ninety-one articles written in Chinese, forty-three articles were from Airiti Library, twenty-three from China Known Network database and

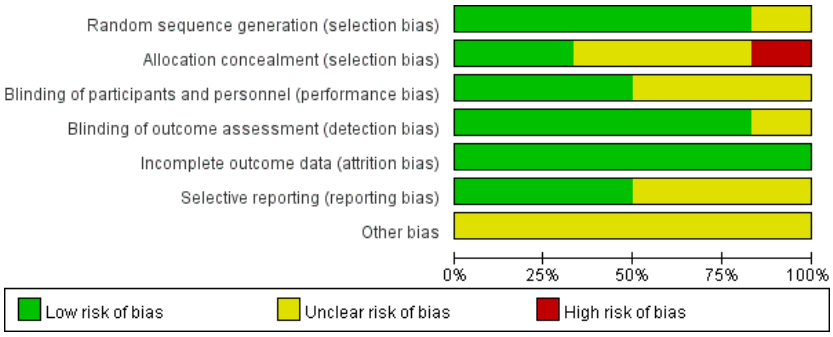

Figure 1 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies. Green for low risk of bias, yellow for unclear risk of bias and red for high risk of bias.

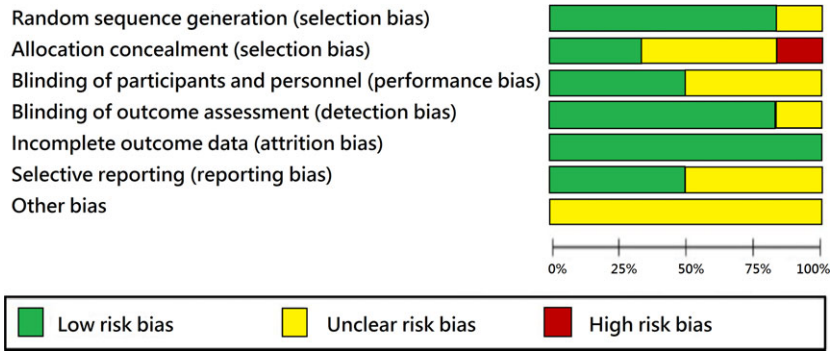

Figure 2 Risk of bias summary: review authors' judgements about each risk of bias item for each included study. Green for low risk of bias, yellow for unclear risk of bias and red for high risk of bias.

twenty-five from Google Scholar. The second stage of search was designed to screen out duplicates, reviewing articles and those unsuitable for meta-analysis. A total of eighteen articles were identified as potentially relevant as a result of the second stage search. During the subsequent full-text review stage (the third stage), all eighteen articles deemed potentially relevant were closely examined by researchers, resulting in ten articles being screened out for not meeting PICO criteria (Fig. 3). Of the remaining eight articles left after aforementioned screening rounds, only two articles were determined to have met the criteria for meta-analysis (Table 1).

A systematic review of the literature

Main observation indexes include types of animals, gender, quantity, methods of interventions, time and body weight (Table 1). After intervention of Pu-erh tea, most studies have shown significant level of blood sugar decrease. One study has focused specifically on the effect of Pu-erh tea on streptozotocin (STZ), substance responsible for inducing diabetes mellitus in rats with blood glucose and lipid conditions. Results of the abovementioned study have shown that Pu-erh tea extract can significantly lower the concentrations of plasma glucose, insulin, triglycerides and free fatty acid, and in turn significantly ameliorate clinical outcomes for subjects in the treatment group (Hsu, 2009). Study by Zhou *et al*. (2009) have also demonstrated that polysaccharide in Pu-erh can significantly reduce blood glucose level of diabetic mice (Zhou *et al*., 2009). Zhou, Kong and Chen have further concluded that, in terms of efficacy in reducing blood sugar, a high dose of Pu-erh tea polysaccharide (160 mg kg^{-1}) was more potent than that of a low dose (80 mg kg^{-1}). Results from study using 8-week-old Wistar spontaneous mutant rats as sample have also shown that Pu-erh tea extract contains catechins, caffeine, polyphenols, amino acids and polysaccharides, ingredients capable of maintaining glucose homeostasis for type 2 diabetes patients (Du *et al*., 2012). Deng *et al*. (2015) used 4-week-old ICR (Institute of Cancer Research) mice to examine the mechanism of PTPS on reducing blood glucose. Results discovered by Deng *et al*. has indicated that carbohydrates in Pu-erh tea polysaccharide can reduce blood glucose via α-glucosidase inhibition, while the inhibition effect on α-amylase was found to be less significant. As part of results derived from their study, Deng *et al*. further concluded that PTPS of Pu-erh tea have a more potent hyperglycaemic effect than acarbose (Deng *et al*., 2015).

Meta-analysis

The authors used data derived from two articles (Du *et al*., 2012; Li *et al*., 2014) to conduct meta-analysis. To better understand the effect of intervening durations, the study has divided the intervention periods into 7, 14, 21 and 28 days, respectively.

Heterogeneity test

A total of sixteen data of two articles were analysed. The RevMan5 software was used to examine whether the time-to-attendance was affected or whether it was heterogeneous (Fig. 4). The statistical results are

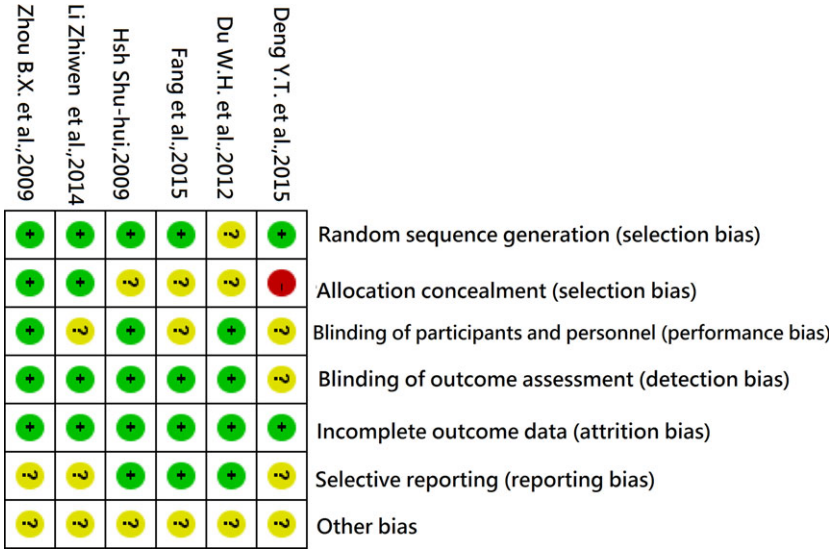

Figure 3 Flowchart of search results and article retrieval.

Table 1 System review results

Study	Animals	Experiment Subjects	Intervention	Experiment duration	Outcome
Cai *et al.* (2017) (Japan)	C57BL/6J male mice, 8 weeks old	Divided into four groups and fed either a normal diet or a high fat diet, with or without 5 mg mL^{-1} Pu-erh tea in the drinking water for 16 weeks	5 mg mL^{-1} Pu-erh tea in the drinking water	16 weeks	Pu'erh tea extract significantly reduced the gain of body weight and subcutaneous adipose tissue(SAT), but not visceral adipose tissue(VAT) adiposity, in mice fed the high-fat diet and induced adipogenesis in VAT. The expression of DNL-related genes, including Glut4, encoding an important insulin-regulated glucose transporter (GLUT4), were highly elevated in VAT. The expression of DNL-related genes in VAT was inversely correlated with hepatosteatosis and systemic insulin resistance.
Deng *et al.* (2015) (Taiwan)	ICR (Institute of Cancer Research) mice(4 weeks old) 18–20 g/Male	Randomly divided into 4 groups of 5	acarbose (5 mg kg^{-1}) TPS of 5-year old Pu-erh tea (1 mg kg^{-1}) TPS of 5-year old Pu-erh tea (5 mg kg^{-1}) Tea extraction acarbose (5 mg kg^{-1}) TPS of 5-year old Pu-erh tea (1 mg kg^{-1}) TPS of 5-year old Pu-erh tea (5 mg kg^{-1})	Blood glucose levels were measured at 0, 0.5, 1, 2 and 3 h	The carbohydrates found in Pu-erh tea polysaccharide (PTPS) inhibit α-glucosidase but have a less inhibitory effect on α-amylase. Mice experiments show that tea polyphenols of PTPS may be better than acarbose on inhabiting blood sugar. It inhibits α-glucosidase, and has a smaller inhibition on α-amylase. It also has fewer side effects.
Du *et al.* (2012) (China)	Spontaneous mutation Wistar rat (8 weeks old)/Male	48 obese diabetic rats and eight non-diabetic rats were randomly divided into six groups	The water extraction and composition of Pu-erh tea: 100 mg kg^{-1} 200 mg kg^{-1} 400 mg kg^{-1} acarbose: 10 mg kg^{-1} rosiglitazone(Avandia):5 mg kg^{-1}	4 weeks	Catechins, caffeine, polyphenols, amino acids and polysaccharides in Pu-erh tea extracts have beneficial effects on glucose homeostasis in type 2 diabetes and in modification of balancing glucose in rats. Pu-erh tea extract inhibits intestinal intestine sucrose, maltase and pancreatic amylase activity in rats. After 4 weeks gavaging of Pu-erh extract, rats had impaired glucose tolerance and improved relief of insulin response. Pu-erh extract has an effect on glucose homeostasis in type 2 diabetes and improves insulin resistance.
Fang *et al.* (2015) (China)	SPF Balb/c rate (4–8 weeks old)/Male	A. Single dose: 48 mice were divided randomly into control group, Pu-erh tea group, green tea group, EGCG, EGCG + caffeine, caffeine B. long-term study: 32 mice were divided randomly into four groups, control, GTE, EGCG, or caffeine	A. Single dose: Pu-erh tea group: Pu-erh tea extract 800 mg kg^{-1} Green tea group: green tea extract 800 mg kg^{-1} EGCG group: epigallocatechin gallate 240 mg kg^{-1} EGCG + caffeine group: EGCG 240 mg kg^{-1}+ caffeine 80 mg kg^{-1} Caffeine group: caffeine 80 mg kg^{-1} B. long-term study: GTE group: green tea extract 5 mg mL^{-1}	A. 2 h B.4 weeks	A. Mice that were given gavage feeding which consumed Green Tea Extract (GTE), Pu-erh Tea Extract (PTE), or Caffeine showed a significant reduction in blood glucose concentration compared to the controlled group. B. Epigallocatechin gallate (EGCG) had no effect on hyperglycaemic activity. After removing caffeine from both GTE and PTE, these groups all lost their effect of reducing blood glucose. After re-adding caffeine, the authors discovered their ability to reduce blood glucose

Table 1 (*Continued*)

Study	Animals	Experiment Subjects	Intervention	Experiment duration	Outcome
			EGCG group: EGCG 1.5 mg mL^{-1} Caffeine group: caffeine 0.5 mg mL^{-1}		
Hsu (2009) (Taiwan)	Sprague Dawley (6 weeks old) SD rate	Two types of experiment: A. Prevention experiment: According to body weight randomly divided into 5 groups of 5. B. Treatment experiment: According to body weight randomly divided into 6 groups of 5.	A. Prevention experiment: Pu-erh tea extracts 0.1 g kg^{-1} B.W. B. Treatment experiment: Pu-erh tea extract 0 0.1 g kg^{-1} B.W 0.2 g kg^{-1} B.W.	A. Prevention experiment: 29 days. B. Treatment experiment: 22 days.	Prevention experiment: the addition of Pu-erh tea extracts before and after evoking diabetes have significantly effect in lower the level of plasma glucose, insulin, triglyceride and free fatty acid. Treatment experiment: the addition of Pu-erh tea extract can significantly improve the clinical symptoms and adipose tissue weight in the treatment group.
Li *et al.* (2014) (China)	C57BL/6J mice, Hereditary diabetic mice: KKAy mice, 70 female, 5–6 weeks old, and Diabetic model by STZ: 70 SD (Sprague-Dawley) male rats, female, 8–10 weeks old	A. Hereditary diabetic experiment: (KKAy) mice 70 KKAy with fasting blood glucose >16 mmol L^{-1} were selected as the experiment samples, were randomly divided into four groups of 15. Four groups: model group, positive drug group, Pu-erh tea group, and green tea group. C57BL/6J mice (n = 11) served as control group. B. Diabetic model by STZ: 70 SD male rats were randomly divided into a normal control group and a diabetic model group. 10 normal control groups and 60 diabetic model groups. The diabetic model was divided into five groups: a positive group, a Pu-erh tea extract group, a green tea extract group, 12 in each group.	A. Hereditary diabetic experiment: a. Model group: distilled water 10 mL kg^{-1} day^{-1} b. Positive drug group: 1.33 mg kg^{-1} day^{-1} c. Pu-erh tea group: Pu-erh tea extract 1.0 g kg^{-1} d. Green tea group: green tea extract 1.0 g kg^{-1} B. Diabetic model by STZ: a. Normal control group: distilled water 10 mL kg^{-1} day^{-1} b. Positive drug group: 1.33 mg kg^{-1} day^{-1} c. Pu-erh tea group: Pu-erh tea extract 1.0 g kg^{-1} d. Green tea group: green tea extract 1.0 g kg^{-1}	4 weeks	Pu-erh tea extract and green tea extract had an effect on lowering fasting blood glucose (FBG) in both KKAy mice and STZ (streptozocin-diabetic) rats. At the same time, both of them reduced the area under the blood glucose curve, but the difference is that the effect of Pu-erh tea extract is superior to green tea extract. While Pu-erh tea extract significantly reduced the fasting serum insulin in the model group. Green tea extract did not significantly make any difference compared to the model group.
Yamashita *et al.* (2012) (Japan)	Male ICR mice, 6 weeks old	The mice divided into four groups to receive oolong tea, black tea, Pu-erh tea, or water	2 g of oolong, black, and Pu-erh tea leaves was extracted in boiled water for 2 min	7 days	Intake of oolong, black, or Pu-erh tea for 7 days enhanced GLUT4 translocation to the plasma membrane of skeletal muscle. Each type of fermented tea stimulated the phosphorylation of phosphoinositide 3-kinase (PI3K), Akt/protein kinase B, and AMP-activated protein kinase (AMPK). Fermented tea also increased the

Table 1 (*Continued*)

Study	Animals	Experiment Subjects	Intervention	Experiment duration	Outcome
					protein expression of insulin receptor. These results strongly suggest that fermented tea activates both PI3K/Akt- and AMPK-dependent signalling pathways to induce GLUT4 translocation and increases the expression of insulin receptor to improve glucose intolerance.
Zhou *et al.* (2009) (China)	Diabetes mellitus mice/ (20 ± 2 g)	The mice divided into four groups: normal control group, diabetes mellitus model for the control group, low dose group, high dose group, six mice in each group	Pu-erh tea extract tea polysaccharides High dose 160 mg kg^{-1} Low dose 80 mg kg^{-1}	3 weeks	Pu-erh tea polysaccharide promoted the recovery of body weight of diabetic mice, but there was not a significant difference in the weight of the mice. Pu-erh tea polysaccharide effectively reduced the blood sugar level in diabetic mice. The high dose of Pu-erh tea polysaccharide had a better hypoglycaemic effect than the low dose of Pu-erh tea polysaccharide, and the statistical difference was significant ($P < 0.05$). The results showed a relationship between dose and response.

highly heterogeneous, and the results indicate that Pu-erh tea has a statistically significant effect on lowering blood glucose.

Meta-analysis results

A total of sixteen data sets from two articles of literatures were included (forty in experimental group and twenty in controlled group). The follow-up time points were 7, 14, 28 days and up. Subsequent population meta-analysis has shown that there was a high heterogeneity of the literature (I^2 = 91.9%, $P < 0.01$). Using random effect model analysis, diabetic mice were continuously fed with Pu-erh tea. After 7, 14, 21 and 28 days, fasting blood glucose was observed to decrease by 0.83 (95% CI: 0.27–1.62), 3.52 (95% CI: −2.80 to 9.84), 3.94 (95% CI: 0.71–7.16) and 6.43 (95% CI: 3.07–9.78) respectively. The study has found that the statistically significant effect of Pu-erh tea on decreasing blood glucose after 21 and 28 consecutive days is equivalent to a decrease in mean fasting glucose of 71 mg dL^{-1} (95% CI: 13–129) and of 116 mg dL^{-1} (95% CI: 55–179) respectively. There was no post error (the *P*-value of Begg's test is = 0.499; Fig. 1).

Meta-regression result

Numerical moderator analysis was used for two variables: intervention period and dose. Per data derived from meta-regression the study has discovered that the longer the intervention period, the more significant effect of reduced fasting blood glucose level (slope: −0.088; 95% CI: −0.142~ −0.0347; $P < 0.05$). Similarly, this study has also revealed that the bigger the dose of Pu-erh, the more significant effect of reduced fasting blood glucose (slope: −0.002; 95% CI: −0.003~ −0.001; $P < 0.05$) (Fig. 5; Table 2).

Other biomarkers results of the two papers that met meta-regression criteria

Du *et al.* (2012) also found that fasting blood insulin levels of diabetic db/db mice given 400 mg kg^{-1} Pu-erh tea (32 mIU mL^{-1}) were lower than those of type 2 diabetic db/db mice (53mIU mL^{-1}) on day 28. Furthermore, the increased blood glucose values of db/db mice given 400 mg kg^{-1} Pu-erh were lower than those of the type 2 diabetic db/db mice group at 1 and 3 h post-OGTT. Li *et al.* (2014) also indicated that fasting blood insulin levels in diabetic mice (34 IU mL^{-1}), given 1000 mg kg^{-1}, The sample group given Pu-erh tea were lower than those (41 IU mL^{-1}) group without Pu-erh tea. These two papers have shown that Pu-erh tea can decrease fasting blood insulin of diabetic mice.

Discussion

A total of eighteen articles (seven in English and the other eleven in Chinese) of literatures were collected

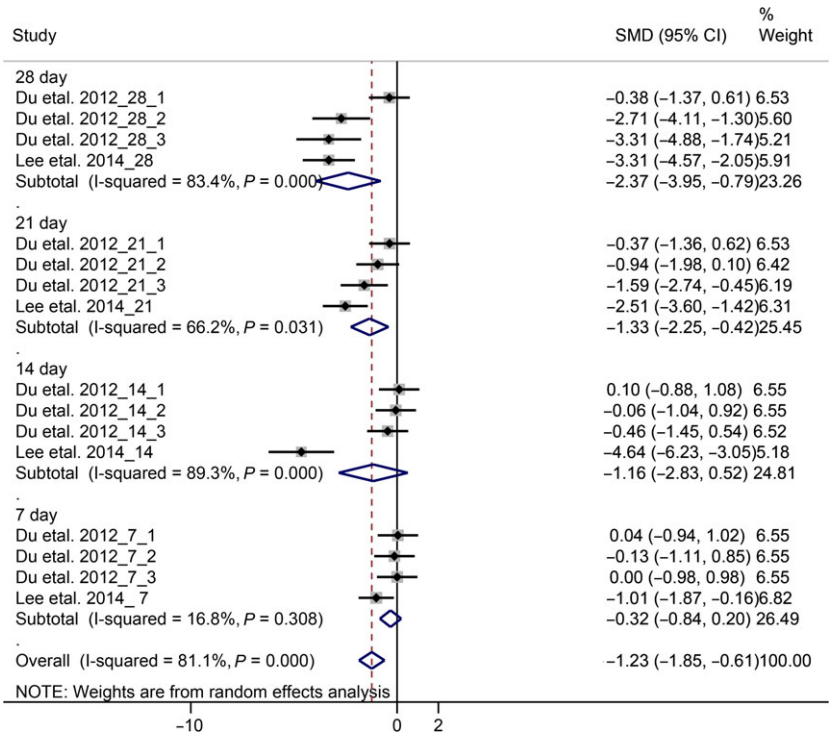

Figure 4 Forest plot of lower blood sugar involved in the timing of mouse trials. The overall I^2 :81.1%.

and included in this study. Among the eighteen articles collected, only two were determined by researchers as adequate for meta-analysis, given that the two studies were pre and post-test designed with 7, 14, 21 and 28 days tracking time. The researchers have conducted meta-analysis and subsequently concluded that Pu-erh tea can effectively control blood sugar in diabetes, based upon data derived from those meta-analyses.

There are four articles related to cell line experiments. Results from those four articles have generally supported that Pu-erh tea extract can significantly lower blood sugar. Consequently, Pu-erh tea can be used as one effective alternative for controlling blood sugar and diabetes. Some studies have focused on the effect of Pu-erh tea ingredients on decreasing both blood glucose and lipid. The results have shown that uracil, gallic acid in peroxisome proliferator-activated receptor gamma (PPARγ), farnesoid X receptor (FXR), liver X receptor (LiXR) each has a noticeable lipid-lowering effect, with gallic acid on PPARγ activation having an effect similar to the positive control agonistic effect of drugs. Regarding hypoglycaemic effect, ethanol extract of Pu-erh tea had the highest hypoglycaemic activity (Zhang *et al.*, 2006, 2009). In recent years, study scope of Pu-erh tea has been shifted from Pu-erh tea types and extraction methods towards the effect of specific Pu-erh tea ingredients. Liu (2013) indicated the mechanism of Pu-erh tea polysaccharide in promoting 3T3-L1 adipocyte differentiation and glucose intake (Liu, 2013). Using molecular biology to investigate the treatment of diabetes, the results showed that Pu-erh polysaccharides simulate PPARγ and glucose transporter type 4 (GLUT4) protein performance to promote adipocyte differentiation and glucose intake. This mechanism improves insulin resistance efficiency and hypoglycaemic effect. The other hypoglycaemic mechanism of PTPS may involve the regulation of PIP3/Akt signal pathway. PTPS was founded to enhance the expression of PI3Kp85/p-Akt/GLUT4 in type 2 Diabetes mellitus mice (Li *et al.*, 2015). The intake in those Diabetes mellitus mice was administered orally with PTPS being dissolved in normal saline at the dose of 200, 400, 800 mg kg^{-1} body weight per day for 28 days. The expression of PI3Kp85, p-Akt and GLUT4 increased with the dose-dependent effect. With the increase of PTPS, glucose level has in turn decreased. Another study indicated that polyphenol-rich and caffeine-rich Pu-erh tea improved diet-induced metabolic syndrome. This effect was likely associated with the remodelling of gut microbiota (Gao *et al.*, 2018).

Regarding results from the systematic review, a majority of the literatures reviewed have shown that Pu-erh tea makes a significant difference in hypoglycaemic effect. Different experiment designs such as intervention concentration of PTPS, intervention time, among other factors will nevertheless affect respective final results of the study. Furthermore, we

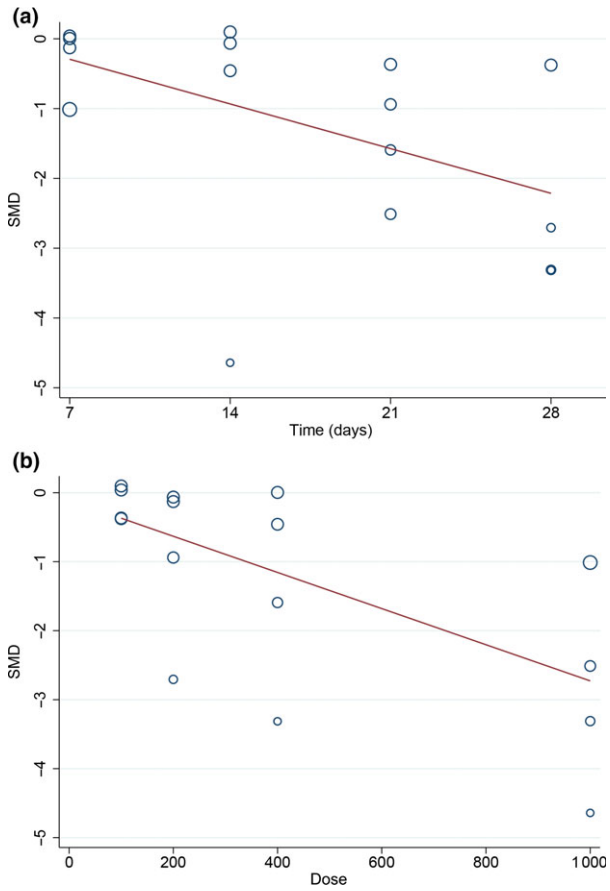

Figure 5 Meta-regression for intervention period (a) and dose (b). (a) Longer intervention period, the effect of reduced fasting blood glucose (slope: −0.088; 95% CI: −0.142~ −0.0347; $P < 0.05$). SMD, standardised mean difference; Intervention periods (time: days). (b) Higher dose of Pu-erh, the effect of reduced fasting blood glucose (slope: −0.002; 95% CI: −0.003~ −0.001; $P < 0.05$, Pu-erh tea dose unit: mg kg^{-1}). SMD: Standardised Mean Difference.

have also examined the effect of intervention duration on heterogeneity, with results showing that heterogeneity exists with different lengths of intervention time.

The results gleaned from meta-analysis have shown that the intake of Pu-erh tea in diabetic mice has a significant effect on lowering blood glucose after 21 days. Based upon conclusive findings derived from this research, the authors recommend with professional confidence that people drink Pu-erh tea for more than 3 weeks in order to maximise the effect on lowering blood sugar.

Conclusions

Research findings from articles selected for systemic review have generally supported that a number of ingredients extracted from Pu-erh tea have the health benefit of lowering blood sugar, with a few studies conducting experiments and validating the argument that drinking Pu-erh tea can help regulate and maintain adequate level of blood sugar. Unfortunately, none of those articles have identified specific ingredient (s) responsible for lowering hyperglycaemia and their actual mechanism(s).

Two factors have restricted the number of literatures selected and included in meta-analyses of our study. Firstly, the difference of Pu-erh tea extraction method and dose might be the important reason for the incongruous results of the Pu-erh tea effect on blood sugar. Inconsistent extraction method might result in different component and concentration in Pu-erh tea extraction. The sample group given low dose of Pu-erh tea extraction compared to the control group show non-significant effect, even the experiment through 1 month. Secondly, different animal model might show unequal response effect to Pu-erh tea extraction.

Via systemic literature reviews and subsequent meta-analyses, using data furnished by those selected articles, our research has validated the claimed health benefits of Pu-erh tea in decreasing blood sugar level with additional evidence-based information derived from statistical analyses. This study has further identified 'intervention concentration' and 'intervention time' as the two key factors in determining the efficacy of Pu-erh tea in decreasing hyperglycaemia. With real-world data from our study, the authors are pleased to validate the widely claimed health benefits of Pu-erh tea in regulating blood sugar.

As all existing researches published and accessible to us still fail to identify the exact ingredient(s) at work, their respective optimal intervention concentration and duration, regulating mechanisms and relationship or interactions with blood biomarkers such as insulin, the authors unanimously agree that more studies are required to explore those unanswered questions,

Table 2 Numerical moderator analysis was used to two variables, including intervention period and dose

_ES	Coef.	Std. Err.	*t*	*P*>|t|	95% Conf. Interval	
Intervention period (days)	0885874	0.0249096	−3.56	0.004	−0.1424013	−0.0347735
Dose (mg kg^{-1})	−0.0024948	0.0005599	−4.46	0.001	−0.0037043	−0.0012853
_cons	1.416394	0.5136038	2.76	0.016	0.3068203	2.525967

*Number of obs = 16 †τ^2 = 0.1734 ‡I-squared_res=43.37% §Adj R-squared=89.59%.

preferably in the forms of animal studies or human clinical trials. We would recommend that interested researchers may consider further studies on tea polysaccharide of Pu-erh tea and its role in lowering blood sugar.

Conflicts of interest

All the authors read and approved the final manuscript. There are no conflicts of interest to declare.

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Ethical approval

This review article did not require informed consent or approval by the ethics committee.

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