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The Anti-hepatotoxic Effect of Ginseng in Rats: Meta-analysis[1)]

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Abstract

The purpose of this meta-analysis was to investigate the anti-hepatotoxic effect of ginseng in rats induced with CC14 or TCDD, the toxicities that cause liver damages. Primary studies were collected from the ScienceDirect database, the DBpia, and the KISS. The data on the effect factors in plasma and in enzyme are listed as many as possible: The effect factors were alanine transaminase (ALT), aspartate transaminase (AST), liver aminopyrine N-demethylase (AD), liver aniline hydroxylase (AH), liver 3,4-Methylenedioxyamphetamine (liver MDA), cytochrome P450 (P450), serum alkaline phosphatase (ALP), serum lactate dehydrogenase (LDH), cytochrome b5 (Cyto b5), glutathione reductase (GR), Liver glutathione S-transferase (GST), liver glutamyltransferase (GT), Liver(ɣ-GCS), serum liver 3,4-Methylenedioxyamphetamine (serum MDA), serum sorbitol dehydrogenase (SDH), serum total protein (TP), and serum ɣ-glutamyltransferase (ɣ-GT). In order to investigate the effect of ginseng, the standard mean difference (HG) between the group of rats induced with toxicity (RH) and the group of rats induced with ginseng (RHG) were combined, and the significance of HGs were tested. The combined HGs checked the biases caused by heterogeneity among studies and the publication biases. Then they were adjusted by using the random effect model and trim and fill method. Although the publication biases were assumed, among all plasma factors the HGs of ALT, AST,

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serum MDA, SDH, TP, and γ-GT were significant, and among all enzyme factors the HGs of liver MDA, Cyto b5, GR, GST, and GT were significant. The treatment with ginseng significantly affected the plasma and enzyme levels in rats induced with toxicity.

Keywords: CCl4; Galactosamine; Hepatoprotective; Meta-analysis; Publication bias.

1. Introduction

Drug-induced liver injury is a common form of adverse effect of drug and accounts for more than 50% of cases of acute liver failure in the United States (Lee (2003)). More than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of fulminant hepatic failure. Drug-induced hepatic injury is the most common reason for the withdrawal of an approved drug. The range of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Medicinal herbs and extracts are widely used in the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite (Cupp (1999)). A number of recent reviews have focused on the adverse effects of herbal products (DeSmet et al. (1997)).

Ginseng, derived from the roots of several plants, is referred to one of herbal remedies. One of the most commonly used and studied of the ginseng is Panax ginseng, also known as Asian or Korean ginseng. The main active component of Panax ginseng is ginsenosides, which has many beneficial effects, such as anti-inflammatory, antioxidant, and anticancer effects, and they are also easily available in its concentration form (Kiefer et al. (2003)). Even though Panax ginseng appears to be well tolerated, a caution is advised about concomitant use with some pharmaceuticals, such as warfarin, oral hypoglycemic agents, insulin, and phenelzine.

The majority of published research on the medicinal effect of Panax ginseng has focused on ginsenosides (World Health Organization. (1999)), which are triterpene saponins. Panax ginseng is known to enhance the release of nitric oxide from endothelial cells in rat aorta and reduces blood pressure in animals (Han et al. (2005)).

Meta-analysis is a tool for summarizing the results of studies in related research hypotheses. Meta-analysis has three steps: deciding the effect measure for difference between groups, summarizing the effect measure, and detecting publication bias. The most often used measure in meta-analysis is the standardized measure of effect size gained from the primary researches. Such measures include the standardized mean difference, the risk difference, and an odds ratio. After deciding the effect measure, the effect measures are combined

and summarized under assumed model. It leads to more convincing conclusions than that obtained from individual studies, because it reduces the random errors in the assessment of treatment (Choi et al. (2007) and Sutton et al. (2000)).

The purpose of this study was to summarize the factors related to the anti-hepatotoxic effects of ginseng on rats induced by toxicity.

2. Methods

2.1 Preparation of dataset for meta-analysis

The studies used in this meta-analysis were found on the Science Direct in English, the DBpia database, and the KISS (Korean studies Information Service System) in Korean. The key words searched were ginseng, panax and hepatotoxicity. This research was limited to five experimental rat studies. From each study, we collected the data of two groups: a group of rats induced by hepatotoxicity such as CCl4 or TCDD (RH) and a group of rats induced by hepatotoxicity with ginseng (RHG). The factors chosen to research the effect of ginseng were the level of alanine transaminase (ALT), aspartate transaminase (AST), liver aminopyrine N-demethylase (AD), liver aniline hydroxylase (AH), liver 3, 4-Methylenedioxyamphetamine (liver MDA), cytochrome P450 (P450), serum alkaline phosphatase (ALP), serum lactate dehydrogenase (LDH), cytochrome b5 (Cyto b5), glutathione reductase (GR), Liver glutathione S-transferase (GST), liver glutamyltransferase (GT), Liver(γ-GCS), serum liver 3, 4-Methylenedioxyamphetamine (serum MDA), serum sorbitol dehydrogenase (SDH), serum total protein (TP), and serum γ-glutamyltransferase (γ-GT). The primary studies were listed as references. The unit of each factor was accordingly changed.

2.2 Measure and models for combining

The Hedge's G statistic (HG) is one method of the standard mean differences between two groups, and is defined as $HG=(\mu E - \mu C)/Sp$ when μE and μC are means of two groups and Sp2 is pooled sample variance. In small sample, the HG is adjusted to HG×coefficient due to the bias. In this study, HG was used as the effect size to measure mean difference about factors between two groups: the RH and RHG. The inverse variance method was used in two models: the fixed effect model and the random effect model. In the study of the fixed effect model, it is assumed that there is no heterogeneity between the study results and the effect measured in the study population has a single value. On the other hand, the studies of the random effect model are heterogeneous and it includes two sources of variation: variation between two studies and variation within the study. If the homogeneity assumption is plausible by a heterogeneity test, the effect size is

estimated by using a variation within the study in the fixed effect model. If not, a variation between studies is estimated and included in a total variation. The fixed effect estimates and the random effect estimates are presented in Table 1.

2.3 Identification of the statistical bias

Publication bias was assessed by using a funnel plot method. The HGs in our study, the calculated association measures of studies about the factor, are plotted against the inverse of the estimated standard error (SE). The results from smaller studies will be more widely spread around the mean effect due to larger random error. If there is no publication bias, the shape of plot is funnel or cone around the mean. To test the asymmetry of a funnel plot, Egger et al. suggested a linear regression test based on a regression analysis of Galbraith's radial plot (Choi et al. (2007) and Whitehead (2002)). Garbraith's radial plots were graphs marked the standardized effect sizes of outcome from each study against the inverse of standard error. The intercept of simple regression is used to measure asymmetry. If the estimated intercept is significantly different from 0, then there may be publication bias. A positive intercept indicates that more studies are associated with bigger effect. Results of Egger's linear regression test are presented in Table 2 about factors. The trim and fill method estimates combined effect sizes by 2-step process, in which the asymmetric data is trimmed and estimated. The trim and fill method is used to compare the original estimates.

3. Results

In Table 1, there are two estimates of combined HGs and three p-value of significant test about mean difference between two groups, RH and RHG, and homogeneity test of primary studies. Two combined HGs were caclulated by inverse variance weighted method on fixed effect model and on random effect model and each paired p-value was the significant probability about the hypothesis that mean difference between RH and RHG is zero. The rest p-value was the significant probability about the hypothesis that the primary studies were homogeneous.

The trim and fill funnel plots of effect factors in Fig. 1 were used to check the publication bias. In addition to funnel plots, the publication bias were checked by Egger's linear regression test in Table 2, and the combined HGs were recalculated by the trim and fill method on the fixed effect model and random effect model in Table 3.

3.1 Standard Means Difference in plasma factors

The studied plasma factors were ALT, AST, ALP, LDH, serum MDA, SDH, TP, and γ-GT. In the fixed effect model, the effect of ginseng on ALP and serum

MDA were significant (<0.05), and the others were very significant (<0.01). The p-values of heterogeneity test among studies on serum MDA, SDH and TP were more than 0.05, but the others were less. In random effect model, the effect of ginseng on ALP and serum MDA were significant (<0.05), and the others were very significant (<0.01).

The mean differences of ALT, AST, ALP, LDH, and γ-GT between RH and RHG were estimated by random effect model for the heterogeneity among studies. And the mean differences of serum MDA, SDH, and TP were estimated by fixed effect model. By the reasons mentioned above, the level of ALT, AST, ALP, LDH, serum MDA, SDH, and γ-GT in plasma were significantly reduced (<0.05), and the level of TP in plasma was increased by treatment with ginseng (<0.05).

Table 1. Combined HGs and Homogeneity test in fixed effect model and random effect model.

	Fixed effect estimate		Heterogeneity	Random effect estimate	
	Estimate	p-value	p-value	Estimate	p-value
ALT	-1.0960	<0.0001	<0.0001	-1.6956	0.0004
AST	-1.9977	<0.0001	<0.0001	-2.9684	<0.0001
ALP	-0.6405	0.0309	<0.0001	-2.5931	0.0302
LDH	-0.9721	<0.0001	<0.0001	-1.7967	0.0050
Serum MDA	-0.6185	0.0367	0.5602	-0.6185	0.0367
SDH	-4.3408	<0.0001	0.8544	-4.3408	<0.0001
TP	0.6944	0.0029	0.7363	0.6944	0.0029
γ-GT	-6.6157	<0.0001	0.0009	-9.8120	0.0011
AD	-1.0850	<0.0001	<0.0001	-2.2768	0.0026
AH	-0.5815	0.0052	0.0002	-0.7953	0.0605
Liver MDA	-2.2904	<0.0001	<0.0001	-3.2861	<0.0001
P450	-0.8009	0.0015	<0.0001	-2.0978	0.0121
Cyto b5	-4.9826	<0.0001	<0.0001	-6.8570	0.0001
GR	4.8851	<0.0001	0.2848	4.9795	<0.0001
GST	2.8763	<0.0001	0.0606	3.1291	<0.0001
GT	4.2373	<0.0001	0.0086	5.0031	<0.0001
γ-GCS	0.8249	0.0028	0.0444	0.9280	0.0421

The data were expressed the combined HGs, p-value about two tests; mean difference between RH and RHG and heterogeneity test. In the fixed effect model, the homogeneity among studies are assumed, but not in the random effect model.

3.2 Standard Means Difference in enzyme factors

The studied enzyme factors were AD, AH, liver MDA, P450, Cyto b5, GR, GST,

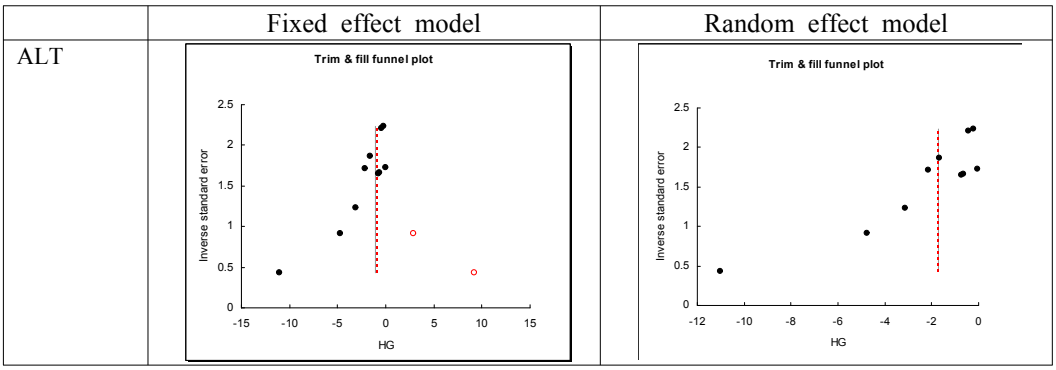
GT and γ-GCS. In the fixed effect model, the effect of ginseng on all enzyme factors were very significant (<0.01). The p-value of heterogeneity test among studies on GR and GST was more than 0.05, and the others were less than 0.05. In random effect model, the effect of ginseng on AH was not significant (0.065), and the others were significant (<0.05).

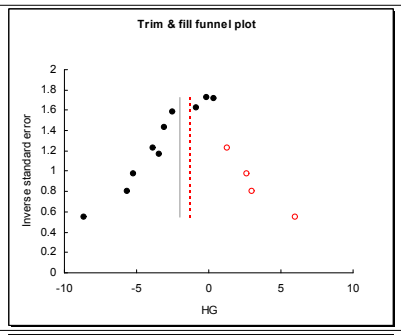
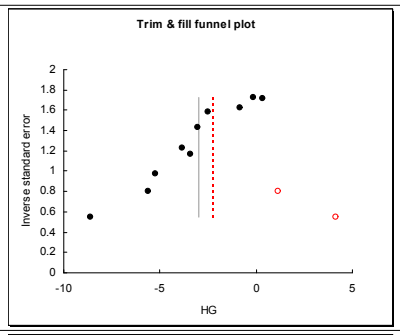
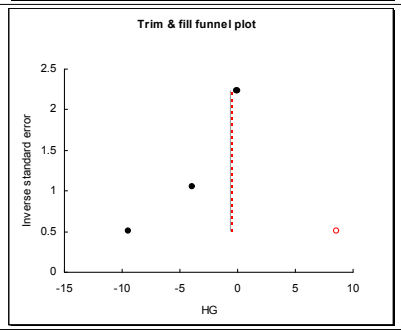
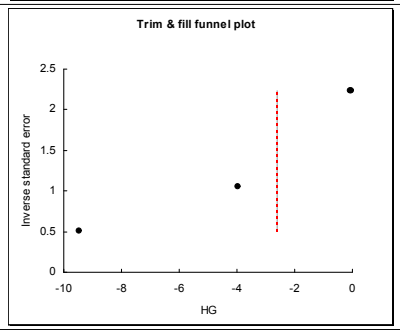
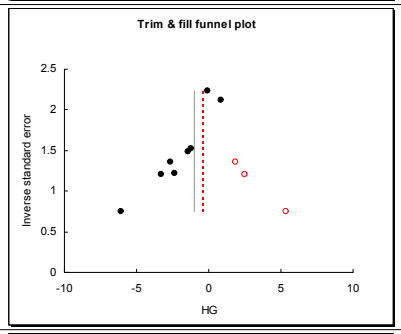
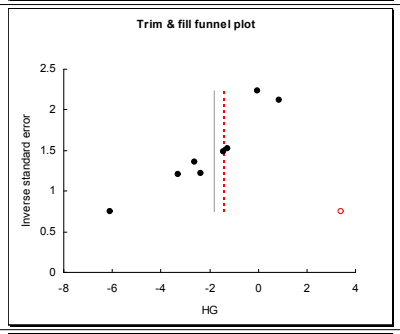
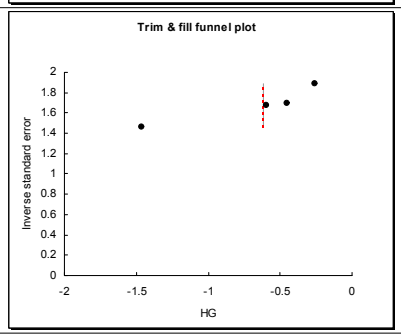
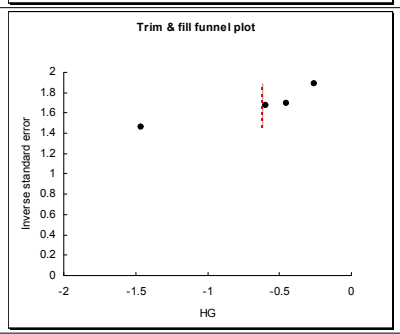
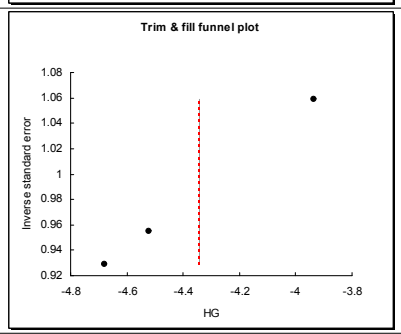
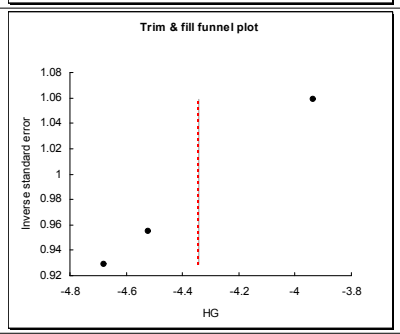
The mean differences of AD, AH, liver MDA, P450, Cyto b5, GT, and γ-GCS between RH and RHG were estimated by random effect model for heterogeneity among studies, and those of GR and GST were by fixed effect model. By reasons previously mentioned, the level of AD, liver MDA, P450, and Cyto b5 was significantly reduced (<0.01), and the level of GR, GST, GT and γ-GCS was increased by treatment with ginseng (<0.05). Especially, the mean of AD, liver MDA, Cyto b5, GR, GST, and GT was very significantly different from those of RHG (<0.01). The mean of AH in RH was not significantly different from those of RHG (0.0605).

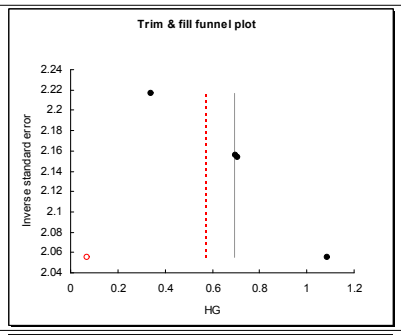
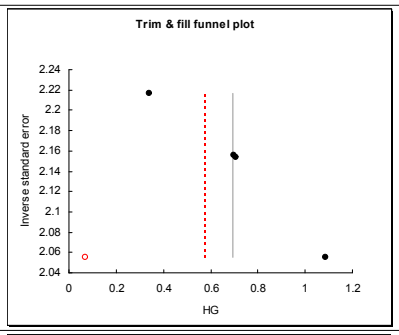
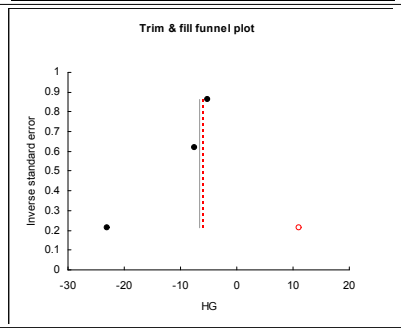
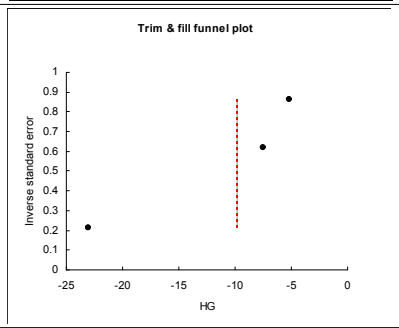
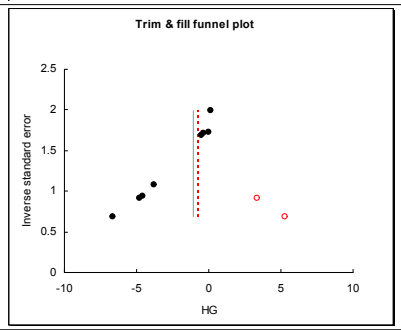
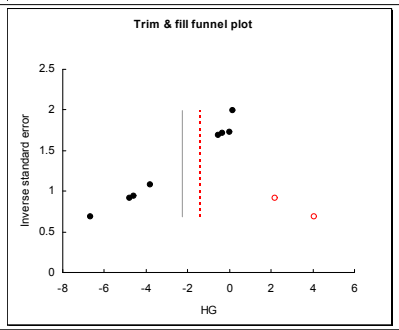
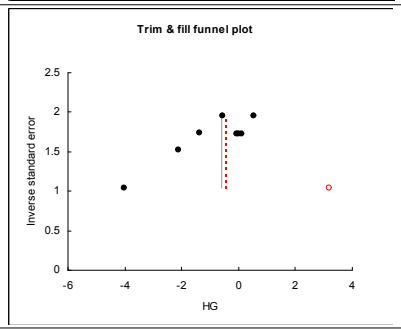
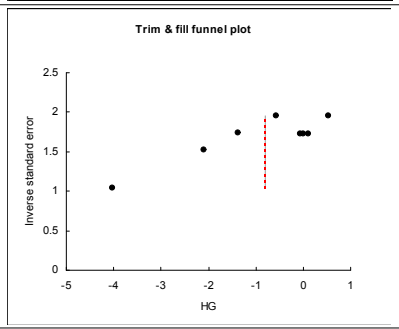
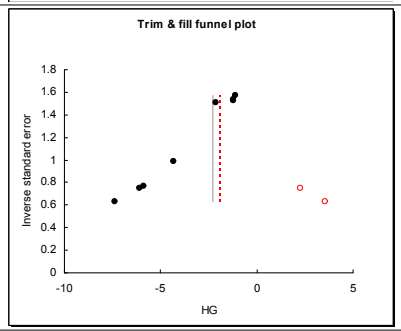
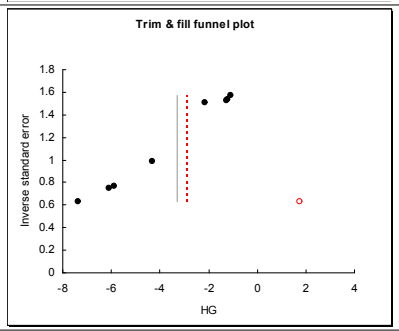
3.3 Publication Biases

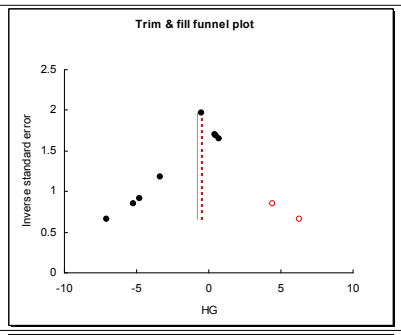
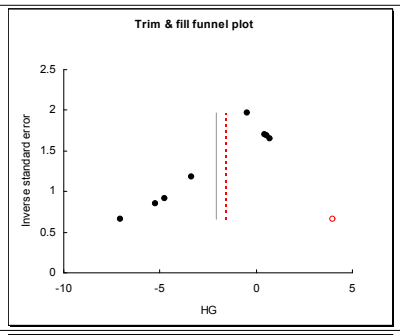
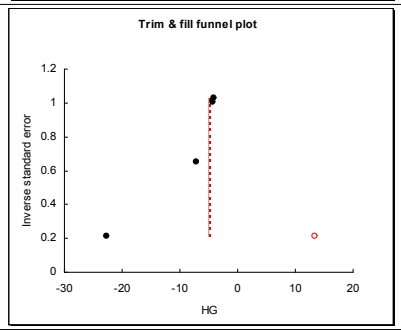
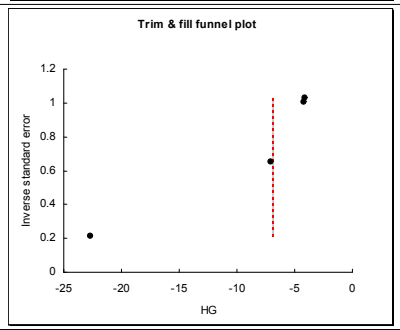
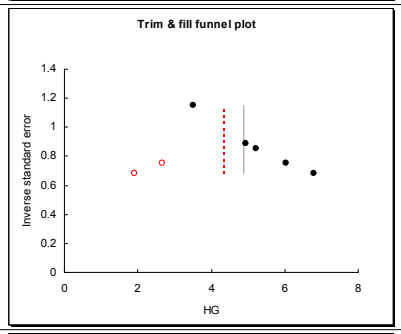
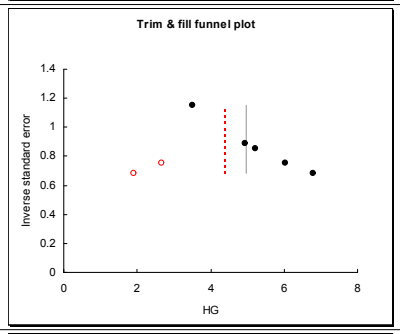
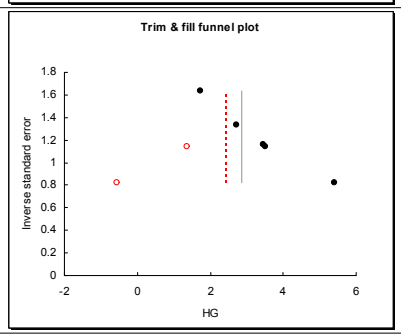
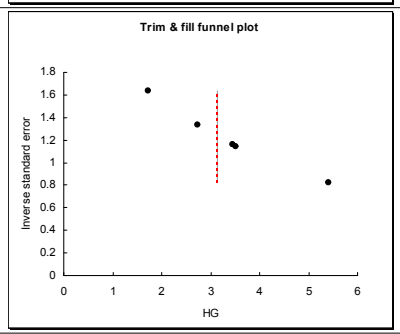
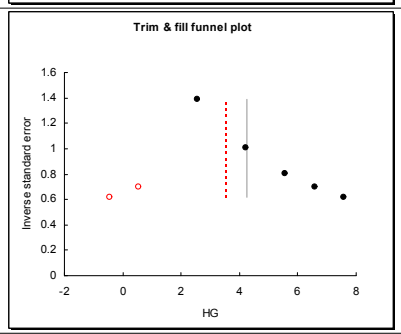
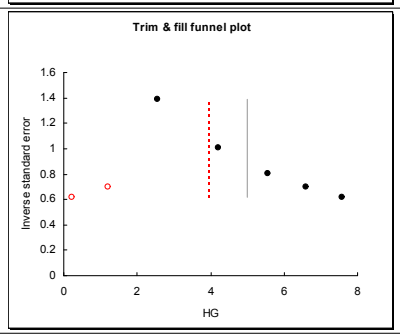
To check the publication bias, the funnel plots were collected in Fig. 1. The dots (●) in funnel plots represent original data of factors and the circles (○) represent filled data allowing 95% confidence interval to be calculated for the adjusted pooled estimate. The results of original data are indicated by a solid line, and the results of the trim and fill method are indicated by a dotted line. Since the dotted plots in funnel plot does not shape a funnel or a cone in Table 1, it suggests that there might be a publication bias.

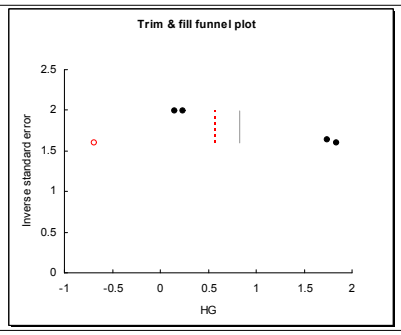
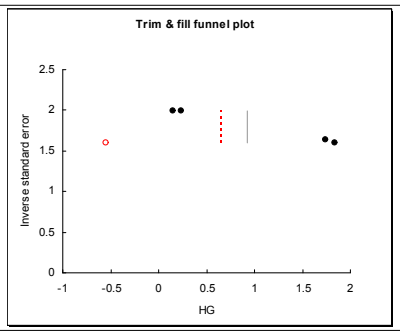
Fig. 1. Trim and fill funnel plot: In fixed effect model and random effect model



	Fixed effect model	Random effect model
AST		
ALP		
LDH		
Serum MDA		
SDH		

	Fixed effect model	Random effect model
TP		
γ -GT		
AD		
AH		
Liver MDA		

	Fixed effect model	Random effect model
P450		
Cyto b5		
GR		
GST		
GT		

	Fixed effect model	Random effect model
γ-GCS		

In addition, the publication bias in estimate on all factors in Table 1 did exist, because the intercept of Egger's linear regression test were significantly different with 0 in Table 2. The intercept and p-value were calculated in Egger's linear regression test for publication bias of combined HG.

Table 2. Estimated intercepts and p-values of Egger's linear regression test

	Egger's linear regression Intercept	p-value
ALT	-6.1915	0.0007
AST	-8.0995	0.0011
ALP	-6.5948	0.0076
LDH	-8.1013	0.0003
Serum MDA	-7.7988	0.0385
SDH	-5.6648	0.0022
TP	20.5978	0.0149
γ-GT	-5.0441	0.0095
AD	-8.2954	<0.0001
AH	-9.6517	0.0117
Liver MDA	-6.8476	<0.0001
P450	-8.3706	0.0013
Cyto b5	-5.1017	0.0003
GR	5.5236	<0.0001
GST	6.2916	0.0002
GT	5.6532	<0.0001
γ-GCS	13.6230	0.0009

The estimates of HG in Table 1 were replaced with estimates recalculated by trim and fill method, and the new estimates and 95% confidence intervals of HG

were presented in Table 3. T-F est were the new estimates and CI_low and CI_up were lower bound and upper bound of 95% confidence intervals. The 95% confidence interval of mean difference about ALT, AST, and γ-GT did not include 0 in the fixed effect model. And the mean difference about serum MDA, SDH, and TP did not have 0 in the random effect model. By trim and fill method on enzyme factors, the 95% confidence interval of mean difference about GR and GST did not include 0 in the fixed effect model, whereas liver MDA, Cyto b5, and GT did not include 0 in the random effect model.

Although the publication biases of estimated HGs between RH and RHG were considered, the ginseng significantly lowered level of ALT, AST, serum MDA, SDH, and γ-GT in plasma factors and liver MDA and Cyto b5 in enzyme factors. The level of TP in plasma factors and GR, GST, and GT in enzyme factors were raised by ginseng.

Table 3. 95% confidence intervals of combined HGs by trim and fill method

	Fixed effect estimate			Random effect estimate		
	T-F est.	CI_low	CI_up	T-F est.	CI_low	CI_up
ALT	-0.9073	-1.2753	-0.1713	-0.9073	-1.2753	-0.1713
AST	-1.2945	-1.7194	-0.4447	-2.2573	-3.6148	-0.8999
ALP	-0.4399	-1.0151	0.7105	-0.4399	-1.0151	0.7105
LDH	-0.3733	-0.7803	0.4407	-1.3692	-2.6506	1.1936
Serum MDA	-0.6185	-1.1987	-0.0382	-0.6185	-1.1987	-0.0382
SDH	-4.3408	-5.4925	-2.0374	-4.3409	-5.4925	-3.1892
TP	0.5776	0.1657	1.4014	0.5776	0.1657	0.9895
γ-GT	-5.9598	-7.7368	-2.4058	-9.8120	-15.7126	-3.9114
AD	-0.6977	-1.1666	0.2401	-1.3621	-2.8265	1.5667
AH	-0.4113	-0.8102	0.3865	-0.4113	-0.8102	0.3865
Liver MDA	-1.9121	-2.4577	-0.8209	-2.8811	-4.2966	-0.0501
P450	-0.3950	-0.8719	0.5588	-1.5615	-3.1889	1.6933
Cyto b5	-4.6551	-5.8721	-3.4380	-6.8570	-10.3328	-3.3811
GR	4.3442	3.4585	5.2298	4.4018	3.2014	5.6021
GST	2.4337	1.8074	3.0600	3.1292	2.0373	4.2210
GT	3.5645	2.7153	5.2629	3.9514	2.1992	5.7035
γ-GCS	0.5796	0.0854	1.5680	0.6583	-0.2087	1.5252

4. Conclusion

This paper studied the anti-hepatotoxity of ginseng by comparing two mean of RH and RHG about plasma factors and plasma factors. The plasma factors were the levels of ALT, AST, ALP, LDH, serum MDA, SDH, TP and γ-GT and the plasma factors were the levels of AD, AH, liver MDA, P450, Cyto b5, GR, GST, GT and γ-GCS. The combined HGs were gained by three steps; estimate HGs on fixed effect model or random effect model after homogeneity test of primary studies, check the publication bias and recalculating HGs about factors which were doubtful of the no publication bias.

In estimating step, the level of only AH in plasma of RH was not significantly different from the level of RHG. The level of ALT, AST, ALP, LDH, serum MDA, SDH, TP, γ-GT, AD, liver MDA, P450, GR, GST, GT and Cyto b5 of RH were significantly different from them of RHG. The calculated estimates of all considered factors might have publication biases, so the estimates in first step were replaced with recalculated estimates by trim-and-fill method. In last recalculating step, the standard mean difference between RH and RHG about ALT, AST, serum MDA, SDH, TP, and γ-GT in plasma factors were significantly meaningful and those about liver MDA, Cyto b5, GR, GST and GT in enzyme factors were significantly meaningful, too.

In conclusion, the effects of the ginseng supplement on ALT, AST, serum MDA, SDH, TP, γ-GT, liver MDA, Cyto b5, GR, GST and GT were clearly shown in the rats fed hepatotoxicity compared with ginseng.

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