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The Effect of Antischemin Preparations on Platelet Hyperactivity in Diabetic Rabbit Model

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Objectives: We aim to investigate how antischemin preparation affects the platelet parameters in a rabbit model with alloxan induced diabetes. **Methods:** Rabbits were divided into 4 groups; group 1 or control group, group 2 or no antischemin group without medication, group 3 or experimental group and group 4 or comparing group. Specific parameters such as PLT, PWD, MPV, P-LCR and PCT concentration were measured on day 3, 7, 14, 21 and 28. **Results:** Treatment of the no antischemin group with 6% alloxan monohydrate only resulted in a significant increase of platelet level and reached the highest value at 28th days (336.5 ± 21.2). When animals pre-treated simultaneously with antischemin for 5 days, the level of platelets did not increase significantly, compared to that in the non-treated group. After treatment of this group with 6% alloxan monohydrate, at the end of the trial (28 day), the platelet level was 274.7 ± 6.2 g/L, and in the clopidogrel pre-treated group, it was 274 ± 13.9 g/L. In the non-treated group, during the first 21 days, alloxan monohydrate caused a slight increase in PDW levels. **Conclusions:** Numerous studies revealed that botanical extracts which have been widely used as medicinal agents show potent anti-diabetic activity. Due to the serious side effects or resistance on synthetic drugs that are used in diabetes, the approach of using herbal remedies could be a successful alternative to the currently available diabetic treatments.

Keywords: Blood Coagulation, Platelet Aggregation, Insulin

Introduction

Diabetes is one of the most common metabolic diseases in the world, affecting an estimated 422 million people, which is around 9.3% of the global adult population [1]. There are different types of diabetes, however the most common are known as Type I and II diabetes. Type I diabetes is a chronic autoimmune disease where the exact cause is still unknown. Here, a patient's immune system destroys the insulin producing cells and is mostly diagnosed in children and young adults. On the other hand, Type II diabetes is non-insulin dependent and several life-style factors are associated with its risk such as overweight, sedentary lifestyle as well as having a family history of diabetes [2].

Type II diabetes often exhibits a strong correlation with cardiovascular disease. Up to 80% of the diabetes mortality results from cardiovascular complications [3]. Therefore, control of hypertension and hyperlipidemia has become highly important to reduce the risk of these complications. Furthermore, recently a prothrombotic state, characterized by increased coagulation, impaired fibrinolysis, as well as platelet hyperreactivity, has been recognized as a component of the insulin resistance syndrome. Insulin, in normal states, is a natural antagonist of the platelet hyperreactivity, while insulin defect in diabetic states creates diminishing endothelial generation of nitric oxide and prostaglandin which results to elevated platelet hyperreactivity [4].

Depending on the types of diabetes, treatments are different from each other in terms of their effects. Patients with Type I diabetes usually inject or inhale insulin, while in Type II diabetes metformin is usually prescribed as a first line for treatment. Metformin inhibits gluconeogenesis in the liver through the activation of the AMP-activated protein kinase, blocking adenylcyclase and mitochondrial glycerol phosphate dehydrogenase [5]. Moreover, Shin et al. demonstrated that metformin increases abundance of the gut *Akkermansia* spp. population which in turn results to the significant improvement of the glycemic profile in mice [6]. In 2018, the American College of Cardiology presented new guideline, in which 2 more types of drugs are recommended to prescribe patients who have cardiovascular disease as well as chronic kidney disease. These are a sodium-glucose cotransporter 2 (SGLT2) inhibitor and glucagon-like peptide-1 (GLP-1) receptor agonists [7].

In addition to diabetes medications, numerous studies showed beneficial effects of the traditional medicines, alternative therapies and natural remedies [8]. There are more than 1000 anti-diabetic herbals which are widely used as oral diabetic medicine now days. For example, *Allium sativum*, commonly known as garlic, may cause a reduction in blood glucose and increase the secretion of insulin. In several studies, a variety of ginseng decreases fasting blood glucose by modulating insulin production/secretion. Ginseng contains numerous valuable components, including saponins, polysaccharides, phenols and alkaloids [9].

It has been suggested that ginseng saponin, ginsenosides, are responsible for the anti-diabetic effect. Further, astragalus (*Astragalus membraneceus*), a member of the legume family, has been studied extensively and identified as a commonly prescribed herb against diabetes. It contains various isoflavonoids, polysaccharides, and saponins as well as important polyamines such as gamma aminobutyric acid. Agyemang et al. revealed that the polysaccharide fraction promotes insulin sensitization and protects pancreatic beta cells from apoptotic death, which has been related to reduced insulin production and increased insulin resistance [10].

Despite the widespread use of herbs as medicine in Asia, little is known about antischemin preparation, composed from medicinal herbs such as *Scutellaria baicalensis*, *Astragalus membranaceus* and *Gingko biloba*, on the treatment of diabetes mellitus. As in our previous studies which were conducted on cerebral ischemic rat model, we found markedly decreasing effects of antischemin preparations on the level of serum triglyceride as well as cholesterol. Moreover, we have determined that antischemin inhibits lipid peroxidation and protects from brain neuron necrosis and degradation. Therefore, in this study, we aim to investigate how antischemin preparation affects the platelet parameters in a rabbit model with alloxan induced diabetes.

Materials and Methods

Study subjects

TThe study was conducted by the Center for Innovation of the New Medical Science University, ELISA laboratory, Gyals center and Pretcilab laboratories. Thirty healthy rabbits weighing 2,3-

2,8 kilograms were selected from the national biotechnology industry in Mongolia.

Experimental groups and procedure

Rabbits were divided into 4 groups; group 1 or control group, group 2 or no antischemin group without medication, group 3 or experimental group with Antischemin (1: 1: 1) at 100 mg/kg, and group 4 or comparing group with Clopidogrel with 2.14 mg/kg orally for 5 days respectively and 5 days after a 6% Alloksan monohydrate (Sigma Chemicals, USA) solution was injected through rabbit ear IV with 60 mg/kg to create a diabetes model which develops a pathogenic disorder for diabetes.

Parameters and measurements

Specific parameters such as Platelets Total (PLT g/l), distribution width-SD (PDW fl), Mean platelet volume (MPV fl), Large platelet ratio (P-LCR %), PCT were measured on day 3, 7, 14, 21 and 28.

Statistical analysis

The PLT, PDW, MPV, P-LCR and PCT values for each group at each time were checked for outliers and missing data. Since our original data did not follow the bell curve, we carried out log transformation to make it as "normal" and used the log transformed data for our analysis. The main effects of time, treatment type and their interaction were determined using a mixed two-way ANOVA with a Greenhouse-Geiser adjustment for lack of sphericity. A critical p-value of < 0.05 was used. The repeated measurements within subjects were then compared to the previous time interval using paired t-tests. The control and study groups' differences at each time interval were tested using one-way ANOVA. A Bonferroni-type correction was applied to all t-test results resulting in a significance level set at $p < 0.017$ (= 0.05/3). SPSS version 24 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethical statement

Ethical approval was obtained from the Ethical Review Committee of Mongolian National University of Medical Sciences (Protocol No.2019/3-07). All efforts were made to minimize the number of animals used and their suffering..

Results

As shown in Table 1, treatment of the no antichemin group with 6% alloxan monohydrate only resulted in a significant increase of platelet level and reached the highest value at 28th days (336.5 ± 21.2). When animals were pre-treated simultaneously with antischemin for 5 days, the level of platelet did not increase significantly, compared to that in non-treated group. After treatment of this group with 6% alloxan monohydrate, at the end of the trial (28 day), platelet level was 274.7 ± 6.2 g/L, and in clopidogrel pre-treated group, it was 274 ± 13.9 g/L. Thus, pre-treatment with antischemin was effective in the suppression of platelet level increase caused by alloxan monohydrate.

Table 1. Grouping of rabbits.

Groups	n	Drug	Dose
Control	6	No alloxan and no antischemin	-
No antischemin	6	Alloxan and no antischemin	60 mg/kg
Antischemin	6	Alloxan and antischemin	60 mg/kg and 100 mg/kg
Clopidogrel	6	Alloxan and clopidorel	60 mg/kg and 2.14 mg/kg

All Alloxan diabetic rabbits were randomly divided into four groups (n=4).

Next, we assessed platelet distribution width in all groups (Table 2). In the non-treated group, during first 21 days, alloxan monohydrate caused a slight increase in PDW levels. However, antischemin as well as clopidogrel significantly inhibited the PDW levels, and this could be observed from the beginning of the pre-treatment. At the end of the trial, these values were more than 10-fold lower compared to that in the non-treated group.

Table 2. Average platelet levels (g/L) at baseline, 7 days, fourteen of all groups.

		Control[a,b,c,d,e]	No antischemin	Antischemin	Clopidogrel[f,g,h]	
Variable		**n = 6**	**n = 6**	**n = 6**	**n = 6**	***p-value**
	Days	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
PLT(g/L)	3	182 ± 7.23	295.4 ± 5.94	279.3 ± 4.9	223.5 ± 8.32	0.051
	7	154 ± 7.42	290 ± 13.1	266 ± 13	224 ± 9.33	0.001
	14	154 ± 11.6	285 ± 3.58	254 ± 12.6	231 ± 18.3	0.041
	21	207 ± 9.81	338.5 ± 13	258 ± 10.5	294 ± 25.9	0.061
	28	198 ± 14.8	336.5 ± 21.2	274.7 ± 6.2	274 ± 13.9	0.006

Two-way mixed ANOVA results: Interaction of time and treatment $F(1.918, 337.59) = 23.195$, $p < 0.001$; Main effect of time $F(1.918, 337.59) = 335.31$, $p < 0.001$; Main effect of treatment $F(1,176) = 0.666$, $p = 0.416$; *One-way ANOVA; Paired t-test in control group: [a]day3 vs. 7, $p = 0.010$; [b]day3 vs. 14, $p = 0.045$; [c]day7 vs. 21, $p = 0.051$; [d]day7 vs. 28, $p = 0.001$; [e]day14 vs. 21, $p = 0.002$. Paired t-test in clopidogrel group: [f]day3 vs. 21, $p = 0.045$; [g]day7 vs. 21, $p = 0.036$; [h]day14 vs. 21, $p = 0.005$.

Table 4 shows average mean platelet volume of all the groups. During 14 days of the trial, antischemin pre-treated group resulted in significant decrease of the platelet volume to the value of 6.85 ± 0.09, while in the control group, this value was 6.8 ± 0.13. However, we have observed decreased effect of the platelet volume in the non-treated group at the end of the trial, which yielded almost the same level compared with the clopidogrel pre-treated group.

Table 3. Average platelet distribution width (fl) of all groups.

		Control[a,b,c,]	No antischemin	Antischemin[d,e,f,g]	Clopidogrel	
Variable		**n = 6**	**n = 6**	**n = 6**	**n = 6**	***p-value**
	Days	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
PDW(fl)	3	6.4 ± 0.1	8.4 ± 0.13	6.06 ± 0.08	6.3 ± 0.16	0.024
	7	6.1 ± 0.22	8.15 ± 0.18	6.06 ± 0.17	5.85 ± 0.05	0.035
	14	6.1 ± 0.15	8 ± 0.30	5.86 ± 0.08	6.0 ± 0.19	0.043
	21	6.6 ± 0.2	8.5 ± 1.35	5.93 ± 0.12	6.25 ± 0.16	0.052
	28	6.5 ± 0.33	65 ± 0.45	6.27 ± 0.16	6.3 ± 0.08	0.081

Two-way mixed ANOVA results: Interaction of time and treatment $F(1.416, 347.59) = 27.115$, $p < 0.021$; Main effect of time $F(1.218, 437.59) = 365.31$, $p < 0.052$; Main effect of treatment $F(1,136) = 0.656$, $p = 0.466$; *One-way ANOVA; Paired t-test in control group: [a]day3 vs. 7, $p = 0.001$; [b]day3 vs. 14, $p = 0.025$; [c]day7 vs. 21, $p = 0.001$. Paired t-test in antieshimy group: [d]day3 vs. 28, $p = 0.024$; [e]day7 vs. 28, $p = 0.011$; [f]day14 vs. 28, $p = 0.001$; [g]day21 vs. 28, $p = 0.045$.

Table 4. Average mean platelet volume (fl) of all groups.

		Control	No antischemin[a,b,c,d,e,f]	Antishemin	Clopidogrel	
Variable		**n = 6**	**n = 6**	**n = 6**	**n = 6**	***p-value**
	Days	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
MPV (fl)	3	6.4 ± 0.10	7.9 ± 0.19	6.43 ± 0.14	6.8 ± 0.14	0.003
	7	6.8 ± 0.18	7.8 ± 0.12	6.53 ± 0.1	6.65 ± 0.05	0.056
	14	6.8 ± 0.13	7.7 ± 0.26	6.6 ± 0.03	6.85 ± 0.09	0.013
	21	6.8 ± 0.16	6.8 ± 0.21	6.63 ± 0.08	7 ± 0.2	0.386
	28	6.9 ± 0.27	7.0 ± 0.23	6.73 ± 0.09	7 ± 0.13	0.655

Two-way mixed ANOVA results: Interaction of time and treatment $F(1.436, 345.59) = 25.115$, $p < 0.041$; Main effect of time $F(1.318, 437.59) = 364.31$, $p < 0.041$; Main effect of treatment $F(1,146) = 0.756$, $p = 0.066$; *One-way ANOVA; Paired t-test in no-antischemin group: [a]day3 vs. 21, $p = 0.002$; [b]day3 vs. 21, $p = 0.045$; [c]day7 vs. 21, $p = 0.040$; [d]day3 vs. 28, $p = 0.001$; [e]day14 vs. 21, $p = 0.006$; [f]day14 vs. 28, $p = 0.008$.

We also examined the platelet large cell ratio (P-LCR) in all groups. As shown in Table 5, from the beginning of the pre-treatment, P-LCR values in control, antischemin as well as clopidogrel pre-treated groups were significantly lower compared to that in non-treated group. In contrast, in control group, 3rd day's P-LCR was 4.6 ± 0.20% and continuously increased through 28 days, reaching a peak of 8.9 ± 0.27% at 14th day ($p < 0.001$). However, in the antischemin pre-treated

group, the highest value was 4.27 ± 0.27% at 21th day, which was significantly lower than the control group. In the non-treated diabetic group, 3rd day's P-LCR was 12.5 ± 0.35% and this was continuously decreased during the trial, reached to 10.5 ± 0.83% at 28th day.

Table 5. Average platelet large cell ratio (%) of all groups.

		Control[a,b,c,d,e,f]	No antischemin	Antischemin	Clopidogrel	
Variable		n = 6	n = 6	n = 6	n = 6	*p-value
	Days	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
P-LCR (%)	3	4.6 ± 0.20	12.5 ± 0.35	3.96 ± 0.28	4.55 ± 0.10	0.061
	7	3.9 ± 0.45	11.5 ± 0.4	3.7 ± 0.53	4.1 ± 0.26	0.025
	14	8.9 ± 0.27	10 ± 0.56	3.76 ± 0.22	5.1 ± 0.36	0.046
	21	8.3 ± 1.24	10.45 ± 0.72	4.27 ± 0.27	5.05 ± 0.44	0.021
	28	8.3 ± 1.11	10.5 ± 0.83	0.18 ± 0.01	5.2 ± 0.21	0.060

Two-way mixed ANOVA results: Interaction of time and treatment F(1.836, 325.59) = 25.115, p < 0.051; Main effect of time F (1.348, 437.59) = 366.31, p < 0.044; Main effect of treatment F(1,196) = 0.786, p = 0.056; *One-way ANOVA; Paired t-test in control group: [a]day3 vs. 14, p = 0.021; [b]day3 vs. 21, p = 0.051; [c]day3 vs. 28, p = 0.001; [d]day7 vs. 14, p = 0.021; [e]day7 vs. 21, p = 0.001; [f]day7 vs. 28, p = 0.002.

Average procalcitonin level (PCT) was shown in Table 6. In the control group, PCT level was not changed significantly, ending with 0.24 ± 0.03%. When treated previously with antischemin, procalcitonin level changed 0.19-0.25% during the trial, which demonstrated that procalcitonin level was unaffected by the antischemin pre-treatment. On the other hand, in the non-treated diabetic group, the level of procalcitonin was significantly higher at the 3rd day (0.33 ± 0.02%), and this value was not significantly changed through the experimental period.

Table 6. Average procalcitonin (%) of all groups.

	Days	Control	No antischemin	Antischemin	Clopidogrel[a,b,c]	
Variable	n = 6	n = 6	n = 6	n = 6	n = 6	*p-value
	Days	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
PCT(%)	3	0.21 ± 0.01	0.33 ± 0.02	0.25 ± 0.02	0.15 ± 0.01	0.002
	7	0.12 ± 0.01	0.31 ± 0.01	0.21 ± 0.01	0.28 ± 0.01	0.041
	14	0.12 ± 0.01	0.31 ± 0.03	0.19 ± 0.1	0.16 ± 0.01	0.057
	21	0.15 ± 0.01	0.30 ± 0.03	0.24 ± 0.1	0.28 ± 0.03	0.029
	28	0.24 ± 0.03	0.31 ± 0.01	-	0.26 ± 0.01	0.015

Two-way mixed ANOVA results: Interaction of time and treatment F(1.736, 325.59) = 26.115, p < 0.061; Main effect of time F (1.388, 437.59) = 376.31, p < 0.051; Main effect of treatment F(1,396) = 0.286, p = 0.046; *One-way ANOVA; Paired t-test in clopidogrel group: [a]day3 vs. 7, p = 0.004; [b]day3 vs. 21, p = 0.041; [c]day7 vs. 21, p = 0.001.

Discussion

Diabetes is a disease in which the blood glucose levels persists very high due to the impairment of the hormone insulin response. Specially, declined insulin secretion is a key pathophysiological feature in Type II diabetes, and is always associated with cardiovascular diseases. The global diabetes prevalence in 2019 is estimated to be 463 million people and will rise to 700 million by 2045. In the Western Pacific region, including Mongolia, 1 in 3 adults has been observed to have diabetes, and the major driver of this statistics comes largely from the high rate of individuals that are overweight and obese [11].

There are several effective treatments that help manage diabetic symptoms. These include metformin, dopamine-2 agonists, as well as dipeptidyl peptidase-4 (DPP-4) inhibitors. Metformin controls liver glucose production and enhances insulin sensitivity in the muscles, whilst dopamine-2 agonists reduce blood glucose following a meal. Further, alogliptin and saxagliptin, which are the DPP-4 inhibitors, support glucagon-like peptide 1 (GLP-1) to remain longer inside the body, which in turn results to the lowering of the blood glucose [12].

In diabetic patients, the CVD is the pivotal cause of morbidity and mortality. Here accelerated atherosclerosis is the main factor which contributes to the high risk of coronary heart disease (CHD) and ischemic stroke in diabetic patients. Furthermore, an early event in the pathogenesis of vascular

complications of diabetes is caused by the alteration of platelet function. The platelets of the diabetic patients are hyperreactive, which therefore yielded increased mean platelet volume (MPV), increased reactive oxygen species (ROS), and increased synthesis of the thromboxane A2 (TXA2). Moreover, the platelets of the diabetic patients show significant adhesive properties [13].

Nowadays, there are three type of drugs prescribed for hyperreactive platelets in diabetes: cyclooxygenase-1 inhibitors (acetylsalicylic acid), thienopyridines (clopidogrel) and platelet glycoprotein inhibitors. Acetylsalicylic acid acetylates the cyclooxygenase-1 enzyme, thereby blocking the formation of TXA2. It has been revealed that acetylsalicylic acid treatment results to approximately 90% decrease in fatal coronary events [14]. On the other hand, there may also be resistance to acetylsalicylic acid in diabetic patients due to other upregulated diabetic signaling pathways which are not blocked by aspirin. Also, clopidogrel is a broadly used drug which inhibits adenosine diphosphate receptor P2Y12 through binding to the P2Y12 receptor in its hydrolyzed form. However, recent studies revealed that clopidogrel shows highly variable effects on diabetic patients, and approximately 40% percent of the treated patients exhibit responses associated with an further increased risk of CVD [15]. In this study, we have demonstrated anti-platelet activity of the antischemin preparation in a diabetic rabbit model. Rabbits were divided into 4 groups: control group, no antichemin group without medication, antischemin group and clopidogrel group (positive control). 6% Alloksan monohydrate (Sigma Chemicals, USA) solution was used to create a diabetic model [16]. We have observed that no the antischemin group with 6% alloxan monohydrate only resulted in a significant increase of platelet levels and reached the highest value at 28th days (336.5 ± 21.2). When animals were pre-treated simultaneously with antischemin for 5 days, the level of platelet did not increase significantly. After treatment this group with 6% alloxan monohydrate, at the end of the trial (28 day), platelet level was 274.7 ± 6.2 g/L, and in the clopidogrel pre-treated group, it was 274 ± 13.9 g/L. Thus, pre-treatment with antischemin was effective in the suppression of platelet level increase caused by alloxan monohydrate. Moreover, in non-treated group, during first 21 days, alloxan monohydrate caused a slight increase in PDW levels [17], while antischemin treatment resulted in significant inhibition of the PDW levels, which were more than 10-fold lower compared to that in the non-treated group. Furthermore, the PCT level was also significantly lower in both control group and the antischemin group, which in turn demonstrates that the procalcitonin level was unaffected by the antischemin pre-treatment. On the other hand, in the non-treated diabetic group, the level of procalcitonin was significantly higher at the 3rd day (0.33 ± 0.02%), and this vale was not significantly changed through the experimental period [18].

Antischemin preparation is an herbal composite from medicinal herbs such as *Scutellaria baicalensis, Astragalus membranaceus and Gingko biloba*. In our previous studies, we have shown that, in the alloxan-induced diabetic rabbit model, antischemin resulted in suppressing effects on blood glucose, serum LDL, TG and cholesterol levels, and enhanced plasma HDL by 11.2-48.5%. The same model experiments was also conducted in order to determine the effects of antischemin on coagulation [19]. In acute, subacute and chronic phase of the diabetic model, thrombocyte activation and aggregation in blood circulation was more significant on days 3-14 [20].

Antischemin pre-treatment resulted in a significant increase in thrombin time (12.35-33.25%), and activated thromboplastin time (10-24.8%). Moreover, fibrinogen decreased by 40.5% indicating that antischemin inhibits thrombocyte aggregation, coagulation and increased blood rheologic properties in blood. Shin et al. demonstrated that the treatment of the Type II patients with *Scutellaria baicalensis* combined with metformin influenced gut microflora and increased the composition of the *Lactobacillus* as well as *Akkermansia* species in gut microbiota [21]. Moreover, this combined treatment improved glucose tolerance and inhibited RNA expression of the inflammatory cytokine TNF-α [22]. *Astragalus membranaceus* is a common Chinese herb which is rich of various biological activities such as saponins, polysaccharides and flavonoids, and is used to treat Type II diabetes. Further, Saleh et al. revealed that the diabetic rats fed *Gingko biloba* extract showed increased pancreatic beta cells and the amount of insulin released by these cells [23-25].

There are some limitations in this study. First, there are not many studies and reports which have investigated antischemin preparations on the treatment of the diabetes mellitus. Second, we have only considered the effects of the whole antischemin preparations on hyperreactivity of the platelet. It has been reported that many medicinal plants contain rich amounts of antioxidants as well as phenolic compounds. Given this fact,

further studies are needed to identify the exact molecular mechanisms of active compounds in antichemin preparations which are involved in reduction of platelet hyperreactivity induced by alloxan.

Conclusions

Numerous studies revealed that botanical extracts which have been widely used as medicinal agents show potent anti-diabetic activity. Due to the serious side effects or resistance on synthetic drugs that are used in diabetes, the approach of using herbal remedies could be a successful alternative to the currently available diabetic treatments. We have confirmed in the present study that antischemin preparation is effective against hyperreactivity of platelets in alloxan-induced diabetes in the rabbit model.

Conflict of Interest

The authors declared no conflict of interest.

References

1. Li M, Ma RN, Li HL, Qu YZ, Gao GD. Astragaloside IV reduces cerebral edema post-ischemia/reperfusion correlating the suppression of MMP-9 and AQP4. Eur J Pharmacol 2013; 5: 189-95.
2. Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos. Ann New York Acad Sci 2012; 4: 18-32.
3. Chapman MJ. The potential role of HDL-and LDL-cholesterol modulation in atheromatous plaque development. Curr Med Res Opin 2005; 21: 17-22.
4. Dang X, Miao JJ, Chen AQ, Li P, Chen L, Liang JR, et al. The antithrombotic effect of RSNK in blood-stasis model rats. J Ethnopharmacol 2015; 15: 266-72.
5. Bowen KK, Naylor M, Vemuganti R. Prevention of inflammation is a mechanism of preconditioning-induced neuroprotection against focal cerebral ischemia. Neurochemical Int 2006; 7: 127-35.
6. De Gracia DJ, Hu BR. Irreversible translation arrest in the reperfused brain. J Cereb Blood Flow Metab 2006; 27: 875-93.
7. Brint S, Jacewicz M, Kiessling M, Tanabe J, Pulsinelli W. Focal brain ischemia in rat: methods for reproducible neocortical infarction using tandem occlusion of the distal middle cerebral and ipsilateral common carotid arteries. J Cereb Blood Flow Metab 1988; 8: 474-85.
8. Lee J, Kim T, Cho W, Ma JY. Antithrombotic and antiplatelet activities of soshiho-tang extract. BMC Complement Altern Med 2013; 6: 137-42.
9. Zhang WD, Zhang C, Wang XH, Gao PJ, Zhu DL, Chen H, et al. Astragaloside IV dilates aortic vessels from normal and spontaneously hypertensive rats through endothelium-dependent and endothelium-independent ways. Planta Med 2006; 6: 621-6.
10. Brown AW. The nature, distribution and earliest stages of anoxic-ischemic nerve cell damage in the rat brain as defined by the optical microscope. Br J Exp Pathol 1968; 4: 87-106.
11. Burkhalter A. Intrinsic connections of rat primary visual cortex: laminar organization of axonal projections. J Comp Neurol 1989; 8: 171-86.
12. Cao F, Liu G, Wang W, Wang B, Wei X, Lu F, et al. Combined treatment with an anticoagulant and a vasodilator prevents steroid-associated osteonecrosis of rabbit femoral heads by improving hypercoagulability. Biomed Res Int 2017; 16: 24-7.
13. Chen SH, Lee J, Yang I, Nassief A, Hsu CY. Combination therapy for ischemic stroke: potential of neuroprotectants plus thrombolytics. Am J Cardiovasc Drugs 2002; 2: 303-13.
14. Chen J, Graham SH, Nakayama M, Zhu RL, Jin K, Stetler RA, et al. Apoptosis repressor genes Bcl-2 and Bcl-x-long are expressed in the rat brain following global ischemia. J Cereb Blood Flow Metab 1997; 1: 2-10.
15. Li N, Feng Li, Tan Y, Xiang Y, Zhang R, Yang M, et al. Preparation, characterization, pharmacokinetics and biodistribution of baicalin-loaded liposome on cerebral ischemia-reperfusion after i.v. administration in rats. Molecules 2018; 17: 1747. doi: 10.3390/molecules 23071747.
16. Reed RL, Bracey AW, Hudson JD, Miller TA, Fischer RP. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. Circ Shock 1990; 10: 141-52.
17. Angelos MG, Hamilton GC. Coagulation studies: prothrombin time, partial thromboplastin time, bleeding time. Emerg Med Clin Nort Am 1986; 2: 95-113.
18. Monroe DM, Hoffman M. The coagulation cascade in cirrho-

sis. Clin Liver Dis 2009; 2: 1-9.

19. Winter WE, Flax SD, Harris NS. Coagulation testing in the core laboratory. Lab Med 2017; 11: 295-313.
20. Schulman S, El-Darzi E, Florido FH, Friesen M, Skoloff GM, Brake MA, et al. A coagulation defect arising from heterozygous premature termination of tissue factor. J Clin Invest 2020; 10: 5302-12.
21. Li M, Li H, Fang F, Deng X, Ma SH. Astragaloside IV attenuates cognitive impairments induced by transient cerebral ischemia and reperfusion in mice via anti-inflammatory mechanisms. Neurosci Lett 2017; 2: 114-9.
22. Wu X, Zhou W, Wei Q, Chen P, Li Y. Cytoprotective effects of the medicinal herb astragalus membranaceus on lipopolysaccharide-exposed cells. Mol Med Rep 2018; 18: 4321-7.
23. Chen A, Xu Y, Yuan J. Ginkgolide B ameliorates NLRP3 inflammasome activation after hypoxic-ischemic brain injury in the neonatal male rat. Int J Dev Neurosci 2018; 69: 106-11.
24. Tang Y, Zhou G, Yao L, Xue P, Yu D, Xu R, et al. Protective effect of ginkgo biloba leaves extract, EGb761, on myocardium injury in ischemia reperfusion rats via regulation of TLR-4/NF-κB signaling pathway. Oncotarget 2017; 9: 86671-80.
25. Xu L, Hu Z, Shen J, Quillan PM. Effects of ginkgo biloba extract on cerebral oxygen and glucose metabolism in elderly patients with pre-existing cerebral ischemia. Complement Ther Med 2015; 4: 220-5.