Investigation on Potential Targets of Fuyuan Xingnao Decoction in the Treatment of Intracerebral Hemorrhage Based on Network Pharmacology

YANQI LUO[1], SHUANGYANG LI[1], YANJIAO LI[1], XUE BAI[1] AND BANGJIANG FANG[1,2]*

Institute of Integrated Chinese and Western Medicine, [1]Department of Neurology, National Traditional Chinese Medicine Clinical Research Base, The Affiliated Hospital of Traditional Chinese Medicine, Southwest Medical University, Luzhou, Sichuan 646000, [2]Emergency Department, Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Xuhui, Shanghai 200232, China

Luo *et al.*: Role of Fuyuan Xingnao Decoction in the Treatment of Intracerebral Hemorrhage

Fuyuan Xingnao decoction has the characteristics of multi-component, multi-pathway and multi-target. Previous studies have found that it has antioxidant, anti-inflammatory and other biological effects, and is now often used to treat neurological diseases such as intracerebral hemorrhage and cerebral infarction. But the specific key ingredients and mechanism are still unclear. Modern bioinformatics and network pharmacology methods were used to predict molecular network mechanisms between intracerebral hemorrhage and Fuyuan Xingnao decoction. Animal experiments were carried out to verify the effect of Fuyuan Xingnao decoction for the treatment of intracerebral hemorrhage, combined with behavior test and pathological detection. 68 active components in Fuyuan Xingnao decoction are involved and 471 gene targets were identified successfully. There are a total of 359 cross-targets between Fuyuan Xingnao decoction and intracerebral hemorrhage. Subsequently, the "disease-drug-active ingredient-target" network diagram was constructed using Cytoscape software and 97 core targets were screened. In addition, the enrichment of gene ontology and Kyoto Encyclopedia of Genes and Genomes suggests that phosphoinositide 3-kinase/protein kinase B and the apoptosis signaling pathway are most likely to be affected for the treatment of intracerebral hemorrhage. Finally, the use of behavioral tests and hematoxylin and eosin staining in animal experiments confirmed the role of Fuyuan Xingnao decoction in the treatment of intracerebral hemorrhage. Based on network pharmacology and experimental validation results, our *in vivo* study indicated that Fuyuan Xingnao decoction is effective for the treatment of intracerebral hemorrhage.

Key words: Fuyuan Xingnao decoction, intracerebral hemorrhage, network pharmacology, hematoxylin and eosin staining

Intracerebral Hemorrhage (ICH) is one of the most common acute and critical conditions in neurology and remains the second leading cause of death worldwide, with a mortality rate of 43 %[1]. Cerebral protection after ICH is a difficult point in clinical treatment. There are two forms of brain injury after ICH, primary brain injury and secondary brain injury. Bleeding from vascular rupture and mass effects are the leading causes of primary brain injury[2]. Hemorrhage-formed hematomas cause compression in brain tissue and induce cerebral edema, leading to more severe secondary brain injury. A large number of studies have proved that after ICH, microglia and macrophages are activated, and most inflammatory factors are released from the cells, triggering a cascade of signal transduction pathways associated with inflammation[3]. During the inflammatory response following ICH, inflammatory cells are activated, the respiratory chain erupts and reactive oxygen species are released, ultimately causing an oxidative stress response[4]. In addition, after ICH, blood releases Red Blood Cells (RBCs) into the brain parenchyma and eventually the red blood cells are lysed into substances such as heme and iron, which exacerbate the inflammatory response and mediate brain damage[5]. Secondary brain injury produces mechanisms related to inflammatory responses, oxidative stress and toxic effects on RBC lysate, all

of which ultimately lead to the death of neuronal cells[6,7]. In the current research reports, the death methods of neuronal cells after ICH mainly include apoptosis, autophagy, necrosis and iron death[8]. Mitochondrial dysfunction, inflammatory response, oxidative stress and other factors caused by ICH can cause apoptosis of neuronal cells.

Fuyuan Xingnao decoction is composed of ginseng, *Panax notoginseng*, leech, *Acorus calamus*, Tiannanxing, rhubarb and motherwort. Our previous study found that in the clinical treatment of acute ischemic stroke, Fuyuan Xingnao decoction effectively improves the coagulation function of patients, enhances vascular endothelial function, reduces secondary inflammatory responses, improves blood rheological indicators and neurological function[9,10], and inhibits the release of inflammatory transmitters[11,12]. In addition, Fuyuan Xingnao decoction can effectively reduce the degree of cerebral edema in rats with cerebral infarction and reduce the permeability of the blood-brain barrier[13]. This Traditional Chinese Medicine (TCM) compound also has the effect of reducing the volume of infarction in brain tissue[14], which is related to the effect of Fuyuan Xingnao decoction in increasing angiogenesis in ischemic brain tissue and promoting blood vessels to regain blood flow perfusion. The mechanism may be used to promote the upregulation of the expression of Vascular Endothelial Growth Factor (VEGF) protein[15]; upregulation of Insulin-like Growth Factor-1 (IGF-1) expression by inhibition of microRNA-320 (miR-320)[16]; upregulates the expression of Cluster of Differentiation 31 (CD31), a neonatal marker in vascular endothelial cells[17]; up-regulation of Cyclin E1 (CCNE1) and Cell Division Cycle 25 A (CDC25A) reduces the expression level of microRNA-503, which inhibits angiogenesis[18]. Furthermore, anti-apoptosis and anti-inflammatory properties have also been reported[19]. These studies highlight the potential function of Fuyuan Xingnao decoction in the prevention and treatment of cerebral infarction. However, the preventive or protective properties of Fuyuan Xingnao decoction for ICH and its underlying molecular mechanism have not been clarified.

MATERIALS AND METHODS

Screening of active ingredients and potential targets of Fuyuan Xingnao decoction:

In the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, the active ingredients of ginseng, *Panax notoginseng*, *Acorus calamus*, motherwort, *Arisaematis rhizoma*, and rhubarb were screened out according to the conditions of Oral Bioavailability (OB)≥30 % and Drug-Likeness (DL)≥0.18. The active ingredient of leeches was selected by setting the score cut off≥20 and p-value≤0.05 through the Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM) database. These targets corresponding to these active ingredients are imported into the Universal Protein Resource (UniProt) database and normalized, and the corresponding human gene names are obtained.

Acquisition of targets related to ICH and screening of intersection targets of Fuyuan Xingnao decoction and ICH:

Using "intracerebral hemorrhage" as the keyword, the target of ICH disease was searched in the GeneCards database. The target of the active ingredient of the standardized name of the Fuyuan Xingnao decoction was intersected with the target of ICH. Finally, the target of the Fuyuan Xingnao decoction acting on ICH was obtained.

Construction of the network diagram of Fuyuan Xingnao decoction-ingredients-target-ICH:

The potential active ingredients contained in the Fuyuan Xingnao decoction and the drug-disease intersection targets were uploaded to the software Cytoscape 3.8.0 software to construct a relationship network of Fuyuan Xingnao decoction-ingredients-target-ICH and visually adjusted.

Construction of Fuyuan Xingnao decoction-ICH Protein-Protein Interaction (PPI) network and screening of core targets:

The intersection target of Fuyuan Xingnao decoction and ICH was imported into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and the species was selected "*Homo sapiens*", constructing a PPI network for Fuyuan Xingnao decoction-ICH. Then, a Tab-Separated Values (TSV)-formatted file will be obtained in the STRING database and the file will be uploaded to the Cytoscape 3.8.0 software for network topology analysis and the core targets will be screened out under the condition that it is greater than the average value of degree.

Gene Ontology (GO) bioprocess analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis:

Enter the above intersecting targets into the Database for Annotation, Visualization and Integrated Discovery (DAVID) database, and the species is selected as "*Homo sapiens*", performing GO bioprocesses and KEGG pathway enrichment analysis. Based on p≤0.05, the top 20 biological processes and KEGG pathways were selected.

Animals:

Healthy male Sprague Dawley (SD) adult rats with a mass of 250±20 g were purchased from the Laboratory Animal Center of Southwest Medical University (production and use license: SCXK (Chuan) 2018-17; SYXK (Chuan) 2018-065). In this experiment, all experimental rats had free access to feed and drinking water. All rats are housed in a clean environment controlled by temperature, humidity and light. The study was approved by the Animal Ethics Committee of the Science and Technology Department of Southwest Medical University (Approval number: 2020904 date: December 30, 2020).

Experimental drugs:

The composition of Fuyuan Xingnao decoction: Fuyuan Xingnao decoction is composed of ginseng 10 g, *Panax notoginseng* 10 g, *Acorus calamus* 12 g, leech 10 g, motherwort 30 g, Tiannanxing 15 g, rhubarb 10 g. All drugs are specially processed and prepared, in which ginseng and leeches are extracted using alcohol and *Acorus calamus* is extracted from volatile oil. Then together with the rest of the medicinal materials, it is prepared into a 2 g/ml concentrate, which is sterilized and placed in a cool and dry place.

Animal grouping and dosing regimen:

Referring to the table of drug dose conversion coefficients for human and animal kilograms of body weight and using the standard adult weight of 60 kg, the formula was obtained. Rat equivalent dose=human dose administered per kg of body weight×6.25. According to the above formula, the low dose group of Fuyuan Xingnao decoction was 5 g/kg/d, the middle dose group of Fuyuan Xingnao decoction was 10 g/kg/d and the high dose group of Fuyuan Xingnao decoction was 20 g/kg/d. Rats in the low, middle and high dose groups of Fuyuan Xingnao decoction were gavaged with corresponding doses of drugs and rats in the sham surgery group and model group were gavaged with the same amount of normal saline for three consecutive days. After the end of gavage, rat brain tissue is removed for subsequent experiments.

Zea Longa score:

Refer to the Zea Longa 5-point method to develop neurological function, scoring standards are 0 points: No symptoms of neurological deficit; 1 point: The left forelimb cannot be fully extended when the tail is upside down; 2 points: Rear-end when walking in a circle to the hemiplegic side and the forelimb on the hemiplegic side drags or curls up; 3 points: Tipping to the side of hemiplegia when walking; 4 points: Impairment of consciousness. Rats with scores in the 1-3 score range are successful rats with a model of ICH. Rats were scored for neurological deficits 6 h after surgery and rats that met the scoring criteria were included in the experiment. Each group of rats were scored again 72 h postoperatively.

Hematoxylin and Eosin (HE) staining:

Brain tissue was fixed with 4 % paraformaldehyde for more than 24 h for paraffin embedding and then sliced. Brain tissue slices were routinely dewaxed and rehydrated, and then stained with hematoxylin for 10 min according to the HE staining kit instructions. Next, the slices were rinsed with tap water for 3 min. Slices were differentiated in 1 % hydrochloric acid, alcohol for 10 s and rinsed with tap water for 1 min. Slices were returned to blue with ammonia water for 15 s and rinsed with tap water for 1 min. The slices were hydrated in 85 % alcohol for 5 min and eosin staining solution was counterstained for 3 min to stain the cytoplasm. Finally, the slices are rinsed with tap water and the gradient alcohol is dehydrated and transparent. Neutral gum seal, after drying, the pathological changes of brain tissue around the hematoma were observed, photographed and saved.

RESULTS AND DISCUSSION

Screening of active components of Fuyuan Xingnao decoction and related targets were explained here. After the active ingredient is retrieved in the database, its duplicate data is removed. The 68 potential active ingredients of 7 flavors of Chinese medicine in the Fuyuan Xingnao decoction were obtained including 17 active ingredients in ginseng, 7 active ingredients

in *Panax notoginseng*, 4 active ingredients in *Acorus calamus*, 8 active ingredients in motherwort, 6 active ingredients in *Arisaematis rhizoma*, 7 active ingredients in *Rheum* and 19 active ingredients in leech (Table 1 and Table 2).

Potential target identification was explained here. The relevant targets of 68 active ingredients were obtained from the database and then the duplicate data were deleted and the gene names were normalized, resulting in a total of 471 relevant targets of the Fuyuan Xingnao decoction. 4228 targets related to ICH were obtained from the GeneCards database. The targets related to Fuyuan Xingnao decoction and the targets related to ICH were intersected and 359 intersection targets were obtained (fig. 1).

TABLE 1: THE ACTIVE COMPONENTS OF GINSENG, SANQI, ACORUS, TATARINOWII, *Herba leonuri*, *Arisaematis rhizoma*, RHUBARB

Drug	Active ingredient	OB (%)	DL
Ginseng	Diop	43.59	0.39
Ginseng	Stigmasterol	43.83	0.76
Ginseng	beta-sitosterol	36.91	0.75
Ginseng	Inermin	65.83	0.54
Ginseng	Kaempferol	41.88	0.24
Ginseng	Aposiopolamine	66.65	0.22
Ginseng	Deoxyharringtonine	39.27	0.81
Ginseng	Dianthramine	40.45	0.2
Ginseng	Arachidonate	45.57	0.2
Ginseng	Frutinone A	65.9	0.34
Ginseng	Ginsenoside Rh2	36.32	0.56
Ginseng	Ginsenoside-Rh4_qt	31.11	0.78
Ginseng	Girinimbin	61.22	0.31
Ginseng	Panaxadiol	33.09	0.79
Ginseng	Suchilactone	57.52	0.56
Ginseng	Alexandrin_qt	36.91	0.75
Ginseng	Fumarine	59.26	0.83
Panax notoginseng	Mandenol	42	0.19
Panax notoginseng	DFV	32.76	0.18
Panax notoginseng	Diop	43.59	0.39
Panax notoginseng	beta-sitosterol	36.91	0.75
Panax notoginseng	Stigmasterol	43.83	0.76
Panax notoginseng	Ginsenoside Rh2	36.32	0.56
Panax notoginseng	Quercetin	46.43	0.28
Acorus calamus	(1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[4,3-c]furan	52.35	0.62
Acorus calamus	8-Isopentenyl-kaempferol	38.04	0.39
Acorus calamus	Cycloartenol	38.69	0.78
Acorus calamus	Kaempferol	41.88	0.24
Motherwort	arachidonic acid	45.57	0.2
Motherwort	Kaempferol	41.88	0.24
Motherwort	Quercetin	46.43	0.28

Motherwort	Isorhamnetin	49.6	0.31
Motherwort	Iso-preleoheterin	66.29	0.33
Motherwort	Preleoheterin	85.97	0.33
Motherwort	Galeopsin	61.02	0.38
Motherwort	ZINC04073977	38	0.76
Arisaematis rhizoma	24-epicampesterol	37.58	0.71
Arisaematis rhizoma	[(2R)-2-[[[(2R)-2-(benzoylamino)-3-phenylpropanoyl]amino]methyl]-3-phenylpropyl]acetate	38.88	0.56
Arisaematis rhizoma	beta-sitosterol	36.91	0.75
Arisaematis rhizoma	Sitosterol	36.91	0.75
Arisaematis rhizoma	Stigmasterol	43.83	0.76
Arisaematis rhizoma	CLR	37.87	0.68
Rheum	Eupatin	50.8	0.41
Rheum	Rhein	47.07	0.28
Rheum	Toralactone	46.46	0.24
Rheum	Daucosterol_qt	35.89	0.7
Rheum	beta-sitosterol	36.91	0.75
Rheum	Aloe-emodin	83.38	0.24
Rheum	(-)-catechin	49.68	0.24

TABLE 2: THE ACTIVE COMPONENTS OF LEECH

Drug	Active ingredient
Leech	Indole-3-carboxaldehyde
Leech	Adenosine
Leech	Adenine
Leech	Uracil
Leech	Xanthine
Leech	2-piperidone
Leech	Cholesterol
Leech	Campesterol
Leech	Nicotinic acid
Leech	Uridine
Leech	Hypoxanthine
Leech	Inosine
Leech	Phenylalanine
Leech	Proline
Leech	Valine
Leech	L-isoleucine
Leech	Glycerol
Leech	Succinic acid
Leech	Palmitic acid
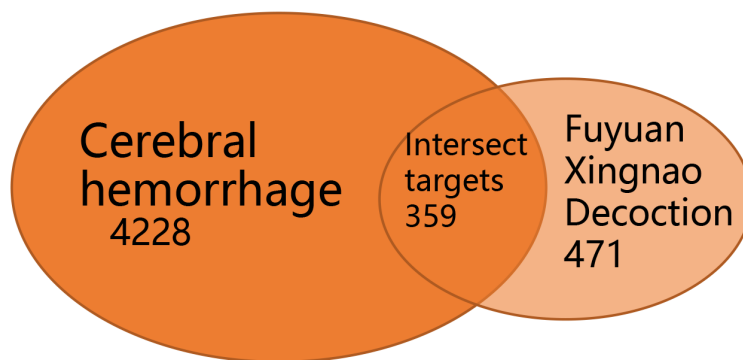
Fig. 1: Common targets of drugs and ICH
Note: The intersection generated between Fuyuan Xingnao decoction-associated targets and ICH related genes

Network diagram of Fuyuan Xingnao decoction-ingredients-target-ICH was shown in fig. 2. The data of the 7 flavors of TCM of the Fuyuan Xingnao decoction, active ingredients and intersection targets were entered into the Cytoscape 3.8.0 software and visualized to obtain the network diagram of Fuyuan Xingnao decoction-ingredients-target-ICH. The red square represents ICH, the hexagons of different colors represent the active ingredients of each Chinese medicine, the green square represents the repeated active ingredients between Chinese medicines, the pink circle represents the constituent drugs in the Fuyuan Xingnao decoction and the dark blue diamond represents the intersection target. In fig. 2, RS stands for ginseng, SQ stands for *Panax notoginseng*, SCP stands for *Acorus calamus*, YMC stands for motherwort, TNX stands for *Arisaematis rhizoma*, DH stands for *Rheum* and SZ stands for leech. The node size is positively correlated with the degree value and the larger the degree value, the larger the node.

PPI network analysis was shown in fig. 3. 359 intersecting targets were imported into the STRING database and the species was set to "*Homo sapiens*" to obtain a PPI network diagram. There are 358 nodes and 2729 edges in the figure, the average value of degree is 15.2 and the average local clustering coefficient is 0.344. The TSV format file was uploaded to Cytoscape 3.8.0 software for network topology analysis and the average value of degree was 38, and 97 core targets were screened out under the condition that degree was greater than 38 (Table 3 and fig. 3).

GO bioprocess and KEGG pathway enrichment was shown in fig. 4 and fig. 5. 359 intersecting targets were entered into the DAVID database for GO bioprocess and KEGG pathway enrichment analysis. According to p≤0.05 as the standard and p value size,

the first 20 bioprocesses and pathways were selected and displayed by enrichment bar plots. The biological processes mainly involve cell response to nitrogen compounds, regulation of neuronal death, positive regulation of cell death, inflammatory response, response to oxygen levels, circulatory system processes, etc. The pathways involved include cancer pathway, Phosphoinositide 3-kinase/Protein kinase B (PI3K-Akt) signaling pathway, Mitogen-Activated Protein Kinase (MAPK) signaling pathway, calcium signaling pathway, sphingolipid signaling pathway, etc.

Findings of animal experiment were shown in fig. 6. Neurological function of rats is assessed at 6 h post-modeling and 72 h post-modeling. At 6 h after modeling, rats in the Sham group had no behavioral disorders and the difference was significant compared with the ICH group ($p < 0.05$) (fig. 6A), confirming the successful establishment of an animal model of ICH. At 72 h after modeling, compared with the ICH group, the scores of the low, middle and high dose groups were significantly reduced ($p < 0.05$). In addition, the neurological function score was most significantly reduced between the three dose groups ($p < 0.05$) (fig. 6B). These results showed that the effect of Fuyuan Xingnao decoction improved the symptoms of neurological deficit after ICH and the effect was more pronounced with the increase of the dose.

HE staining evaluated the effect of Fuyuan Xingnao decoction on the surrounding tissues of cerebral hematoma. In the ICH group, we observed a decrease in the number of nerve cells, swelling and degeneration. Moreover, nerve cells are poorly structured and arranged out of order. After the treatment of Fuyuan Xingnao decoction, the degree of swelling of nerve cells in brain tissue was reduced and the cells were arranged relatively closely (fig. 7).
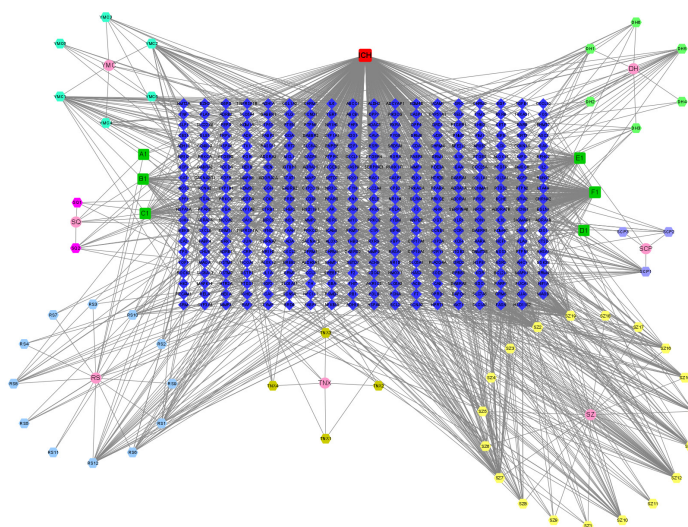
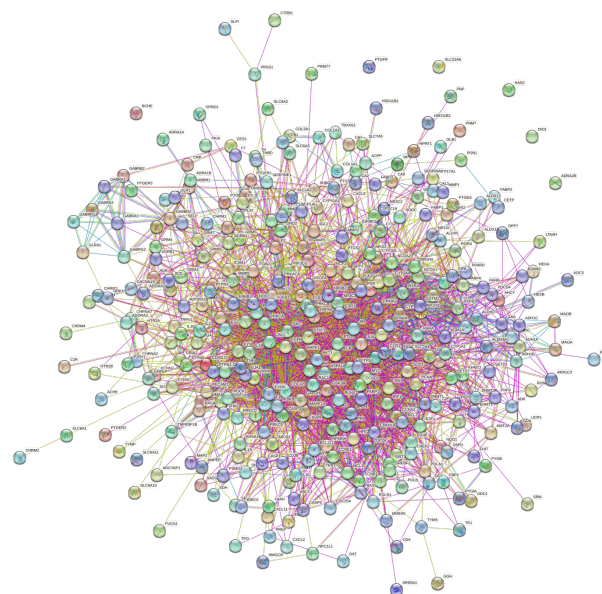Fig. 2: Network diagram of Fuyuan Xingnao decoction-component-target-ICH

Fig. 3: PPI network of active components of ICH

TABLE 3: CORE TARGETS OF "DRUG-ICH"

Target	Degree value	Target	Degree value	Target	Degree value
AKT1	158	IL2	72	CASP1	50
TNF	147	PTPRC	72	NOS2	49
IL6	146	BCL2L1	71	PARP1	49
TP53	133	IFNG	70	CXCL10	48
IL1B	127	PECAM1	69	CDK2	47
VEGFA	126	SERPINE1	68	SELP	46
JUN	120	CRP	68	F2	46
CASP3	119	MAPK14	68	PRKCD	45
EGFR	113	STAT1	68	CCNB1	45

MYC	111	MAPK8	66	RUNX2	45
PPARG	110	CAV1	63	IGFBP3	44
ESR1	109	ACE	63	SLC2A4	43
PTGS2	107	TGFB1	63	RAF1	42
HIF1A	104	AR	62	RB1	42
MMP9	104	MPO	60	HSPB1	41
EGF	102	NR3C1	59	CHUK	41
FOS	95	REN	58	PLAU	41
PTEN	94	KDR	57	CYP3A4	40
CXCL8	94	CASP9	57	DPP4	40
CCL2	93	PLG	57	ESR2	40
CCND1	92	CDKN1A	57	IGF2	40
IL10	92	TNFRSF1A	56	PRKCB	39
ERBB2	85	SPP1	55	F3	39
NOS3	82	MMP3	55	CXCL2	39
ICAM1	77	IKBKB	55	ABCB1	38
PPARA	77	SELE	54	SOD1	38
HMOX1	76	IL1A	54	GJA1	38
MMP2	76	PGR	54	NCF1	38
VCAM1	75	PRKCA	53	IRF1	38
MAPK1	75	MMP1	52	CCNA2	38
NFKBIA	75	GSK3B	52	FYN	38
CASP8	75	NFE2L2	52		
RELA	73	CD40LG	50		
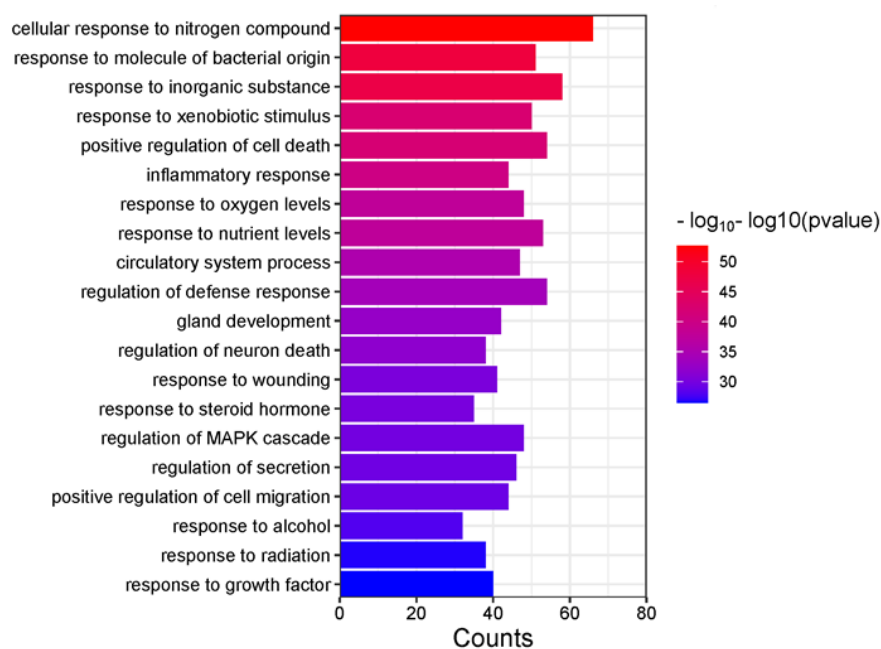
Fig. 4: GO biological process analysis
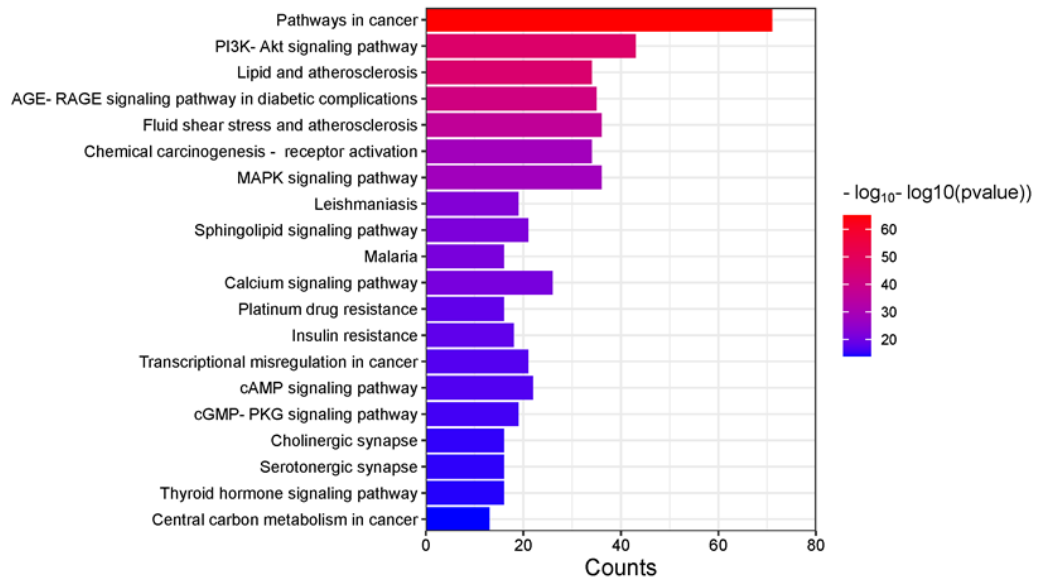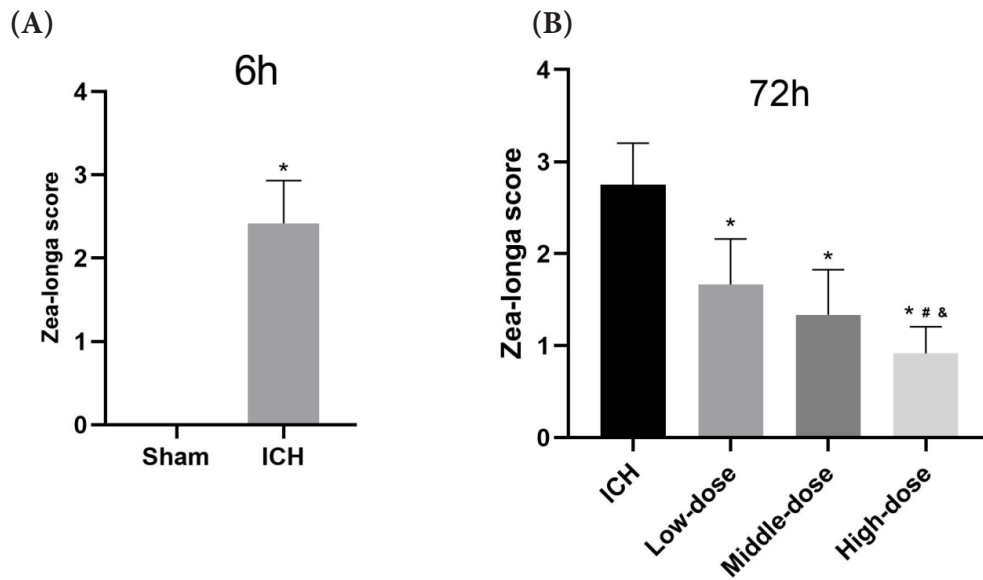
Fig. 5: KEGG enrichment analysis

Fig. 6: Neurological functional results
Note: Data of neurological deficit scores are shown as mean±Standard Deviation (SD) in each group, (A) Neurological function scores of rats 6 h post-operatively (n=12), *p<0.05 compared with Sham group and (B) Neurological function scores of rats 72 h postoperatively (n=12), *p<0.05 compared with ICH group; #p<0.05 compared with low-dose group and &p<0.05 compared with middle-dose group
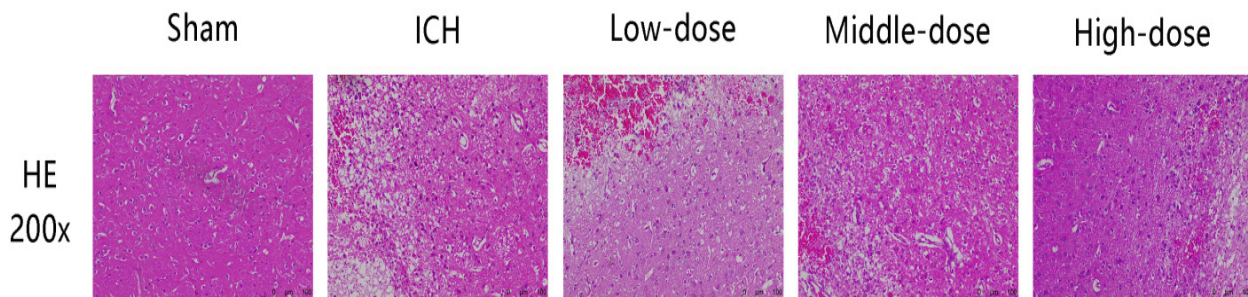

Fig. 7: Representative images showing the surroundings of a hematoma in brain tissue stained with HE as observed by microscopy

TCM is a complementary or alternative medical system used to treat various diseases[20] and is also a potential source of candidate resources for ICH treatment. Fuyuan Xingnao decoction is a commonly used TCM prescription in clinical practice, which has been used to treat diabetes, cerebral infarction, ICH and other diseases[21]. In recent years, through clinical experiments and animal experimental studies, the effect of Fuyuan Xingnao decoction has been extensively studied and it has been proven to have anti-inflammatory[12], antioxidant, anti-apoptotic effects[19]. Fuyuan Xingnao decoction also has the effect of promoting cerebral angiogenesis[17] and neuroprotection[22]. In our previous study, we confirmed the improved effect of this prescription on ICH[23], but the underlying mechanism is unclear. The theory of network pharmacology is basically consistent with the overall principle of TCM, which is very suitable for the analysis of multi-component and multi-target TCM prescriptions[24,25]. Therefore, we used network pharmacology methods, combined with active ingredient screening, drug targeting, bioprocess analysis and pathway analysis, to explore the potential mechanism of Fuyuan Xingnao decoction in the treatment of ICH, and further verified the therapeutic effect of Fuyuan Xingnao decoction in the treatment of ICH *in vivo* experiments.

We screened a total of 68 active ingredients from Fuyuan Xingnao decoction with 471 corresponding targets and 359 intersecting targets corresponding to ICH. Through the analysis of these intersecting targets, it was found that AKT Serine/Threonine Kinase 1 (AKT1) may be a key target. The analysis results of GO biological processes showed that the regulation of neuronal apoptosis was enriched and our KEGG pathway analysis showed that these targets were significantly enriched in the PI3K-Akt signaling pathway. There are three highly homologous subtypes in the AKT family, AKT1, AKT2 and AKT3. AKT1 participates in the cell survival pathway by inhibiting the apoptosis process, which can prevent apoptosis and promote cell survival. In addition, Akt is an important effector molecule downstream of PI3K. The PI3K/Akt pathway is related to cell proliferation and apoptosis[26]. PI3K is divided into three categories according to its structure and function, and type I PI3K is currently the most widely studied[27]. When PI3K is activated, PI3K acts as a second messenger to transmit the activation signal to Akt, thereby activating Akt phosphorylation and inducing a cascade reaction

with downstream molecules. Studies have found that the use of PI3K/Akt pathway inhibitors inhibits the beneficial effect of drugs on the expression of apoptosis markers after ICH. This suggests that the mechanism of action of drugs to improve neuronal apoptosis is related to the activation of the PI3K/Akt pathway to mediate the expression of downstream apoptosis-related proteins[28]. Some researchers have studied the mechanism of action of PI3K/Akt pathway in ICH and used baicalein to intervene in rats with ICH model, and found that baicalein can activate the PI3K/Akt signaling pathway to inhibit the expression of Nuclear Factor kappa B (NF-κB) protein and improve neuronal apoptosis in rats with ICH[29]. The results of our network pharmacological predictions have similarities with previous studies.

Fuyuan Xingnao decoction contains a long list of active compounds effective for stroke. Ginseng is one of the most important drugs in the prescription, whose main active ingredient was ginsenoside and it has anti-inflammatory, antioxidant and anti-apoptosis on cerebral ischemia and hypoxia[30]. It has been shown that ginsenosides Rg3 and Rh2 inhibit neuronal apoptosis and neuroinflammation by upregulating PI3K/Akt and inhibiting extracellular signal-regulating kinases[31]. In addition, the main active ingredient of the important Chinese medicine *Panax notoginseng* is a saponin, which has been confirmed to reduce cerebral edema, reduce Malondialdehyde (MDA) levels, reduce the expression of inflammatory factors, Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-α) and improve Superoxide Dismutases (SOD) activity in rats with ICH[32]. In addition, *Arisaematis rhizoma* may reduce cerebral edema by inhibiting Matrix Metalloproteinase-2 (MMP-2) expression[33]. *Acorus calamus* can also reduce cerebral edema and brain cell swelling after ICH[34]. Chrysophanol, the active ingredient of *Rheum*, inhibits apoptosis of nerve cells by regulating the PI3K/Akt/mammalian Target of Rapamycin (mTOR) signaling pathway, reducing the expression of inflammatory factors as well as the level of markers of oxidative stress, thereby improving brain injury in rats with ICH[35]. All these evidences supported the potential of Fuyuan Xingnao decoction in treatment of ICH.

At present, network pharmacology provides a powerful tool for exploring the compatibility and action mechanism of TCM prescriptions. However, there are some limitations. For example, main active ingredients should be considered in animal medical

research of Fuyuan Xingnao decoction. We will verify the efficacy of active ingredients in treating ICH through *in vivo* experiments. The results show that Fuyuan Xingnao decoction can effectively improve pathological changes and restore damaged nerve function in rats with ICH.

Author's contributions:

Bangjiang Fang is the corresponding author of the study; Xue Bai is the co-corresponding author of the study; Yanqi Luo and Yanjiao Li are the first authors who contributed equally to this work. Shuangyang Li helped in organizing the information. All authors read and approved the final manuscript.

Conflict of interests:

The authors declared no conflict of interest.

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