Modern research progress on pharmacological effects of paeoniflorin

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Abstract. Paeoniflorin is a monoterpenyl glycoside compound commonly found in Paeoniaceae plants. It has anti-inflammatory, analgesic, anti-tumor, immune regulation, nerve protection, anti-depression, improvement of diabetic nephropathy, anti-oxidation, and inhibition of intracellular Calcium overload, improve mitochondrial function, reduce dyslipidemia, inhibit apoptosis and inhibit cell autophagy and other biological activities. In recent years, research on paeoniflorin at home and abroad has been extensive and profound. This article focuses on the research progress of paeoniflorin in pharmacological effects.

1. Introduction
Paeoniflorin (PF), also known as Peonidin, is a glycoside compound. It is a pinane monoterpenyl picroside extracted from the root of the Ranunculaceae plant Paeonia lactiflora. A large number of studies have shown that paeoniflorin has a wide range of biological activities, such as anti-inflammatory, analgesic, anti-tumor, immune regulation, nerve protection, anti-depression, improvement of diabetic nephropathy, anti-oxidation, inhibition of intracellular calcium overload, improvement of mitochondrial function, and relaxation. Reduce abnormal blood lipids, inhibit apoptosis and inhibit autophagy and other effects. This paper mainly summarizes the research status of paeoniflorin's pharmacological effects and mechanism in recent years.

2. The structure and physicochemical properties of paeoniflorin
Paeoniflorin is an effective monomer component extracted from traditional Chinese medicines such as Paeoniae Radix Alba, Paeoniae Radix Rubra, and Paeonia suffruticosa Andr. It is a hygroscopic amorphous powder with a chemical formula of C_{23}H_{28}O_{11} and a molecular weight of 480.50, melting point 196°C. This product has very low toxicity and unstable chemical properties. It needs to be stored in a low temperature and frozen environment, and it is stable in an acidic environment (pH 2~6), but unstable in an alkaline environment [1]. It’s structure and physicochemical properties are listed in Table 1.

Table 1. Structure and physicochemical properties of Paeoniflorin

| Compound     | Molecular formula | Molecular weight | Molecular Structure | Attributes             | Identification method |
|--------------|-------------------|------------------|---------------------|------------------------|-----------------------|
| Paeoniflorin | C_{23}H_{28}O_{11} | 480.45           |                     | Hygroscopic, amorphous powder | NMR; MS               |
3. Experimental research on pharmacological effects

3.1. Anti-inflammatory effect

Paeoniflorin has anti-inflammatory effects. Studies have shown that paeoniflorin has a certain protective effect on acute inflammatory brain injury in mice caused by lipopolysaccharide (LPS). Related researches showed that in mice brain tissues with brain damage caused by endotoxin, the activities of myeloperoxidase (MPO) and inducible nitric oxide synthase (iNOS) decreased, NO content decreased in brain tissue, serum TNF-α (tumor necrosis factor-alpha), IL-1β (Interleukin-1β) and iNOS mRNA expression were down-regulated. It is speculated that the mechanism may be related to the inhibition of nuclear translocation of NF-kB (nuclear factor kappa-B), thereby inhibiting the expression of inflammatory factors [2].

Paeoniflorin can down-regulate the expression of pro-inflammatory factors IL-2 and IL-6 in ulcerative colitis (UC) rats, significantly decrease serum IL-1β and TNF-α levels, and up-regulate the expression of anti-inflammatory factors IL-10 and IL-4, accelerate the balance of anti-inflammatory and anti-inflammatory factors, regulate the abnormal immune response of the intestines, suppress the inflammatory response, and repair ulcers [3, 4].

It can be seen that paeoniflorin has a significant anti-inflammatory effect, and its mechanism of action may be achieved by inhibiting the expression of inflammatory factors and regulating the balance of inflammatory factors (Table 2).

| Administration Route | Dose | Model | Indicators | Results |
|----------------------|------|-------|------------|---------|
| Intraperitoneal injection | Low: 10mg/kg; High: 30mg/kg | LPS-induced acute brain injury model in mice | Serum TNF-α, IL-1β, MPO in brain tissue, iNOS activity, NO content | MPO, iNOS activity, NO content gets low, TNF-α, IL-1β, iNOS mRNA down-regulated |
| Gavage | 2.5 g/kg | UC rat model | IL-2, IL-6, IL-10 | IL-2 and IL-6 down-regulated, IL-10 up-regulated |
| Gavage | 2.5 g/kg | UC rat model | IL-1β, TNF-α, IL-4 | IL-1β, TNF-α down-regulated, IL-4 up-regulated |

3.2. Analgesic effect

Paeoniflorin has a strong analgesic effect, which can significantly increase the pain threshold of the mouse model of inflammatory pain induced by Freund's complete adjuvant. Further studies have found that paeoniflorin can inhibit the release of peripheral inflammatory factors, reduce the synthesis of NO in the spinal cord, return the expression of iNOS to normal, and inhibit the activation of spinal microglia. This process may signal Akt-NF-κB, participate. It is inferred that paeoniflorin analgesia may be achieved by inhibiting the Akt-NF-κB signaling pathway, inhibiting the release of inflammatory factors and the activation of spinal microglia [5]. Its analgesic effect may also be related to increasing serum β-endorphin (β-EP) and cerebral cortex β-EP levels, and reducing the production or release of prostaglandin E2 (PGE2) in the cerebral cortex [6]. In addition, paeoniflorin can inhibit ASK1 (Apoptosis signal-regulating kinase 1) activation, effectively inhibit the response of glial cells and CCI-induced neuroinflammation, and play an analgesic effect (Table 3) [7].
3.3. Anti-tumor effect
Paeoniflorin has anti-tumor effects. It can inhibit the phosphorylation of p65 and IκBα, and at the same time activate the activity of Caspase3 to induce tumor cell transformation [8]. Paeoniflorin can inhibit the proliferation and invasion of breast cancer cells by inhibiting the NOTCH-1 signaling pathway [9]. Paeoniflorin also upregulates the expression of HRTA3, promotes HTRA3-mediated apoptosis, and inhibits the growth of pancreatic cancer cells [10]. Paeoniflorin has good anti-cancer activity on a variety of tumors. At present, the anti-cancer activity of paeoniflorin has been comprehensively studied. The study of various in vivo and in vitro cancer activities and related molecular mechanisms will be beneficial to this natural compound as a clinical anti-cancer. Further development and utilization of drugs (Table 4).

Table 3. Analgesic effect of paeoniflorin

| Administration Route | Dose       | Model                                                                 | Indicators                                                                 | Results                                                                                           |
|---------------------|------------|----------------------------------------------------------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Intraperitoneal     | 80, 40,    | Acetic acid writhing method-induced pain model in mice                | Number of twists                                                          | Decreased number of twists                                                                        |
|                     | 20 mg/kg   |                                                                      |                                                                            |                                                                                                  |
| Intraperitoneal     | 80 mg/kg   | Freund's complete adjuvant induced inflammation pain model in mice   | Mechanical pain threshold, hot plate pain threshold, TNF-α, NO, spinal cord i NOS | Mouse mechanical pain threshold, the mean threshold of hot plate pain rises, inhibit the release of TNF-α et al., NO synthesis decreased, i NOS expression returned to normal |

3.4. Immunomodulation
Paeoniflorin can significantly increase the number of peripheral blood white blood cells in mice with blood deficiency syndrome caused by radiation, colony forming unit of bone marrow macrophages (CFU-GM), colony forming unit of burst red blood cell (BFU-E), colony forming unit of erythrocyte (CFU-E) and mixed colony forming unit (CFU-M). At the same time, it can up-regulate the expression of hematopoietic growth factors, Epo (erythropoietin) and granulocyte colony-stimulating factor (G-CSF) genes, and promote the recovery of bone marrow hematopoietic function [11].

In addition, experimental results show that paeoniflorin can inhibit the proliferation of Th1 cells (IFN-γ+CD4+) and Th17 cells (IL-17+CD4+), while increasing the expression of Th2 cells (IL-4+CD4+) [12]. All these results have revealed the immunomodulatory effect of paeoniflorin (Table 5).

Table 4. Antitumor effect of paeoniflorin

| Effective concentration | Model                                                                 | Indicators          | Results                                                                                           |
|-------------------------|----------------------------------------------------------------------|---------------------|--------------------------------------------------------------------------------------------------|
| 0.5, 1.0, 2.0 mg/mL     | Hepatoma cell line: HepG2                                             | Caspase3, NF-kB p65 | Activates Caspase-3, inhibits p65, Phosphorylation of NF-kB and IκBα induces apoptosis of cancer cell HepG2 |
| 0, 7.5, 15, 30 μmol/L   | Breast cancer cell line: MCF-7                                       | NOTCH-1, HES-1 expression level | Through the inhibitory pathway Notch-1 to inhibit breast cancer cell proliferation and invasion |
| 0–1000 μmol/L           | Pancreatic cancer cell line: Capan-1, MIAPaCa-2                      | HTRA3               | Up-regulation of HTRA3 expression, promote HTRA3-mediated apoptosis, and inhibit the growth of pancreatic cancer cells |

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Table 5. Immunomodulatory effect of paeoniflorin

| Administration Route | Dose     | Model                                      | Indicators       | Results                                                                 |
|----------------------|----------|--------------------------------------------|------------------|-------------------------------------------------------------------------|
| Gavage               | 26.54 mg / kg | Radiation-induced blood deficiency model in mice | Epo, G-CSF       | Promote the expression of bone marrow hematopoietic growth factors Epo and G-CSF to recover hematopoietic function |
| Gavage               | 100 mg / kg  | Candida albicans systemic infection mouse model | IFN-γ⁺CD4⁺, IL-17⁺CD4⁺, IL-4⁺CD4⁺ | Inhibit the proliferation of Th1 cells and Th17 cells, increase Th2 cell expression |

3.5. Neuroprotection
Paeoniflorin can effectively improve the learning and memory impairment of APP/PS1 double transgenic mice. After treatment with paeoniflorin, the neuronal apoptosis of AD (Alzheimer's disease) model decreased significantly. The mechanism of action may be through activation of PI3K/Akt pathway, thereby up-regulating Bcl-2 and down-regulating the proteins of Caspase-3, Caspase-9 and Bax. The expression level inhibits the apoptosis of nerve cells, thereby protecting nerve cells and achieving the goal of treating neurodegenerative diseases [13].

Vascular dementia (VD) is a severe cognitive dysfunction syndrome caused by cerebrovascular disease. Microglia (MG) are not only the key immune cells in the central nervous system (CNS), but also the important effector cells in the neuroinflammatory response. After stress or injury, the phenotype and polarization of MG (M1 or M2) play a key role in slowing down the inflammatory response of CNS diseases and accelerating the repair of nerve cells. It is concluded that paeoniflorin has a neuroprotective effect and can activate CB2R to regulate the M1/M2 phenotypic polarization transition of microglia in vascular dementia rats. It is speculated that its mechanism may be related to the signaling pathways regulating mTOR/NF-κB and PI3K/Akt (Table 6) [14].

Table 6. Protective Effect of Paeoniflorin on Nerve

| Administration Route | Dose     | Model                                      | Indicators       | Results                                                                 |
|----------------------|----------|--------------------------------------------|------------------|-------------------------------------------------------------------------|
| Intraperitoneal injection | 5 mg / kg | APP / PS1 transgenic mice                  | Caspase-3, Bcl-2, Caspase-9, Bax | Bcl-2 up-regulated, Caspase-9, Bax, and Caspase-3 protein expression down-regulated |
| Intraperitoneal injection | 10, 20, 40 mg/kg | VD rat                                    | M1 phenotype marker CD68, IL-1β, TNF-α, IL-6, iNOS, NO, M2 phenotype marker CD206, IL-10, arginase-1, TGF-β1, Ym1 | Inhibit M1 phenotype markers, and further increase the expression of M2 phenotype markers |

3.6. Antidepressant effect
Paeoniflorin can improve depression induced by long-term high-dose interferon-α (IFN-α). Experimental studies have shown that long-term high-dose use of IFN-α can induce depression-like behavior, which is related to mPFC, vHi, and interleukins (IL-1β, IL-6, IL-9, IL-10, IL-12), Tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) are related, especially the obvious neuroinflammation in the amygdala. Paeoniflorin has a certain antidepressant and anti-neuro-
inflammation effect on the behavior and specific emotion-related areas of interferon-α-induced depression mice (C57BL/6J), can reduce neuroinflammation in the brain area, and exert an anti-depressant effect [15]. Experiments have shown that paeoniflorin can significantly improve the depressive symptoms of depression model rats, and effectively improve the expression rhythm of circadian clock genes in the hippocampus of depression model rats. It is speculated that the antidepressant effect of paeoniflorin may be related to the changes in the expression of circadian clock gene proteins and mRNA in the hippocampus of depression model rats (Table 8) [16].

**Table 8. Effect of paeoniflorin on diabetic complications**

| Administration Route | Dose       | Model       | Indicators          | Results                                                                 |
|----------------------|------------|-------------|---------------------|------------------------------------------------------------------------|
| Gavage               | 20 mg/kg   | Diabetic rat| GFAP, glutamic acid, GLAST, GS | GLAST, GS expression is up-regulated, GFAP expression and glutamate content are significantly decreased |
| Intraperitoneal injection | 25,50,100mg/kg | Diabetic mice | CD68, TNF-α, IL-1β, MCP-1, i NOS mRNA | PF diabetic mice i NOS expression down-regulated, TNF-α, IL-1β, MCP-1, CD68 down |

3.7. **Other effects**

In recent years, a large number of studies have found that paeoniflorin has a significant antioxidant effect [19], mainly by inhibiting hyperoxidation, improving antioxidant capacity [20], inhibiting calcium ion overload [21], improving mitochondrial function [22] and many other ways and means. Targets play a role and have special advantages in anti-oxidative stress. Studies have also found that paeoniflorin has the effects of anti-platelet aggregation, lowering blood viscosity, regulating blood lipids, dilating blood vessels, and reducing dyslipidemia (Table 9) [23].

**Table 9. Other Functions of Paeoniflorin**

| Dose              | Model       | Indicators          | Results                                                                 |
|-------------------|-------------|---------------------|------------------------------------------------------------------------|
| 10^{-7}, 10^{-6}, 10^{-5} mol / L (20μL/50mg) | —           | MDA, T - AOC       | MDA levels fell, T – AOC Increased                                     |
| 0, 2, 10, 50 μM PC12 cell | cytochrome C, Caspase-3, Caspase-9 | Mitochondrial membrane potential decreases, increasing the release of cytochrome c and the activity of Caspase-3 and Caspase-9 |
| 10/20/30 mg/kg/day ApoE⁻/⁻ mice | LDL-C, TC, TG | After PF treatment, TC, LDL-C and TG levels were significantly reduced |

4. **Clinical research on pharmacological effects**

In recent years, there have been many studies on Chinese medicine, including zoology and clinical researches on pharmacological effects and pharmacology [24-34], studies on germplasm resources and component identification of medicinal plants [35-38], and researches on drug delivery and Chinese medicine formulation [39-42]. These studies have made in-depth analysis from various aspects, more in-depth elucidation of the ingredients and mechanism of Chinese medicine, and laid a foundation for better development and utilization of Chinese medicine.
In clinical application, paeoniflorin can significantly slow down the consumption of morphine in patients with cancer pain, and can reduce the side effects of morphine. The results of clinical controlled trials showed that after morphine was combined with paeoniflorin, the patient's morphine consumption was significantly reduced. Compared with other patients with similar pain levels, their morphine consumption was also significantly reduced, and paeoniflorin could also reduce the side effects caused by morphine. It is speculated that this effect of paeoniflorin may be related to reducing neuroinflammation [43]. Paeoniflorin is one of the main active substances in Danggui Shaoyao San (DSS) that can inhibit neuroinflammatory response. It can inhibit neuroinflammatory response through MAPK and NF-KB signaling pathways. A large number of clinical studies have shown that DSS can improve Moderate and severe Alzheimer's disease (AD) patients have dyskinesias and cognitive decline [44]. In other clinical trials, it is believed that paeoniflorin may be one of the key active ingredients of the prescription for soothing the liver and strengthening the spleen (the content of paeoniflorin is 25.34mg/g). Shugan Jianpi Decoction can improve the menstrual cycle, menstrual quality, etc., can significantly improve the six levels of serum sex hormones in patients with liver depression and spleen deficiency infertility, and treat liver depression and spleen deficiency infertility [45].

Table 10. Clinical study on paeoniflorin

| Ingredient          | Source                                  | Case                      | Concentration range | Medicinal effect                                                                 |
|---------------------|-----------------------------------------|---------------------------|---------------------|---------------------------------------------------------------------------------|
| Paeoniflorin with morphine | Paeonia total glycosides capsules | Patients with advanced cancer | (0.3 g capsules, with the content of paeoniflorin 104 mg) 0.6 g tid | Paeoniflorin can significantly reduce the patient's dosage of morphine, and reduced the side effects of morphine |
| Paeoniflorin         | Shugan Jianpi (White Peony Root, Bupleurum Root, Angelica, Atractylodes macrocephala, Fuling) | Infertility patients with liver depression and spleen deficiency | 25.34mg/g | Significantly improve the six levels of serum sex hormones in patients with liver depression and spleen deficiency infertility, and treat liver depression and spleen deficiency infertility |

5. Conclusion
In summary, paeoniflorin not only has the effects of anti-inflammatory, analgesic, anti-tumor, immune regulation, nerve protection, anti-depression, and improvement of diabetic nephropathy, but also anti-oxidation, inhibits intracellular calcium overload, improves mitochondrial function, and slows down Dyslipidemia, inhibition of apoptosis and other biological activities. It can be seen that paeoniflorin has a wide range of biological activities, can act on multiple targets, multiple pathways, and treat multiple diseases, and is expected to become a drug for the prevention and treatment of multiple diseases. However, it was also found that the research on paeoniflorin mainly focused on anti-inflammation, anti-tumor, immune regulation, anti-apoptosis, etc. Although the mechanism researches were extensive but not in-depth, more potential pharmacological effects and targets may not have been discovered yet. Therefore, the research on the pharmacological effects and mechanism of paeoniflorin should be further studied, so as to better realize its medicinal value.

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