Study on the Property Change of Rhizoma Coptidis and Its Ginger Juice Processed Products Based on 5-Ht Level and Brain Tissues Morphology of Rats

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Abstract. According to the theory of traditional Chinese Medicine (TCM), all Chinese materia medica need to be processed using Pao zhi which is a processing technology before being used in clinic. Ginger juice, made from dried or fresh ginger, is one of the main TCM processing accessories and always used to help change some Chinese materia medica's properties for its warm or hot nature. The purpose of this paper is to discuss the influence of ginger juice on Rhizoma Coptidis (RC) by determining 5–hydroxytryptamine (5-HT) content and observing morphological changes in the harns tissue of rats. Raw Rhizoma Coptidis (RRC), fresh ginger juice processed Rhizoma Coptidis (FGJPRC), dried juice processed Rhizoma Coptidis (DGJPRC), dried ginger juice (DGJ) and fresh ginger juice (FGJ) were prepared using appropriate methods. Immunohistochemical staining was used to observe the distribution of 5-HT and fluorescence spectrophotometry was applied to determine 5-hydroxytryptamine content in the brain tissue of rats. 5 - HT in brain tissue of the rats of RRC group was distributed most densely, with the highest content. Compared to the blank group, RRC and different ginger processed RC groups could lead to increasing content of 5-HT in rat encephalon, and significant differences in RRC. Compared with the RRC, the 5-HT content in rat encephalon in DGJPRC, FGJPRC, FGJ and DGJ groups reduced, and DGJPRC, FGJPRC groups showed significant difference, FGJ and DGJ groups showed extreme significant differences. The research showed that processing with hot, warm accessories would moderate the cold nature of RC. The cold and hot nature of Traditional Chinese Materia Medica could be expressed by the difference of 5-HT contents and morphological changes of rats' brain tissue. Simultaneously, the research showed the different excipient of ginger juice would have different effects on the processing of RC.

1. Introduction

The medicinal properties of TCM are known as drug biased. Drugs through processing can change biased. RC is the dried rhizome which comes from *Ranunculaceae Coptis chinensis Franch., Coptis teeta Wall., Coptisdeltoidea C.Y.Cheng* et Hsiao., often called *"Coptis chinensis*"," *Coptis teeta*", and "*Radix Coptidis Deltoideae*". It is mainly distributed in Sichuan, Hubei and Yunnan province, etc. The nature of RC is bitter and cold. Its medical effects are always considered as on the heart, spleen, liver, stomach, gallbladder and large intestine meridian. It has the function of clearing away heat , eliminating damp evil and removing toxic material [1]. 5-HT is an significant monoamine neurotransmitter within the cerebrospinal axis. 5-HT neuron cell bodies located mainly in the Raphe

nuclei of the brainstem and its adjacent areas, their projection fibers are widely distributed in the cerebral cortex, subcortical region, brainstem, cerebellum and spinal cord, the brain region. 5-HT transmitters with different receptor subtypes activate second messengers and ion channels which have effects on the cardiovascular and respiratory systems, body temperature, apetite, sexual behavior, sleep, pain and emotional regulation of a variety of complex physiological and pathological process [2-5]. In addition, 5-HT transmitter also play an important role in learning and memory [6]. Research shows that Chinese herbal compound has different effects in central monoamine neurotransmitter [7], such as 5-HT, prompted by the central monoamine neurotransmitter and cold-heat nature exist correlation. In this article, through different dosages on groups of rats, the effect on brain morphological and distribution and the change of the content of 5-HT research is discussed the influence of different ginger systems of RC properties.

2. Material and equipment

2.1. Reagents

Serotonin standard was purchased from NICPBP and its NICPBP was 111656-200401.The n-Butanol, n-Heptane and Ether were obtained from Tianjin Hengxing chemical reagents company. L–cysteine and o-Phthalaldehyde were obtained from the Shanghai Kaiyang biotechnology company. SP-Histostain TM-Plus kits, the condensed reagents kits together with the serotonin antibody were obtained from Wuhan doctor de sheng bioengineering company and the Hcl, paraformaldehyde, glutaraldehyde were from Sinopharm Group chemical reagents company. Sucrose was prepared too.

2.2. Apparatus

The Cary Eclipse Fluoscence was obtained from the American varian Technology Corporation, China. Desktop low temperature and high speed refrigerated centrifuge purchased from Sartorius (Germany), an Electro-Thermostatic Water Bath from Shanghai Instrument Company was used along with pipettes from Shanghai tuo mo si scientific instrument company. A manual homogenizer from Beijing Younikang biotechnology scientific company, Raynger ST infrared thermometer from American Raytek, herb grinder from Changsha Xulang grinding mechanical company, RE52CS rotary evaporator from Shanghai Yarong bio-chemical instrument company, SHZ-D (III) rotary water vacuum pump from Gongyi Yuhua instrument company, HH-1508R III computer rapid cold-tome from Huahai scientific and teaching instrument company, Jinhua, Zhejiang, E220LED biologic microscope from Xiamen MOTIC company was used for the experimental analysis of samples.

3. Experimental methods

3.1. Preparation of Rhizoma Coptidis and its processed product

Rhizoma Coptidis was purchased from the Tianqi Hall medicine plants and produced from Sichuan. The preparation of FGIPRC contained mixing 100g RC Tablets with 10% FGJ moistening and stir frying 15 minutes at 120 ˚C in stir fry- container, sieving and allowing debris to cool. DGJPRC was prepared with a same method, where 100 g of RRC and 10% DGJ were mixed with each other, moistening and stir frying 15 minutes at 120 ˚C in stir fry- container, sieving and allowing debris to cool.

3.2. Sample preparation

RRC, FGJPRC and DGJPRC powders (150 g each) were sifted through a 5 mm mesh screen, added 10 times the bulk of water and allowed to soak for 60 minutes and decanted after that. The solutions filtered after boiled for 30 minutes. Residues were decanted again by putting in 8 times the bulk of water, filtering after boiling for 30 minutes. Both percolates were gathered and concentrated to 1g•ml-1. RRC, FGJPRC and DGJPRC were extracted separately by 150 ml, the concentration decantation was kept at 4 ˚C before use.

4. Observation of the morphology of cerebrum tissues

4.1. Grouping and medicine administration

There had 72 female SD rats weighing 180–220 g offered by Experimental Animals Co., Ltd of Cavins in Changzhou (Certificate No.SCXK2011-0003) were grouped into six groups randomly, and each group including 12 rats as follows: blank control group, RRC, FGJPRC, DGJPRC, FGJ and DGJ groups. The groups were treated with RRC, FGJPRC, DGJPRC, FGJ and DGJ at dosage of 3 ml (about containing crude drug 3g) once a day for 3 weeks. In each group, eight rats were put into death based on medicine administration to take cerebrum tissues for the mensuration of 5-HT content. And the rest four rats were used in morphological researches.

4.2. Preparation of section

Cerebrums were harvested (foramen magnum with scissors transection, carefully in the foramen magnum inclined insert the scissors to cut open the parietal bone, hemostatic forceps disc parietal bone broken on both sides, the olfactory bulb was shangdi parietal carefully removed, one side with scissors to cut the optic nerve and agent to the base of skull, the entire block of brain tissue is removed) 1h after the RRC, FGJPR, DGJPRC, FGJ, DGJ, blank groups rats in 22 days for 1 hour were sacrificed by cardiac perfusion-fixation under chloral hydrate anesthesia (fixed liquid: 10% paraformaldehyde, 0. 1% glutaraldehyde, and 0. 1 M phosphate buffer into 250 ml), and were cleaned with ice normal saline. After washing, brain tissues were placed in post-fixation fluid (to remove the glutaraldehyde from the fixing solution) for 3-4 hours; the liquor was substituted by phosphate buffer containing 30% sugar and kept at 4 ˚C past a night. After being organization fully at the bottom of the sink solution, from the optic decussation to the cortex, a vertical dissection was executed to excise the prosencephalon. Another vertical dissection was executed to cut off a section of the cortex from the hinder boundary of the hypothalamus to the cortex. After the spinal cord and epencephalon were cleared away, the remainder was the stem of brain (including the quadrigeminal body, pons, and medulla oblongata) was removed. The stem of brain was cut into coronal sections and a series of cryostat sections 35 μm thick were used for immunohistochemical staining.

4.3. Operating method of Rat Cardiac perfusion fixation

Two syringes separately filled with normal saline and fixative were adjusted to make the tube free of bubbles. The rats relaxed their front and hind legs after anesthetized, and were laid on the autopsy table with their legs fixed. Abdominal skin was raised with tweezers and a small incision in the right hand side of the abdomen made with scissors. The skin was cut from the incision to the jaw along midabdominal centre line and xiphoid centre line for separation of subcutaneous tissue, and then the skin turned to two sides. The sternum was cut upwards along the midabdominal centre line and xiphoid centre line again, and cut along the two sides of the diaphragm muscle using a hemostat to clamp tightly the skin of sternum and chest, which was rolled outwards to expose the heart fully. The pericardium was opened with the tweezers saline solution used to keep the heart moist. The aorta was isolated the aorta prepared for ligation and infusion using silk thread. A small incision was cut in the left ventricle with ophthalic scissors, then an infusion needle inserted into the left ventricle with a silk thread ligation whilst infusing with the physiological saline at a rate around 20 ml/min. At the same time, the right atrium was cut to discharge the blood. The liver was observed until it gradually became white. After the fixed liquid went into the blood vessels of rats, the rats, limbs appeared to twitch gradually, confirming that the perfusion fluid had gone into the brain

4.4. Immunohistochemical Staining

Staining and color development were performed by ASP kit and concentrated DAB kit, Each tissue parts were put on poly-L-lysine-Prep slides and dried with fan at temperature 58 ˚C~ 60 ˚C for 1 hour, fixed with 4% paraformaldehyde for 30 min and incubated in 3% (v/v) H_2O_2 deionizer water for 5–10 minutes to dispel endogenous peroxidase activity. Then, Samples were washed 1 ~ 2 times with water

and 5% BSA sealing fluid, rinsed with tap water for 20 minutes at room temperature, washed in distilled water for 2 min, followed by soaking in 75% ethanol, 95% ethanol for 3 minutes then anhydrous ethanol soak for 3 min, This was followed by washing in xylene 1 for 5 min, xylene 2 soak for 5 min.

4.5 .Microscopic observation of section
A multiple lensed microscope adjusted to times 40 magnification was used. The brain sections obtained from the RRC, FGJMRC, DGJMRC, FGJ, DGJ and blank groups were observed after color development and several micrographs were taken from each group at biological microscope.

5 Effection of 5-HT concentraction in rats cerebrum tissues

5.1. Configuration of solutions for processing of cerebrum tissue needed
Prepared Acidic n-butanol by mixing 0.85 ml concentrated HCl with 1L n-butanol. Cysteine solution (0.25%) was newly prepared with 0.1 M HCL. 0.002%o-phthalaldehyde-10 M HCl solution was newly prepared before use.

5.2. Preparation of 5-HT standard solution
5-HT standard solutions were prepared by putting 2 mg concentrated 0.01 M HCl in 200ml and mixed. Take solution 0.5 ml, 1.0ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, respectively with 0.01 mol/l HCL constant volume to 10 ml, at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0μg/ml and set aside.

5.3. Brain tissue processing and 5 - HT content determination
The rat brains of RRC, FGJPRC, ZGJPRC, FGJ, DGJ and blank groups were fast obtained after putting rats to death. The cerebrum tissues were washed with saline of ice-cold tempreture to clear away epencephalon, the olfactory bulb and blood components. Weighed Half of the cerebrum tissue after filter paper absorbed excess water. Put ten times bulk of the pre-cooled acidic n-butanol into whole cerebrum tissues. Centrifuged samples for 5 minutes and 2.5 ml of supernatant were sub-packed and put in 1 ml 0.1 M HCl. Afterwards vibrated samples for 5 minutes, centrifuged for 5 minutes and probably 0.4 ml of the bottom phase was received. To this liquor, 0.1 ml of newly prepared 0.25% cysteine and 3 ml 0.002% o-phthalaldehyde-10 M HCl was added, mixed and boiled for 10 minutes. This mixed liquor was used to mensurate the concentration of 5-HT after chilled down. The fluorescence at 360 nm was mensurated in a Cary Eclipse fluorescence spectrophotometer (Varian Inc., USA) by using an excitation wavelength of 480 nm [8]. 0.5ml 5-HT standard solutions with different concentration were disposed with ten times of acidic n-butanol and mixed as standard. The remaining steps taken were similar as those used in the samples. Another tube applied as blank was full of 0.5ml 0.01 M HCl, with 5 ml acidic n-butanol put in and mixed each other. The rest steps were used for the samples as a blank pipe were used for zero.

6. Results

6.1. Brain tissue morphology
Comparing with blank control group, the 5-HT-immunopositive neurons in the brain stem raphe nuclei showed denser distribution in RRC, FGJPRC and DGJPRC groups, but compared with RRC group, FGJPRC and DGJPRC stained more sparsely and showed lighter coloring. Compared with the blank group, 5-HT neurons in cell showed lighter coloring and only found in the brainstem raphe nucleus in FGJ and DGJ groups, other areas appeared to be circular and blank. Due to less immune response, and dyeing, DGJ group was less stained than that FGJ group. The results was shown in Figure 1.

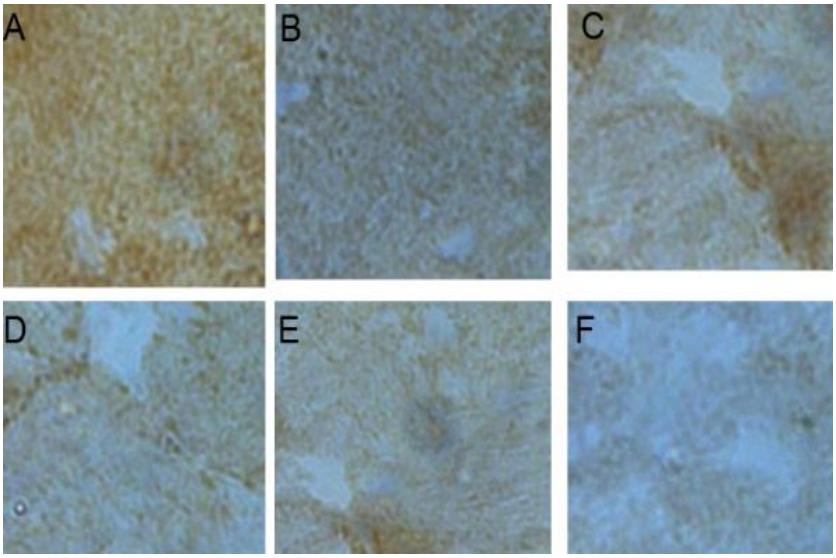

Figure.1 Brain tissue sections treated by six products. A: RRC group; B: Blank group; C: FGJPRC group; D: DGJPRC group; E: FGJ group; F: DGJ group.

6.2. The change of brain 5 - HT content

Using the blank tube to set the spectrophotometer to zero and then fluorescent intensity of the known concentration of 5-HT standard solutions was determined.

The fluorescent intensity of each group measured value were taken into 5-HT standard linear regression equation and used to calculate the content of 5-HT in different samples.

The results showed that, compared to the blank group, RRC and different ginger processed RC groups could lead to increasing content of 5-HT in rat harns, and significant differences in RRC. DGJ and FGJ groups could decrease the content of 5-HT in the harns of rats, without significant difference. Compared with the RRC, the content of 5-HT in rat brain in DGJPRC, FGJPRC, FGJ and DGJ groups reduced, and DGJPRC, FGJPRC groups showed significant difference, FGJ and DGJ groups showed extreme significant differences. FGJ compared with DGJ, DGJPRC compared with FGJPRC, although there were no significant difference, but there was a trend of reduce in the content of 5-HT. The results were shown in table 1, table 2 and figure 2

Table 1. The concentration and fluorescence intensity 5 - HT standard solution

Label	Solution[μg/ml]	Fluorescent intensity
1	0.5	7.421
2	1.0	13.572
3	1.5	20.135
4	2.0	28.98
5	2.5	39.341
6	3.0	43.245

Table 2. 5-HT contents in different samples.

Group	5-HT content[μg/g][$\bar{x} \pm s$]
Blank	19.05 ± 1.15
RRC	21.11 ± 1.78^{a}
DGJPRC	20.02 ± 1.15^{b}

FGJPRC	20.11 ± 1.10^{b}
FGJ	$18.68 \pm 1.50^{c,d,e}$
DGJ	$18.38 \pm 1.98^{c,d,e}$

[a] compared with blank group $P < 0.05$.
[b] compared with RRC group $P < 0.05$.
[c] compared with RRC group $P<0.01$.
[d] compared with FGJPRC group $P<0.05$.
[e] compared with DGJPRC group $P<0.05$.

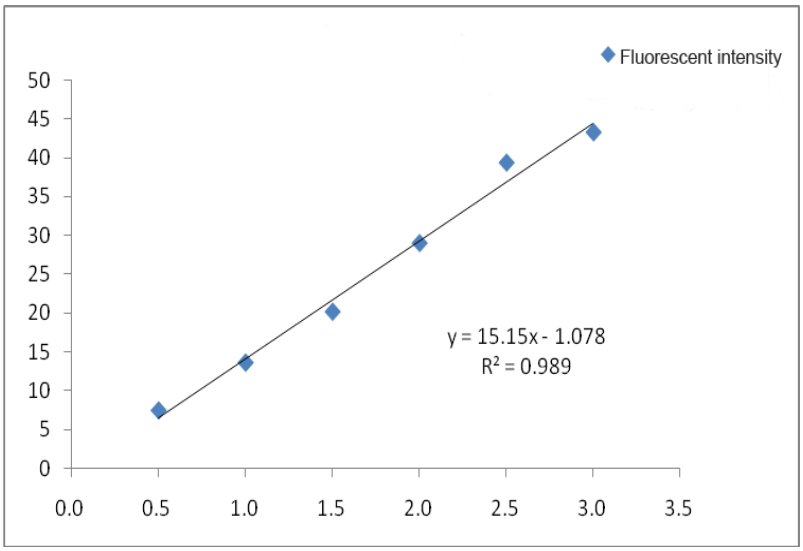

Figure. 2 5-HT standard curve

7. Discussion

As neurotransmitters, 5-HT is mainly distributed in the pineal gland and the hypothalamus, and functions an inhibitory neurotransmitter. The study of Huang etc [9] found that Cold syndrome in brains of rats, NE and DA content decrease, and 5-HT content increased. Wu et al. [10] and Huang et al [11] had demonstrated that, warm medicine could make the brain NE, DA content increase with decreasing 5 - HT content and enhanced excitability. This paper investigated that the FGJ, DGJ, FGJPRC and DGJPRC affect the distribution of brain tissue. Its content was determined to prove the difference of different ginger juices and as well as the processing accessories under the influence of drugs.

The results showed that, 5-HT in brain tissue of the rats of RRC group was distributed most densely, with the highest content. But after processing with different ginger juices, distribution and 5-HT content had the varying degree changes. These results showed that cold coptidis after warm medicinal properties of the ginger processing were eased with the cold eased; among them DGJPRC was less than FGJPRC on 5-HT distribution and content was also reduced. Schematic diagram is shown in Figure. 1,Figure. 2, Table.1 and Table.2

8. Conclusions

RRC by DGJ and FGJ of the two different hot degree as accessories was processing, medicinal properties of two processing varieties were changed obviously, after 5-HT content and changes of brain morphosis of rats to reflect the degree of the two changes, The results “ginger broil coptis by hot resisting cold”, “dried ginger is hot in nature” , and “fresh ginger is cold in nature ” are consistent with the traditional theory.

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Conflict of interest
None.

References
[1] Teng H, Choi YH: Optimization of ultrasonic-assisted extraction of bioactive alkaloid compounds from rhizoma coptidis (Coptis chinensis Franch.) using response surface methodology. *Food Chem* **142**:299-305, 2014.
[2] Kaumann AJ: Surprises from a cardiac 5-HT_4TG mouse: spontaneous atrial arrhythmias by endogenous 5-HT of atrial origin Different mechanism of arrhythmias through 5-HT_4 receptors and β-adrenoceptors. *Naunyn Schmiedebergs Arch Pharmacol* **386**:353-6, 2013.
[3] Navailles S, Lagière M, Le Moine C, De Deurwaerdère P: Role of 5-HT2C receptors in the enhancement of c-Fos expression induced by a 5-HT2B/2C inverse agonist and 5-HT 2 agonists in the rat basal ganglia. *Exp Brain Res* **230**:525-35, 2013.
[4] Aronsen D, Bukholt N1, Schenk S: Repeated administration of the 5-HT1B/1A agonist, RU 24969, facilitates the acquisition of MDMA self-administration: role of 5-HT1A and 5-HT1B receptor mechanisms. *Psychopharmacology (Berl)* **233**:1339-47, 2016.
[5] Seyedabadi M, Fakhfouri G, Ramezani V, Mehr SE, Rahimian R: The role of serotonin in memory: interactions with neurotransmitters and downstream signaling. *Exp Brain Res* **232**:723-38, 2014.
[6] Bombardi C, Di Giovanni G: Functional anatomy of 5-HT2A receptors in the amygdala and hippocampal complex: relevance to memory functions. *Exp Brain Res* **230**:427-39, 2013.
[7] Szyndler J, Maciejak P, Turzyńska D, Sobolewska A, Bidziński A, Płaźnik A: Time course of changes in the concentrations of monoamines in the brain structures of pentylenetetrazole-kindled rats. *J Neural Transm (Vienna)* **117**:707-18, 2010.
[8] Möller M, Du Preez JL, Viljoen FP, Berk M, Harvey BH: N-Acetyl cysteine reverses social isolation rearing induced changes in cortico-striatal monoamines in rats. *Metab Brain Dis* **28**:687-96, 2013.
[9] Huang YJ, Qin HZ, Li SY, Shi B, Liang YJ, Liu JH, Liu L, Zheng ZW: 3 kinds of Cloves extracts on the effect of neurotransmitter, cAMP, cGMP in brain of rats with cold. *Zhongguo Shiyanfangjixue* **16**:153-155, 2010.
[10] Wu Y, Pan X, Xu Y, Lu X, He S, He R, Gong M: Optimization of combinations of ginsenoside-Rg1, ginsenoside-Rb1, evodiamine and rutaecarpine for effective therapy of mouse migraine. *J Nat Med* **70**:207-16, 2016.
[11] Huang YQ, Qing HZ,Li SY, Shi B, Liang YJ, Liu JH, Liu L, Zheng ZW: The effect of 3 kinds of clove extract on cold syndrome rat brain neurotransmitter and cyclic adenosine monophosphate, Cgmp. *Zhongguo Shiyan Fangjixue Zazhi* **16**:153-155, 2010.