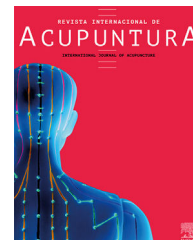




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ORIGINAL ARTICLE

Comparative analysis of the anti-depression effects of lorazepam, acupuncture, and curcumin utilizing the forced swimming test in experimental rats models



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KEYWORDS

Accupuncture;
Depression;
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Abstract

Introduction: lorazepam is one of benzodiazepine medication that is usually used for its sedative and anxiolytic effect and has anti-depressant like effects. Turmeric is a plant that contain curcumin as main active compound that exert its antidepressant action inhibit monoamine oxidase and increase levels of dopamine and serotonin in brain.

Aim: the present study investigate the depression effect of curcumin (natural selective serotonin inhibitors) in comparison with acupuncture and lorazepam.

Method: 32 adult rats weighing (150 g–280 g) were divided equally into 8 groups each group contain 4 rats. The first 3 group of rats were treated with lorazepam, mixture of (curcumin, black pepper and olive oil) and pricked with insulin needle at point gb39, respectively, while second 4 groups of rats were received lorazepam and mixture, lorazepam and acupuncture, mixture and acupuncture, and lorazepam, mixture and acupuncture, separately, and the eighth group is control group don't receive any drugs. After 35 days, forced swimming test was made for all rats.

Results: Liver parameters: - GOT increased in all group, GPT increased in rat's group that received lorazepam, mixture group and lorazepam + mixture group, This indicates the existence of problems previously with the liver of the rat used, and what remains is the high levels of GOT and GPT in the control group, while TSB is within the normal range for all groups. Kidney parameters: - blood urea levels increased in all groups and S. creatinine levels within normal range for all groups.

Conclusion: the evaluation of natural SERMs in the forced swimming test showed promising antidepressant-like effects, comparable to lorazepam and acupuncture. Further research is needed to understand the mechanisms and determine optimal dosage and safety. Natural selective serotonin inhibitors hold potential as alternative or complementary treatments for depression.

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PALABRAS CLAVE

Acupuntura;
Depresión;
Curcumina;
Prueba de natación
forzada

Análisis comparativo de los efectos antidepresivos del lorazepam, la acupuntura y la curcumina utilizando la prueba de natación forzada en modelos experimentales de ratas

Resumen

Introducción: El lorazepam es uno de los medicamentos benzodiazepínicos que generalmente se utiliza por sus efectos sedantes y ansiolíticos, y también se utiliza para tratar el insomnio y las convulsiones. La cúrcuma es una planta que contiene curcumina como compuesto activo principal, que ejerce su acción antidepresiva al inhibir la monoaminoxidasa y aumentar los niveles de dopamina y serotonina en el cerebro.

Objetivo: El presente estudio investiga el efecto antidepresivo de la curcumina (SRI natural), en comparación con la acupuntura y el lorazepam.

Método: Se dividieron 32 ratas adultas con un peso de (150 g-280 g) en 8 grupos, cada grupo con 4 ratas. Los primeros 3 grupos de ratas fueron tratados con lorazepam, una mezcla de (curcumina, pimienta negra y aceite de oliva) y fueron pinchados con una aguja de insulina en el punto gb39, respectivamente. Mientras que los otros 4 grupos de ratas recibieron lorazepam y la mezcla, lorazepam y acupuntura, la mezcla y acupuntura, y lorazepam, mezcla y acupuntura, por separado. El octavo grupo fue el grupo de control que no recibió ningún medicamento. Después de 35 días, se realizó una prueba de natación forzada para todas las ratas.

Resultados: Parámetros hepáticos: los niveles de GOT aumentaron en todos los grupos, los niveles de GPT aumentaron en el grupo de ratas que recibieron lorazepam, el grupo de la mezcla y el grupo de lorazepam + mezcla. Esto indica la existencia de problemas previos en el hígado de las ratas utilizadas, y lo que queda es la alta concentración de GOT y GPT en el grupo de control, mientras que los niveles de TSB se encuentran dentro del rango normal para todos los grupos. Parámetros renales: los niveles de urea en sangre aumentaron en todos los grupos y los niveles de creatinina sérica se mantuvieron dentro del rango normal para todos los grupos.

Conclusión: La evaluación de los SRI naturales en la prueba de natación forzada mostró efectos antidepresivos prometedores, comparables al lorazepam y la acupuntura. Se necesita más investigación para comprender los mecanismos y determinar la dosis óptima y la seguridad. Los SRI naturales tienen potencial como tratamientos alternativos o complementarios para la depresión.

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Introduction

Acupuncture is recommended by the world health organization as promising therapeutic strategy.¹ In order to encourage the release of endorphins (known as happy Hermon) and other naturally accruing chemicals, acupuncture stimulates the acupoints in different parts of the body which promote relaxation and rest by increasing level of serotonin and GABA in the brain.² Acupuncture enhances patients overall mental

and physical health by increasing their sleep duration and quality, additionally its decreased the likelihood of depressed insomnia.³ Acupuncture at acupoints GB39 (xuanzhong), which is located at the point where the lateral knee and the lateral malleolus of the tibiofibular^{4,5} as show in Fig. 1.

Selective serotonin reuptake inhibitors is considered the most antidepressant used due to their effectiveness in treating numerous of psychiatric diseases such as

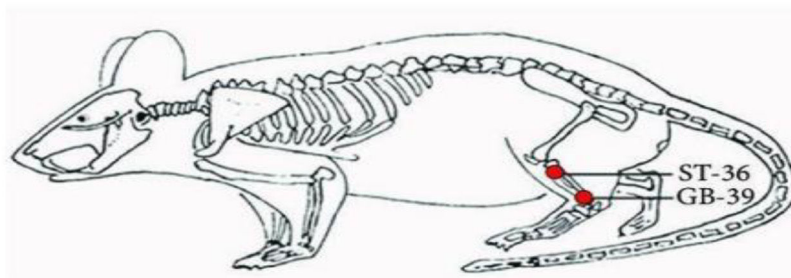


Fig. 1 Acupoints GB39 location in rat.⁶

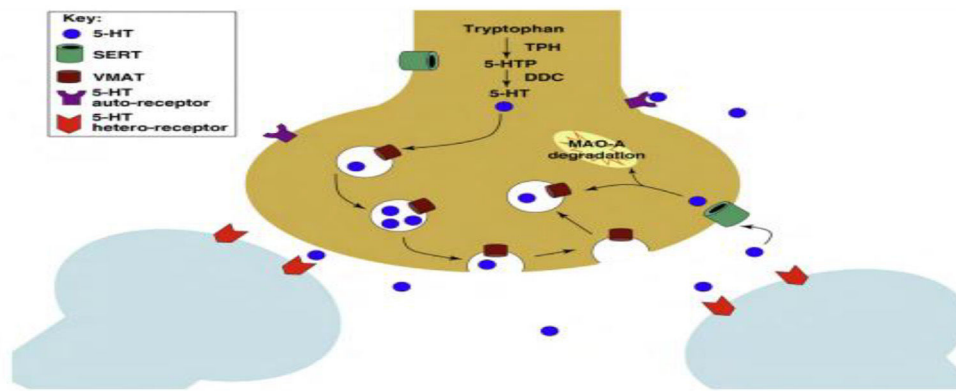


Fig. 2 Selective serotonin reuptake inhibitors mechanism of action.¹⁰

depression, anxiety and some behavioral disorder include bulimia nervosa, obsessive compulsive disorder and panic disorder.^{7,8} SSRIs exert their action by interfering with serotonin reuptake transporter that cause influx of serotonin at the neurotransmitter junction.⁹ As illustrated in Fig. 2.

Curcumin as a natural material present in turmeric (*curcuma longa*) may have antidepressant – like effect in rat and mouse model of depression, with results resembling those of traditional antidepressant like imipramine and fluoxetine. According to many preclinical studies indicate the potential use of curcumin for the treatment of depressive illnesses.^{11–13}

This study focused on acupoints Gb39 to evaluate its antidepressant effect in comparison with curcumin and lorazepam in depressed wistar rats' model.

Materials and methods

Lab animals

Eight groups of four wistar male rats each were formed from 32 total range of weight (150–280 kg), with the first group serving as control. The rats in the second and third group received lorazepam and mixture contain curcumin, black pepper and olive oil, respectively, while fourth group received simply acupuncture treatment. The fifth group received lorazepam and mixture, sixth group treated with lorazepam and acupuncture, the seventh group treated with mixture and acupuncture and finally eighth group received lorazepam, mixture and treated with acupuncture at the same time.

All animal experiments comply with the ethical guide line for the use of animals in research given by the national committee for research ethics in science and technology.

Drugs and reagents dosing

Lorazepam dose was 0.25 ml (1 tab. of lorazepam dissolved in 4 ml of distilled water) administered via oral route once daily. Mixture of 4 g curcumin, 0.2 g black pepper and 16 ml of olive oil used in dose 1 ml via oral route once daily.

Acupuncture

For 30 days, there was one acupuncture session every day. The acupuncture was done on Gb 39 acupoints. The concept of traditional Chinese medicine, the rat skeleton atlas and acupoints, and the anatomical location described in previous studies^{4,6} and were used to estimate the depth and placement of acupuncture. Needles were placed perpendicularly as deep 2–3 mm at Gb39. Moreover, the acupuncture needles were left in for two minutes.⁵

Forced swimming test

A forced swimming test made for all rats in each group after 30 days of treatment by using glass water tanks (20 cm diameters * 30 cm height) with water level 15 cm from bottom and each animal left in water tank for 5–10 min.¹⁴

Liver and kidney function monitoring

After 30 day, blood was drawn from heart of all rats included in the study and serum analysis was made to measure the liver enzyme (GOT, GPT, ALP) and kidney enzyme (blood urea and serum creatinine).

Results

Liver function test

Table 1 show the mean \pm SD for (AST\GOT), (AST\GOT) and (TSB) which are liver enzyme as markers to discover whether liver was damage or not due to use treatments, as show in the table there were no significant differences between them.

Kidney function test

Table 2 show the mean \pm SD for (SCr) and (B.U.) which are kidney enzyme as markers to discover whether kidney was damage or not due to use treatments, as show in the table there were no significant differences between them except when used Lorazepam, curcumin and acupuncture

Table 1 Liver enzyme value in the experimental rats.

parameters	Aspartate aminotransferase (AST\GOT)	Alanine aminotransferase (AST\GOT)	Total serum bilirubin (TSB)
Groups			
Control	147 ± 7.095 ^a	36 ± 1.000 ^a	0.5 ± 0.1 ^a
Lorazepam	162 ± 24.68 ^a	46 ± 5.292 ^a	0.6 ± 0.1 ^a
Curcumin	160 ± 11.37 ^a	57 ± 22.01 ^a	0.6 ± 0.3 ^a
Acupuncture	162 ± 21.08 ^a	39 ± 2517 ^a	0.5 ± 0.2 ^a
Lorazepam and curcumin	198 ± 10.58 ^a	40 ± 1.000 ^a	0.7 ± 0.2 ^a
Lorazepam and acupuncture	192 ± 33.13 ^a	44 ± 6.557 ^a	0.6 ± 0.2 ^a
Curcumin and acupuncture	162 ± 48.51 ^a	48 ± 3.215 ^a	0.6 ± 0.2 ^a
Lorazepam, curcumin and acupuncture	194 ± 5.000 ^a	64 ± 7.506 ^a	0.6 ± 0.1 ^a

Values were expressed as mean ± SD, different small letters mean significant difference ($p \leq 0.05$).

together represent the significant difference with all other treatments.

Swimming test

Swimming test made as indicator to antidepressant effect for treatments used in study, and show there were significant difference between treatments depended on time that rats spend in water, as show in Table 3.

Discussion

The fundamental mechanisms for acupuncture's substantial effectiveness in TCM are yet unknown. The purpose of acupuncture was examined, and more information needs to be thoroughly evaluated in the future. It is becoming more and more common to use it to treat a variety of illnesses, but the mechanism has not been fully understood because it does not have a specific treatment for all illnesses and because there are too many sites of action.¹⁵

In cases of drug-induced liver and renal disease, biomarkers constitute a helpful diagnostic criterion. Biomarkers can be used to assess cell damage, impairment severity, disease prognosis, and harm kind in cases of organ injury.¹⁶ Increased serum AST and ALT activity are a sign of cellular permeability and loss of the membrane of the liver cell's structural integrity. The degree of hepatotoxicity brought on

by drugs is measured by the hepatocyte membrane's release of these intracellular enzymes.¹⁷

The liver converts lorazepam to inactive metabolites. The toxic effects of an infrequently formed intermediate metabolite are likely to blame for the liver damage caused by lorazepam. According to several research, preparing curcumin into nanoparticles increases its levels in rat livers and has a hepatoprotective impact on rats¹⁸, and other research demonstrated the impact of curcumin/turmeric on patients with non-alcoholic fatty liver disease's liver enzymes. This study indicated a subgroup receiving less than 1000 mg/day of curcumin supplementation had a significant tendency toward lower ALT blood values. AST was significantly reduced in studies after 8 weeks of treatment.¹⁹

In our study, As show in Table 1 GOT increased in all groups while GPT increased in the mixture and Lorazepam+ mixed groups, likely as a result of earlier issues such liver inflammation or injury in the rats that were used in the study. Elevated liver values in the control group compared to the other groups are, in my opinion, proof of this. The level of TSB is within the normal range in all groups except for the lorazepam group because curcumin has no impact on bilirubin. There is a minor increase in TSP, but this increase has few adverse effects.

The indicators of renal function that are most frequently examined are creatinine and blood urea nitrogen (BUN). A waste product of muscle metabolism called creatinine is removed from the blood by the kidneys. Increased blood creatinine levels may be a sign of compromised renal function. Urea nitrogen, another waste product that the

Table 2 Kidney biomarker in experimental rats.

parameters	Serum creatinine (SCr)	Blood urea (B.U.)
Groups		
Control	0.77 ± 0.061 ^a	33 ± 1.665 ^a
Lorazepam	0.43 ± 0.225 ^a	39 ± 1.952 ^a
Curcumin	0.59 ± 0.172 ^a	28 ± 11.40 ^a
Acupuncture	0.72 ± 0.076 ^a	31 ± 0.702 ^a
Lorazepam and curcumin	0.54 ± 0.096 ^a	31 ± 4.729 ^a
Lorazepam and acupuncture	0.71 ± 0.249 ^a	35 ± 0.751 ^a
Curcumin and acupuncture	0.76 ± 0.100 ^a	28 ± 1.908 ^a
Lorazepam, curcumin and acupuncture	0.79 ± 0.177 ^a	64 ± 7.506 ^b

Table 3 Swimming test of experimental rats.

parameters	Swimming time (min.)
Groups	
Control	2.9 ± 0.36 ^a
Lorazepam	2.3 ± 0.03 ^a
Curcumin	1.5 ± 0.03 ^{b,c}
Acupuncture	1.2 ± 0.22 ^{b,c}
Lorazepam and curcumin	2.5 ± 0.04 ^{a,d,e}
Lorazepam and acupuncture	2.3 ± 0.03 ^{a,d,e}
Curcumin and acupuncture	1.5 ± 0.05 ^{b,c,f}
Lorazepam, curcumin and acupuncture	2.5 ± 0.05 ^{a,d,e,g}

kidneys typically filter out of the blood, is measured by the BUN. Increased BUN levels may be a sign of renal disease or other health issues.¹⁶ Blood urea nitrogen levels are high in all groups and creatinine levels are within the normal range, which points to renal failure or other health issues.

(FST), one of the most used methods for examining depressive-like behavior in rodents. The FST is predicated on the idea that when an animal is placed in a container filled with water, it will first try to escape but eventually show immobility that could be interpreted as a sign of behavioral despair. Due to the fact that this test involves subjecting the animals to stress, which has been demonstrated to play a part in the likelihood for serious depression, it has been widely employed.²⁰ The antidepressant effects of lorazepam caused the group that got it to resist treatment within 4 min of reaching the despair phase. Within 4 min, the group receiving curcumin experienced dejection, and the cause of this Curcuma's antidepressant impact is resistant. Within two minutes of being poked with insulin needles, the group experienced hopelessness. Perhaps this is due to the fact that pricking's antidepressant effects are unclear.

Conclusion

Lorazepam has no significant effect on the kidneys and liver at normal doses, and curcumin acts as a hepatoprotector and improves liver and kidney parameters, but in our research, some liver and kidney parameters were increased. This indicates pre-existing problems in the liver and kidneys of the rats used.

Conflict of interest

No conflict of interest.

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