EXPLORING THE EFFECT OF THYMOQUINONE ON NEUROPSYCHOLOGICAL IMPAIRMENT IN EXPERIMENTALLY INDUCED FIBROMYALGIA

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Abstract - In the present investigation, the effect of thymoquinone on neuropsychological impairment in reserpine induced fibromyalgia has been explored. Fibromyalgia was induced by the administration of 1 mg/kg reserpine to mice for 3 consecutive days. The effect of thymoquinone (10 and 20 mg/Kg) on fibromyalgia was explored. Fibromyalgia was evaluated by using different models such as Eddy's hot plate test, tail immersion test for evaluating hyperalgesia; forced swim test for depression; inclined plane test for evaluating motor activities and Morris water maze, elevated plus maze test for evaluating acquisition and retention of memory using standard methods. Reserpine administration was found to mimic the effects and symptoms of fibromyalgia. Maximum effect was evident on day 4 and 6 of reserpine treatment as observed by an increase in thermal hyperalgesia, depression, impaired motor- coordination and memory. Pretreatment with the standard drug duloxetine and thymoquinone was found to significantly attenuate reserpine effect in neuropsychological impairment in reserpine induced fibromyalgia from day 2 to 5. However on 5th day of treatment there was 100% mortality in both the treatment groups. The results of study indicate that thymoquinone does not produce any protection in reserpine induced fibromyalgia.

Keywords - Fibromyalgia, Pain Reserpine, Thymoquinone

I. INTRODUCTION

Fibromyalgia or fibrositis is a disorder characterized by widespread musculoskeletal pain and tenderness that is associated with an organic disease varied etiopathogenesis [1]. The incidence of fibromyalgia is very high with approximately comparable rates of 7.3% to 12.9% across different countries [2]. Symptoms are varied in patients such as pain, joint stiffness, fatigue, non- restorative sleep, cognitive impairment and mood disorders. The patients also suffer with loss of appetite leading to weight loss and muscle wasting. The main aim of fibromyalgia treatment is to alleviate pain and depression along with reduction in other symptoms. Studies have revealed that non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen are similar in efficacy to placebo. The currently used treatments involve risk of seizures and serotoninergic syndrome when it is combined with selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenalin reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs). But it is also having many side-effects like serotonin syndrome, itching, drowsiness, decreased appetite, anxiety [3]. Thymoquinone (TQ) is the main constituent of the volatile oil from *Nigella sativa* (black cumin) seeds and has different biological activities in various systems [4]. The current study was designed to investigate the effect of thymoquinone in experimentally induced fibromyalgia.

II. MATERIALS AND METHODS

Induction of fibromyalgia

Swiss albino mice (female) with body weight ranging from 25 to 35 g were used. Reserpine (1 mg/ Kg, s.c.) was given to the animals for 3 consecutive days and observations were taken on models demonstrating hyperalgesia, depression, cognition and learning and motor coordination on 2nd, 4th, 6th and 10th day.

The following animal groups were employed (n=5)

Group I Control: Normal control Distilled water p.o

Group II (Resp control): Reserpine Control (1mg/Kg, s.c.)

Group III (Dul + Res): Duloxetine (30 mg/Kg p.o) + Reserpine

Group IV (TQ20 + Res): Thymoquinone (20 mg/Kg p.o) + Reserpine

Group V (TQ20 + Res): Thymoquinone (10 mg/Kg p.o) + Reserpine

III. ASSESMENT OF FIBROMYALGIA

Test for hyperalgesia

Eddy's hot plate test

Animals were placed originally on hot plate (55^{0}C) and reaction time (s) for hind paw licking, jumping was recorded. A cut off time of 15 s was used to prevent any thermal injury to mice. The latency of response was taken [5].

Tail immersion

The terminal 3 cm of tail was put in hot water maintained at a temperature of 55˚C±1˚C. Reaction

time in seconds for flicking of tail indicating sign of pain was recorded. A cut off time of 15 s was used. The tail flicking latency of response was taken as the end point [6].

Test for depression

Forced swimming test

The apparatus consists of a transparent reservoir of 50 cm height × 20 cm width filled to 30 cm depth with water at room temperature. The duration of immobility was recorded during the 5 min cut off time. A mouse was judged to be immobile, when it starts to float in a vertical position, keeping his head above the water and making only small movements [7].

Test for motor co-ordination

Inclined plane test

Briefly, the mice were placed at the wooden board connected at one end by a hinge at an angle of 60 ^{o} and the fall- off time from the plane was noted. The cut-off time period for the test was 30 s [8].

Test for cognition and memory

Morris water maze test

The animals were placed into the water tank at the same starting point and had to swim to the platform placed below the water rendered translucent with white non-toxic color. After climbing the platform, the animal was allowed to stay there for 20 s. The latency to find the platform was measured as the time of placement of the mouse in the water to the time it finds the platform. The cut off time was 2 min. The animals were trained for 6 consecutive days [9].

Elevated plus maze test

Briefly, mouse was placed on the open arm and as soon as it entered the closed arm a buzzer with shrilling sound was pressed. The animal was trained till it learned to correlate buzzer with closed arm. A period of 5 min was observed as the cut-off time. 24 h after training, memory retention was measured. Time spent in open arm was recorded [10].

STATISTICAL ANALYSIS

All results were expressed as mean ± SEM. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Reserpine induced Fibromyalgia

Reserpine administration is found to mimic the effects and symptoms of fibromyalgia on consecutive daily administration at a dose of 1 mg/Kg per day for a period of three days. Maximum effect was evident on day 4 and 6 of reserpine treatment. Administration of reserpine resulted in an increase in thermal hyperalgesia as indicated by significant decrease in reaction time as compared to control in hot plate and tail immersion test (Fig 1a & b), depression was observed by an increase in immobility time in forced swim test as compared to control (fig 1c), impaired motor-coordination as indicated by significant decrease in fall off time as compared to control mice in inclined plane test (Fig 1d) and memory as indicated by significant increase in latency time as compared to control in Morris water maze test (Fig 1 e) and time spent in closed arm in elevated plus maze test (Fig 1 f). Similar findings have been documented by earlier studies with reserpine [11]. Reserpine is a monoamine depletor and causes oxidative stress in the body by degradation of biogenic amines and blocking vesicular monoamine transporter that transmits and stores dopamine and also affects muscles leading to muscular damage [12]. A dose of 1mg/Kg reserpine administered subcutaneously is reported to produce effects similar to clinical fibromyalgia in rodents. It also leads to auto-oxidation by increased production of reactive oxygen species [13].

Effect of thymoquinone on fibromyalgia in mice

Pretreatment with the thymoquinone and standard drug duloxetine, was found to significantly attenuate reserpine effect in neuropsychological impairment in reserpine induced fibromyalgia as evidenced by an increase in response time in case of Eddy's hot plate method and tail immersion test as compared to reserpine treatment symbolizing attenuation of hyperalgesia (Fig 1 a & b) and decreased the immobility time as compared to reserpine treated mice in the forced swimming test that is a marker of anti- depressant activity (Fig 1c). Thymoquinone treatment was found to significantly improve motor co-ordination as evidenced by a decrease in the fall off time as compared to reserpine treatment in inclined plane test (Fig 1 d) but all the animals died on 5th day of the study. Also, a decrease in escape latency in Morris water maze and time spent in the closed arm of EPM (Fig 1 e & f) indicating memory and cognition enhancement but mortality rate was 100% on 5th day of the study. These observations indicate that although treatment of thymoquione significantly alleviated the symptoms of fibromyalgia on day 4 as compared to reserpine treated animals but could not improve mortality. Duloxetine is used for clinical management of fibromyalgia. Thymoquinone is reported to have significant effects against nitrosative stress in brain tissue, nociception, anxiety and memory impairment [14] however in current investigation % mortality rates were very high and all the animals died by 5th day of the study. Furthermore, fibromyalgia is associated with increased oxidative and nitrosative stress as evidenced by an increase in the level of free radicals [15] Thymoquinone is known to have its neuroprotective and anti- oxidant potential in rats, reduction of oxidative stress with thymoquinone treatment could contribute towards the

alleviation of symptoms of fibromyalgia [16]. Although thymoquinone treatment attenuated fibromyalgia in mice but its toxicity was observed as evidenced by animal mortality on 5th day of treatment. It was earlier reported that acute oral treatment of thymoquinone resulted in signs of toxicity such as hypoactivity and difficult in respiration followed by death in mice. It may be because of increased tissue oxidative stress due to reduction in reduced glutathione [17]. Further administration of thymoquinone supplemented with an antioxidant can be beneficial for the treatment of fibromyalgia.

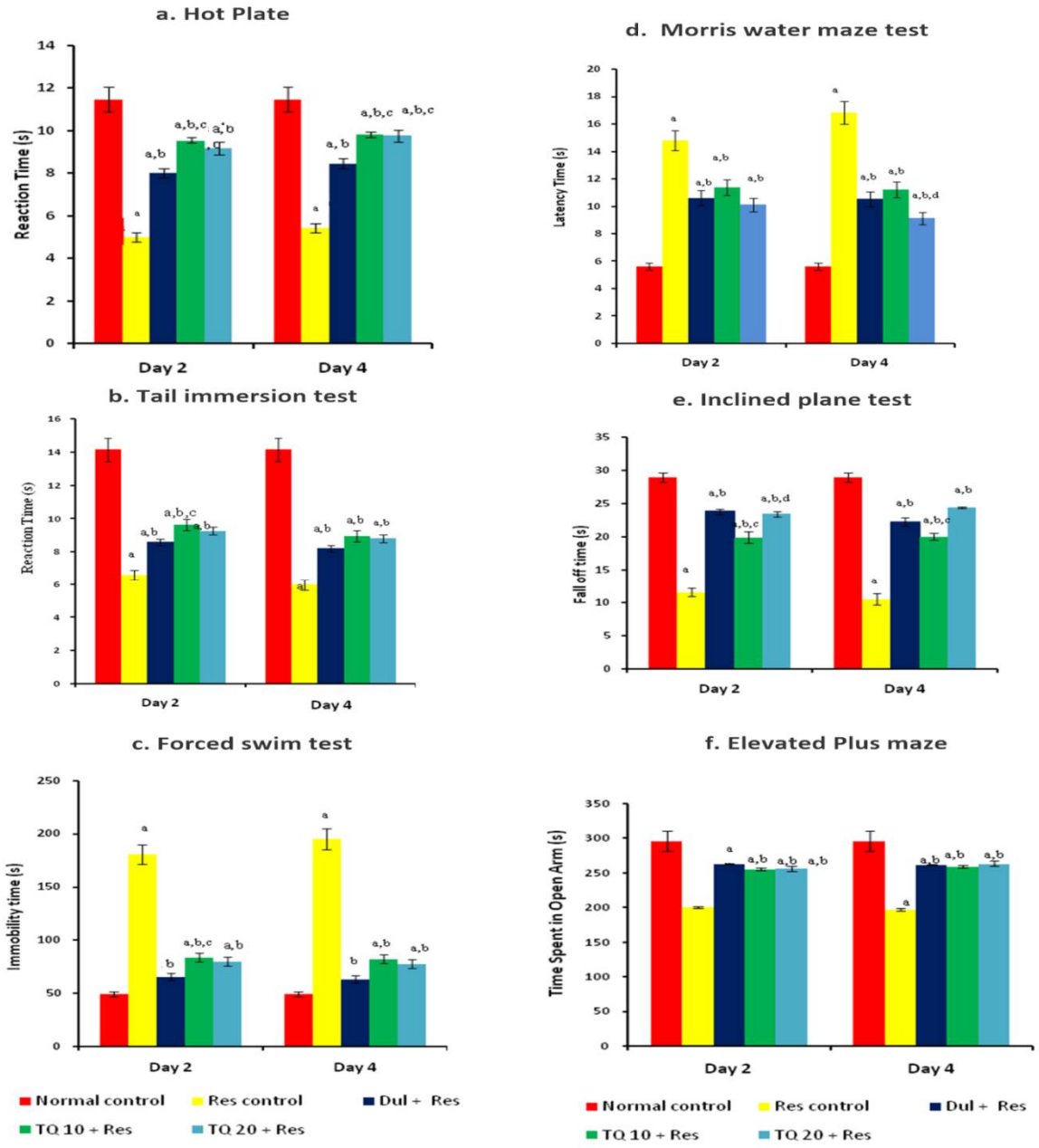


Fig 1. Effect of Various pharmacological interventions on reaction time in hot plate test [a]; tail immersion test [b]; immobility time in forced swim test [c]; latency time in Morris water maze test [d]; fall off time in inclined plane test [e]; Time spent in open arm in elevated plus maze test [f]. Values are expressed as mean ± SEM; a is p<0.05 vs Normal control; b is p<0.05 vs Res control; c is p<0.05 vs Dul + Res (where Res and Dul is denoted as Reserpine and Duloxetine respectively). Values are expressed as mean ± SEM; a is p<0.05 vs Normal control; b is p<0.05 vs Res control; c is p<0.05 vs Dul + Res (where Res and Dul is denoted as Reserpine and Duloxetine respectively).

CONCLUSION

It may be concluded that daily thymoquinone treatment helps in improving symptoms of fibromyalgia. Thymoquinone at a dose of 10 and 20 mg/kg is toxic. However since some behavioral effects of reserpine treatment were improved, a lower dose of thymoquinone may be studied for its effects on fibromyalgia.

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