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EFFECT OF ASTAXANTHIN IN ANIMAL MODELS OF ANXIETY AND DEPRESSION

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ABSTRACT

To study anxiolytic and antidepressant effect of Astaxanthin as an adjuvant to standard drug.In this study, to investigate the effects of astaxanthin on anxiety and depression,we performed some behavioural trials including elevated plus maze test, hole-board test, forced swim test, and tail suspension test.Astaxanthin (3 mg/kg/day for 15 days, orally) significantly increased the time spent in open arms in the elevated plus maze test and increased the head-dipping count in the hole-board test and significantly decreased the immobility time in the forced swim test and the tail suspension test.In rats, Astaxanthin exerted anxiolytic effects, and also antidepressant activity when given as an adjuvant with standard drugs.

KEY WORDS: **Astaxanthin, Diazepam, Amitryptyline, Anxiety, Depression,**

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INTRODUCTION

Astaxanthin is a naturally occurring carotenoid found in algae, bacteria and fungi which impart red color to the organisms.[1] Humans obtain astaxanthin from marine animals feeding on the algae.[2]Astaxanthin is a strong antioxidant and antiinflammatory agent. It has a unique property of transmembrane location because of its polar and nonpolar structure. Polar side has inone ring which is a strong free radical scavenger while the nonpolar transmembrane domain helps in moving the free radicals rapidly outside the cell membrane.[3,4] Strong antioxidant property of astaxanthin is responsible for the hosting of beneficial effects in human including decreasing oxidative DNA damage,[5] decreasing biomarkers of inflammation,[6] positive effects on lipid profile,[7] boosting of immunity and improvement in cognition.[8] Apart from this, astaxanthin also showed improved memory performance in mice[9] and protection of nerve cells against oxidative stress in various in-vitro studies.[10] Certain CNS disorders such as anxiety and depression in humans have been shown to have multifactorial causes and one of the cause could be the oxidative stress.[11] There are studies which have tried to analyze the role of astaxanthin as an treatment option in cases of anxiety and depression against the standard treatment.[12] But in this study we tried to find out the benefits of astaxanthin as an adjuvant to the standard treatment. This is probably first study of its kind which compared the role of astaxanthin as an adjuvant to the standard treatment thereby seeking the information on enhancing the effect of standard drugs for anxiety and depression in rat models.

2. MATERIALS AND METHODS

2.1 Animals

The experiments were performed on four weeks old male rats, weighing 150-250 gms. The experimental protocol was approved by the Institutional animal ethics committee and was executed according to the guidelines of the Committee for the Purpose of Control and Supervision for Experiments on Animals (CPCSEA) India. The animals were obtained from the animal house of Peoples College of Medical Sciences & Research Centre Bhopal and care was taken to perform the experiments within the norms prescribed by the competent agencies and minimal suffering to the animals. Animals were housed at 24˚C to 30˚C under a 12 hours light ,dark cycle and ad libitum access to water and food. Behavioural experiments were undertaken between 9:00am to 4pm.

2.2 Administration of Astaxanthin

Astaxanthin was obtained from MyNutraMart, Bangalore and was mixed with wheat flour. In evaluation of anxiety and depression like behaviours, astaxanthin was orally administrated at the dose of 3mg/kg/day for 15 days. 1 hour after the last administration, the animals were subjected to the experiment.Animals were divided into 3 groups of 6 animals each. Three groups made for each test were controlgroup, standard drug group and standard drug with astaxanthin group. Tests performed for anxiety studies were Elevated plus maze and Hole board test. For studies on depression, tests performed were Forced swim test and Tail suspension test.

2.3 Elevated plus maze

Apparatus made consisted of two open and two closed arms 50 cm × 10 cm×40cm made with open roof. The arms were connected with a central square (10 cm × 10 cm) to give the apparatus a plus sign appearance. The maze was kept elevated 50 cm above the floor in a dimly-lit room.[13]The animals were placed individually on the central square of the plus maze facing on enclosed arm. As a positive standard, rats were intraperitoneally (ip) administered diazepam 1mg/kg. During the 5 min test preference for the animal for the first entry, the number of entries into the open or closed arm and the time spent in each arm of the maze were noted.

2.4 Hole Board test

After giving diazepam 1mg/kg/ip as standard or diazepam along with astaxanthin the rats were placed in the Hole Board apparatus consisting of an open board (30cmx30cmx16cm) with four equidistant holes each having a diameter of 2cm in the floor. Head dip duration and counts were recorded manually for a 6 minutes test.[14]

2.5 Forced swim test

Following oral administration of standard drug Amitryptyline hydrochloride (20mg/kg in wheat flour rolled into pellets) and Amitryptyline along with astaxanthin, the animals were placed in a glass cylinder (30cm diameter) filled with water reaching a height of 10cm from the base of the glass over a period of 6 minutes at 28˚c±1˚c and the immobility time was recorded. Rats were judged immobile only when they were floating passively in the water while making no or little effort to keep their head out of water.[15]

2.6 Tail suspension test

After giving the standard drug Amitryptyline hydrochloride (20mg/kg in wheat flour) and Amitryptyline along with astaxanthin the rats were suspended 50cm above the ground from their tail using an adhesive tape and their behaviour was noted for 6 minutes. Immobility time was noted for the last 5 min.of the test.[16]

2.7 Statistical Analysis

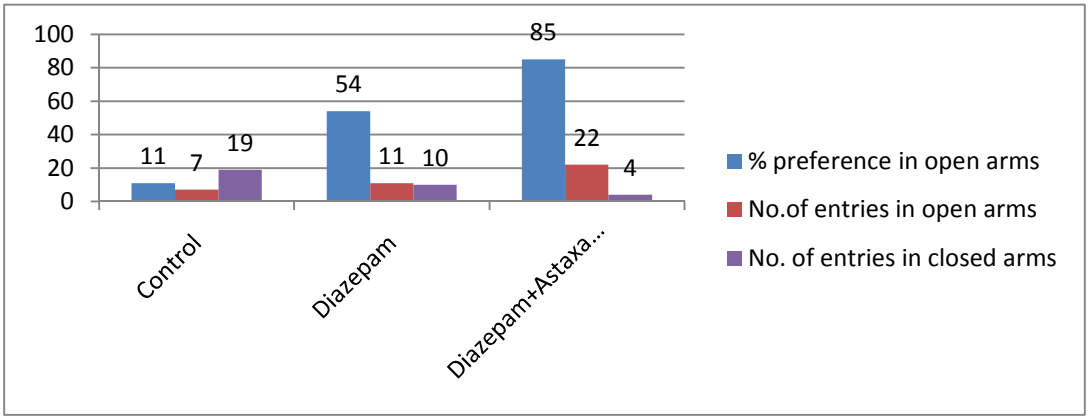
Statistical comparisons were made by one-way ANOVA, followed by Tukey HSD test , with $P < 0.05$ being considered as statistical significant.

3. RESULTS AND DISCUSSION

3.1 Elevated Plus Maze Test

The elevated plus maze is commonly used to assess anxiety related behaviour. In the elevated plus maze, avoidance of the open arms, an increase in the time spent in the closed arms and a decrease in rearing indicates anxiety.[17, 18] In the elevated plus maze model, the number of entries and the preference for open arms by astaxanthin treated rats was significantly increased ($P< 0.05$) as compared to control. The number of entries in the closed arm by astaxanthin treated rats significantly decreased($P<0.05$) as compared to control. (Figure 1)

Figure 1
Elevated Plus Maze Test

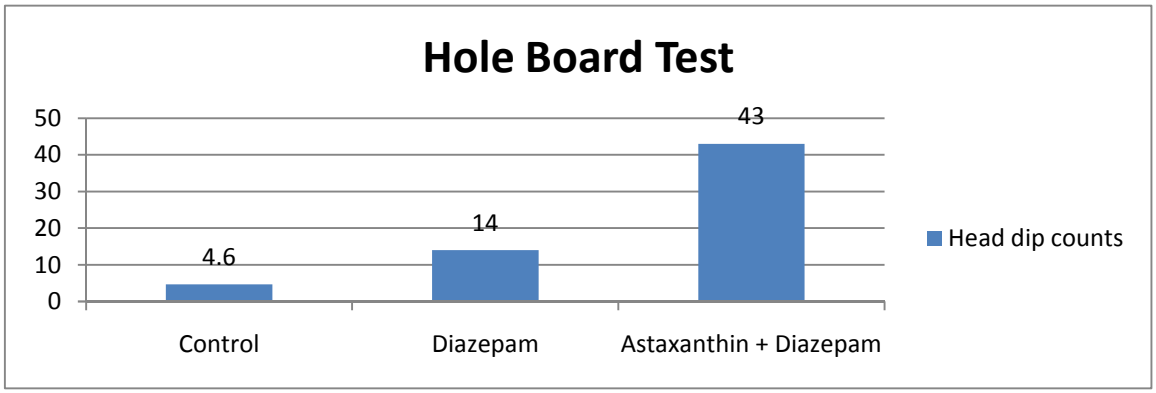


3.2 Hole Board test

Head dipping behaviour of the animals in the Hole Board test is considered to be an indicator of anxiety.[19] Increased frequency and duration of the head dipping is an indicator of anxiolytic state. As a positive standard, Diazepam significantly increases both the frequency and duration of head dipping ($p<0.01$) but in comparison with astaxanthin (3mg/kg/day) administered along with the standard drug, it produced an even more significant antianxiety effect ($p <0.01$) and figure 2 shows that the addition

of astaxanthin greatly enhances the activity of diazepam to produce the antianxiety effect. (Figure No.2)

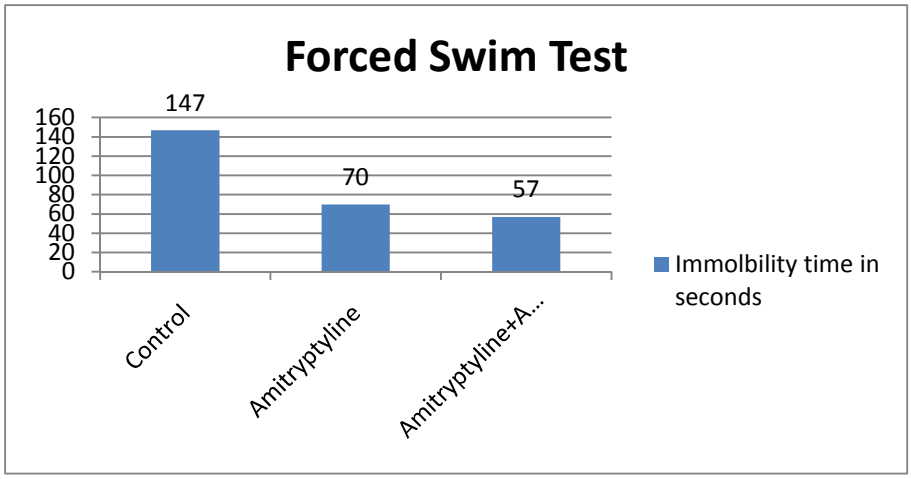
Figure 2
Hole Board Test



3.3 Forced Swim test

When an animal is forced to swim it makes all the efforts to come out of the stress by making rigorous swimming movements. When it unable to do so, it gives into the situation and becomes immobile making only very small swimming movements. Antidepressant drug will make animals to make more sustained efforts and there is a reduction in immobility period. In this study standard drug and astaxanthin combination was found superior to the standard drug (Amitryptyline) in decreasing the immobility time ($p<0.01$) (Figure 3)

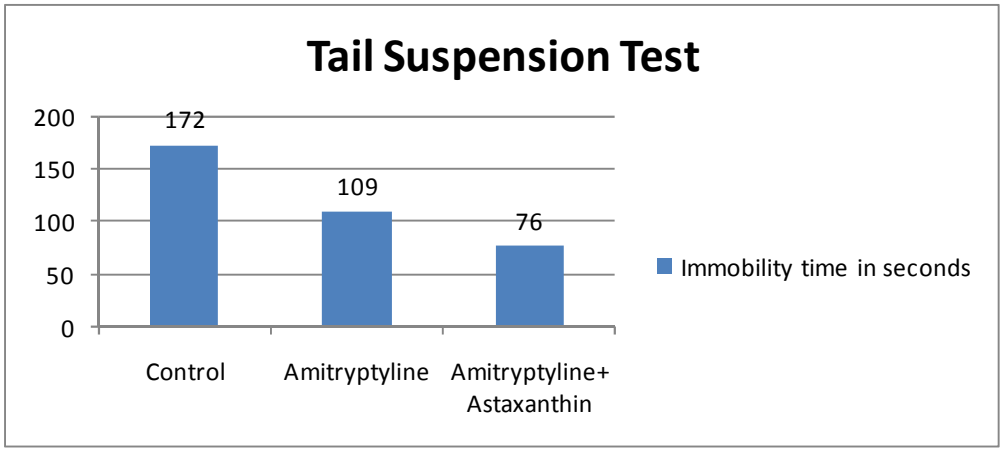
Figure 3
Forced Swim Test



3.4 Tail suspension test

When the rat is suspended by its tail, it undergoes severe stress and it tries to escape by making several struggling movements. When it realizes that there is no escape, it becomes immobile. The immobility time is recorded. In our test again the combination of standard drug along with astaxanthin significantly reduced the immobility period ($p<0.01$). (Figure no.4)

Figure 4
Tail Suspension Test



4. DISCUSSION

Anxiety and depression are the neurological disorders having multiple etiology and mechanisms. In one of a small open label trial in 10 healthy men complaining increasing forgetfulness, astaxanthin improved the attention, memory and the reaction time. Other effects of astaxanthin on CNS as discussed earlier could be because of its strong antioxidant nature and its ability to scavenge free radicals which might offer significant neuroprotection.[20, 21, 22] There are several studies linking oxidative stress as one of the causes of anxiety and depression in rat model of psychiatric disorder and reversal of oxidative stress induced anxiety by inhibition of phosphodiesterase 2.[11] There is also a study which correlates between peripheral blood granulocyte oxidative status and the anxiety in mice.[23] Although precise molecular mechanism of the beneficial effects of astaxanthin in the anxiety and depression are not known but most probably its antioxidative and free radical scavenging mechanism could be attributed. In our study we tried to seek the information on any beneficial role of astaxanthin as an adjuvant to the standard drug prescribed for the treatment of anxiety and depression. In both the experiments on anxiety and depression, astaxanthin has shown remarkable adjunctive value in terms of increase in effectiveness of the standard treatment. As such, astaxanthin treated rats were more healthier and active looking on a visual scale in comparison to the control and standard drug groups. There is one study which compared astaxanthin with the standard treatment for anxiety and depression in mice and found antianxiety effect of astaxanthin but no antidepressant effect.[12] But in our study we found strong adjunctive antianxiety as well as antidepressant role of astaxanthin along with the standard treatments. Diazepam produces its anxiolytic effects through GABA channels causing CNS depression and anxiolysis by increasing conduction of Cl^- ions.[24] Amitryptyline is a tricyclic antidepressant and acts by inhibiting reuptake of serotonin and norepinephrine with strong effect on 5-HT and moderate effect on NE transporters.[25] Astaxanthin with its novel mechanism of neuroprotection and prevention of neurodegeneration might aid the standard drugs, as these disorders have multifactorial origin.

5. CONCLUSION

Anxiety and depression form a large part of psychological disorders prevalent in the society with considerable burden both in terms of cost and duration of the treatment and adverse

effects caused by these drugs remains a challenge. We tried to use the astaxanthin as an adjuvant to the standard treatment in the rat models of anxiety and depression. Idea of this study was to establish the adjunctive value of the astaxanthin for increasing the effectiveness of the treatment in terms of the reduction of the dose of the standard drug. Analysis of the results showed marked improvement in the effectiveness of the standard drug. We would like to recommend further studies in larger animal groups or if possible conduction of human trials to establish the role of Astaxanthin as an adjuvant to the standard therapy of anxiety and depression.

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