

Effect of astaxanthin on Diuresis and body composition in experimentalnephrolithiasis

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ABSTRACT: Nephrolithiasis one of the most prevalent and widely studied of renal diseases. No remedy has proved to be completely effective in alleviating the renal damage caused by kidney stones. The most common type of kidney stones is calcium oxalate stones which are composed mainly of calcium oxy monohydrate crystals. This study has been carried out to evaluate the effect of the xanthophyll carotenoid astaxanthin on diuresis and body composition in male Wistar albino rats in which experimental calcium oxalate nephrolithiasis has been induced using ethylene glycol and 1, 25-dihydroxycholecalciferol. Astaxanthin extracted from the microalga *Haematococcus pluvialis* at two doses 25mg/kg b.wt. and 50mg/kg b.wt. is fed orally and the food and water intake, urine output, body weight and relative weights of liver, kidney and brain were assessed. It was found that both the doses of astaxanthin in a similar manner stimulate food and water intake, increase the urinary output and help in regaining the body weight all of which were significantly reduced in the nephrolithiatic group. The relative organ weights of liver and kidney which were increased in the nephrolithiatic group were also brought to normal levels. These results indicate a protective effect of astaxanthin on calcium oxalate nephrolithiasis.

KEYWORDS: astaxanthin, body composition, calcium oxy monohydrate, diuretic, nephrolithiasis

I. INTRODUCTION

Nephrolithiasis is a renal disease that has worldwide prevalence and high recurrence rate. It is common and is estimated to occur in approximately 12% of the population with a recurrence rate of 70-80% in males and 40-60% in females [1]. Once afflicted, the relapse rate is high and the recurrence interval is shortened [2]. The "stone belt" regions in India comprise of parts of Maharashtra, Gujarat, Punjab, Haryana, Delhi and Rajasthan. In these regions, the disease is so prevalent that most members of a family will suffer from kidney stones sometime in their lives [3]. 80% of kidney stones are composed of calcium oxy monohydrate crystals [4]. Several invasive and non invasive strategies are used for the clinical management of nephrolithiasis but are limited by their adverse side effects and high recurrence rates [5]. Natural products have thus occupied an important part of research in this context due to their minimum side effects. Astaxanthin (3, 3'-dihydroxy-beta, beta-carotene-4, 4'-dione, CAS 472-61-7), is a natural xanthophyll carotenoid credited with several pharmacological effects such as antioxidant, anti inflammatory, immunomodulatory and anti cancer effects [6]. The microalga *Haematococcus pluvialis* is found to accumulate up to 5% of astaxanthin, the maximum amount as per reported sources [7]. The objective of the present study was to evaluate the diuretic potential and the effect in body composition of astaxanthin from *H. pluvialis* at two doses 25 and 50 mg/Kg b.wt on experimentally induced calcium oxalate nephrolithiasis in male Wistar albino rats. The food and water intake, the urine output, the weekly difference in body weight and relative kidney, liver and brain weights were studied to evaluate the potential beneficial effect of astaxanthin.

II. MATERIALS AND METHODS

2.1 Animal treatment

Ethylene glycol and 1, 25-dihydroxycholecalciferol were obtained from Merck India Ltd. Freeze dried *Haematococcus pluvialis* biomass was purchased from Ambe phytoextracts Pvt. Ltd., New Delhi, India. The amount of astaxanthin was based on (1.5% w/w) content of astaxanthin to powder. To evaluate the effect of astaxanthin on nephrolithiasis, the animals were randomly divided into six groups of six rats each. Animal care and treatment were in conformity with the guidelines of Institutional Animal Ethical Committee, School of Biosciences, Mahatma Gandhi University, Kerala, India (Approval No: B1662009/3). All animals were housed in a constant temperature room (25 ± 2 °C) with 12 h light/dark cycle, fed standard diet (Amrut Rat Pellet, Pranav Agro Industries Ltd., Pune, India) and given water *ad libitum* throughout the experimental period. Each group underwent a different treatment for 21 days. The groups were designated as follows:
Group I –Control, fed on normal lab diet only.

Group II - Astaxanthin 25 mg/kg b.wt control, fed with astaxanthin at 25 mg/kg b.wt.

Group III - Astaxanthin 50 mg/kg b.wt control, fed with astaxanthin at 50 mg/kg b.wt.

Group IV- Nephrolithiatic, the rats were administered 0.75% ethylene glycol daily to raise the oxalate levels and 0.5µg 1, 25-dihydroxycholecalciferol, the active metabolite of Vitamin D₃ every other day for 28 days to raise the calcium levels to induce calcium oxy monohydrate nephrolithiasis. Nephrolithiasis was also induced in groups V and VI as per the above method.

Group V - Nephrolithiatic treated with astaxanthin 25 mg/kg b.wt. for 21 days.

Group VI - Nephrolithiatic treated with astaxanthin at 50 mg/kg b.wt for 21 days.

All rats were given the treatments by oral intubation. The animals were monitored daily for signs of weakness or illness. Each cage was loaded with 30g of food and 150ml of water daily. The leftovers were measured daily at a fixed time. The difference between the loading quantities and the leftovers was taken as the daily food and water intake. 24 h urine samples were collected by placing the animals in individual metabolic cages. The body weights were recorded, the animals were euthanized, the organs (brain, kidney and liver) were excised following necropsy, dissected free of fat and weighed using calibrated balance. Relative kidney weight was calculated dividing the absolute weight of the kidney by weight of the animal on the 21st day.

2.2 Statistical Analysis

One way ANOVA followed by Tukey's post hoc multiple comparison test was used for comparison among the groups. SPSS/PC+ version 18 (SPSS Inc. Chicago, Illinois, USA) was used and results were presented as mean \pm standard deviation (SD). $p < 0.05$ were considered statistically significant.

III. RESULTS AND DISCUSSION

3.1 Effect of astaxanthin on food intake

Food is vital for existence. The physiological effects of experimental compounds are reflected on the food intake pattern. The food intake in the nephrolithiatic animals treated with astaxanthin at 25 mg/kg b.wt. and 50 mg/kg b.wt. for 21 days are averaged and shown in Fig.1. It was found that during the induction of calcium oxalate nephrolithiasis, there is a progressive decrease in the amount of food taken and it becomes minimum during the last week of induction. The treatment with astaxanthin brings about a measurable increase in the consumption of food reaching normal levels towards the end of the experimental period. There is a marked increase in the food intake in the 25 mg/kg b.wt. treated group than the 50 mg/kg b.wt. treated group during the course of treatment but they reach almost same levels during the last three days of the study. Since all the control groups exhibit a normal food intake pattern, the results for these groups are not represented.

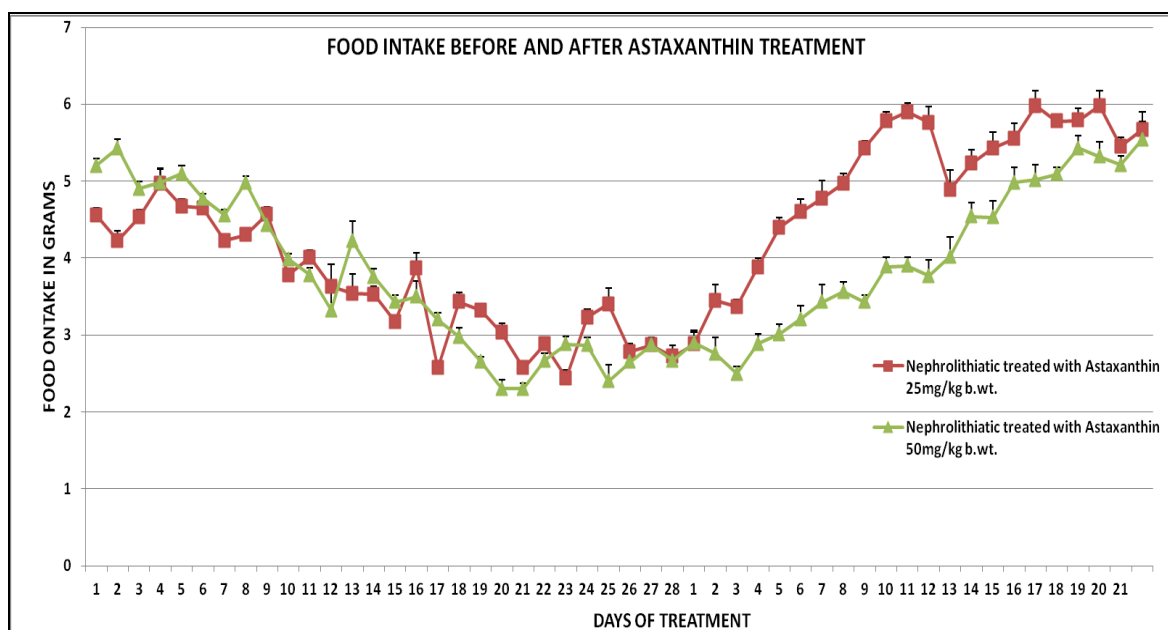


Figure 1. Food intake during nephrolithiasis induction and treatment with 25 mg/kg b.wt. and 50 mg/kg b.wt. astaxanthin for 21 days. The values are mean \pm standard deviation for each group.

3.2 Effect of astaxanthin on body weights

The weekly difference in body weights during the three week experimental duration is shown in Fig. 2. The body weights of all animals were more or less similar at the beginning of the experiment. A very slow increase in the body weights were noted in the two groups induced with nephrolithiasis, which are to be treated with the two doses, till the fourth week. These astaxanthin treated nephrolithiatic groups however showed a regaining of body weights as the treatment progressed and their body weights were similar to that of the controls at the end of the experiment. Both the doses exhibited similar efficacy in weight gain.

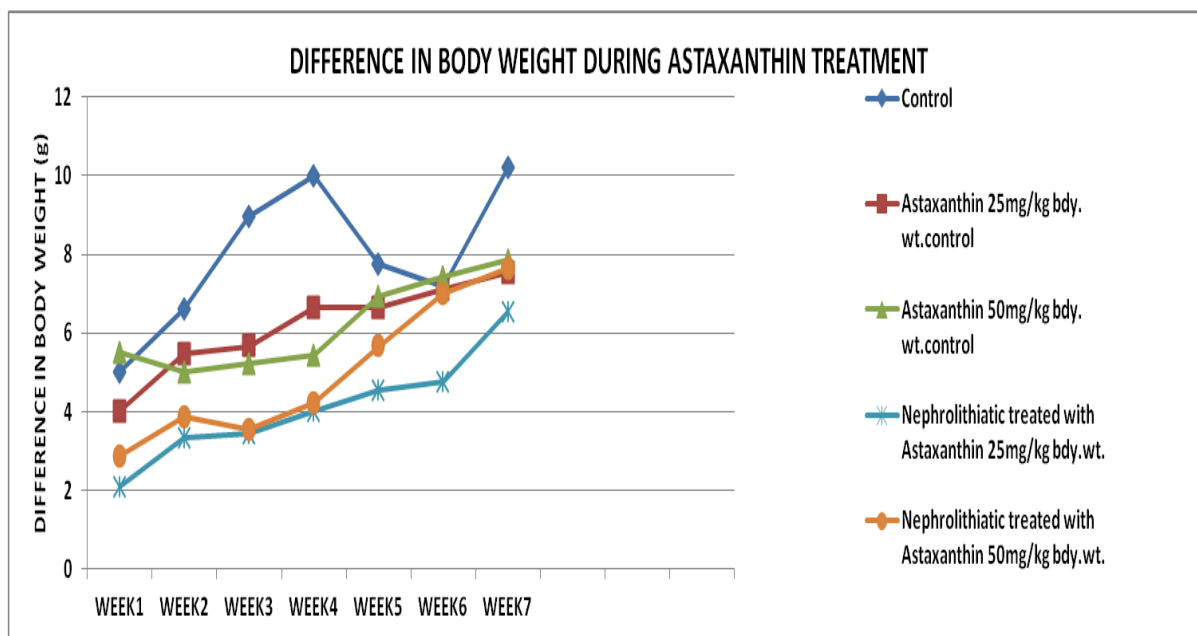


Figure2. Difference in body weight recorded weekly during nephrolithiasis induction and treatment with 25 mg/kg b.wt. and 50 mg/kg b.wt. astaxanthin for 21days. The values are mean±standard deviation for each group.

The reduction in body weight in the nephrolithiasis induced animals reflects the effect of these compounds on the general metabolism of rats. In a previous toxicological study on ethylene glycol administration also, a decreased food intake and body weight reduction had been observed [8]. The physiological changes resulting from the increased oxalate and calcium metabolism and the accumulation of calcium oxalate and the depressed food consumption during the period of calcium oxalate nephrolithiasis might be the contributing factors for the reduction in weight. Astaxanthin administration at both doses was found to revive food consumption probably by generating appetite and hence leading to weight gain.

3.3 Effect of astaxanthin on water intake

There was a decrease in water intake in the animals during the induction of nephrolithiasis with the least consumption during the last eight days reaching as low as 0.98 ± 0.006 ml. Treatment with astaxanthin brings about an improvement in the daily water intake. There is a constant increase which reaches 5.5 ± 0.34 ml on the twenty first day of treatment (Fig.3). On comparison with the 25 mg/kg b.wt. treated group, the 50 mg/kg b.wt. treated group is found to be more effective in stimulating the water intake.

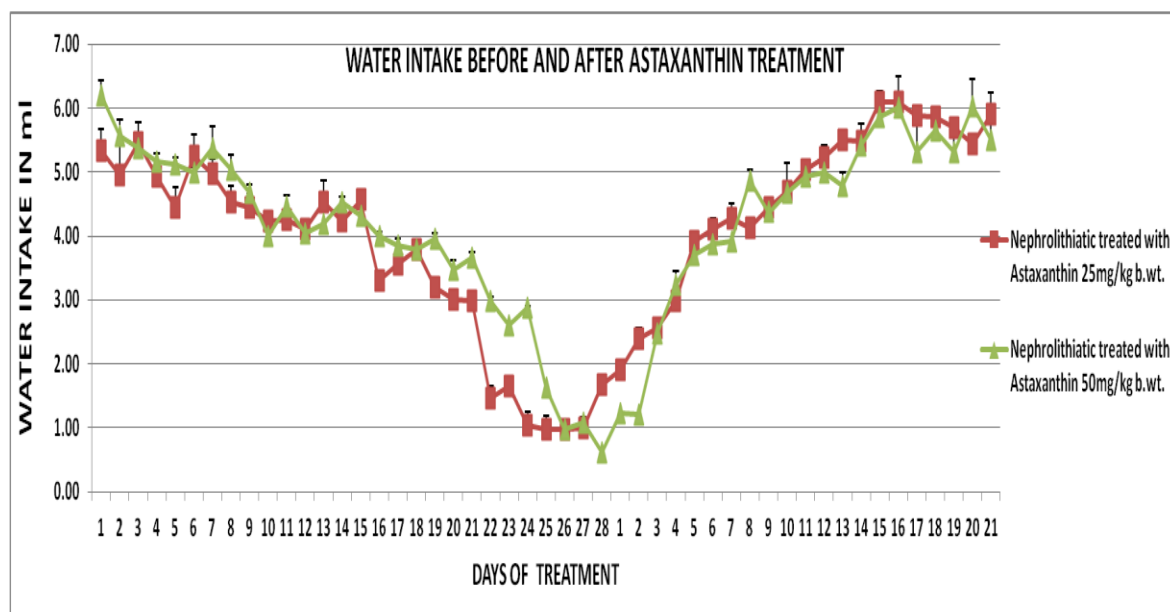


Figure 3. Water intake during nephrolithiasis induction and treatment with 25 mg/kg b.wt. and 50 mg/kg b.wt. astaxanthin for 21 days. The values are mean \pm standard deviation for each group.

3.4 Effect of astaxanthin on urine volume

There was a reduction in the 24h urine volume with duration during the induction period and the urine output was minimum towards the last few days of calculi induction. However treatment with astaxanthin was found to have a beneficial effect promoting urine output. Initially during the treatment period, the effect of both the doses was found to be almost same. However, the 50mg/kg b.wt. treated group was found to be stimulating more urine output than the 25mg/kg b.wt. treated group but towards the last week of treatment (Fig. 4). Since all the control groups exhibit a normal pattern, the results for the controls are not represented for water intake and urine volume.

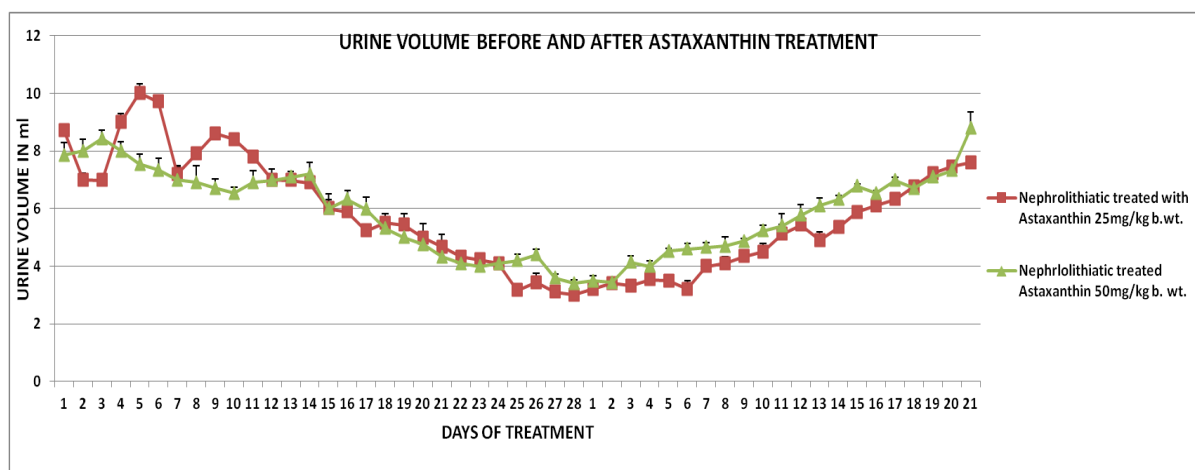


Figure 4. 24h Urine volume during nephrolithiasis induction and treatment with 25 mg/kg b.wt. and 50 mg/kg b.wt. astaxanthin for 21 days. The values are mean \pm standard deviation for each group.

The calcium oxalate crystals cause obstruction to the urinary tubules and reduce the outflow of urine [10]. This must be the reason for the reduction in urinary output in the nephrolithiatic group. Astaxanthin promotes crystal solubilisation and exerts a diuretic effect as evident from the increased urine output in both the treated groups.

3.5 Effect of astaxanthin on vital organ weights

Organ weights can be the most sensitive indicators of the effect of an experimental compound. Significant differences can occur in the organ weights even in the absence of morphological alterations [11]. The ratio of organ weight to the body weight is calculated here and indicated as relative organ weights to

account for the differences in the body weight in the animals used in the study. The relative weights of liver and kidney increase in the nephrolithiatic group when compared to the control groups. Results from animals treated with both the doses of astaxanthin demonstrate that the organ to body weight ratio decreases and reaches values not different from the control groups at the end of the 21 day treatment. The relative brain weights however exhibit a similar value for all the experimental groups irrespective of the treatment modality (Table 1).

Table 1 Effect of astaxanthin at 25mg/kg b.wt. and 50 mg/kg b.wt. on absolute organ weights and relative organ weights (%) for 21 days

Groups	Liver (g)	Relative liver weight(%)	Kidney (g)	Relative kidney weight (%)	Brain (g)	Relative brain weight (%)
Control	8.07±0.81	3.62±0.40	1.52±0.13	0.76±0.08	1.90±0.15	0.87±0.012
Astaxanthin 25mg/kg b.wt. control	7.77±0.85	3.35±0.50	1.48±0.27	0.68±0.10	1.81±0.30	0.79±0.09
Astaxanthin 50mg/kg b.wt. control	7.42±0.67	3.33±0.30	1.57±0.21	0.71±0.09	1.77±0.12	0.8±0.05
Nephrolithiatic	12.67±0.20 ^{abc}	7.22±0.80	1.99±0.12 ^{abc}	1.13±0.08	1.70±0.15	0.82±0.06
Nephrolithiatic treated astaxanthin 25mg/kg b.wt.	7.65±1.30 ^d	3.46±0.10	1.60±0.20 ^d	0.73±0.01	1.72±0.06	0.86±0.03
Nephrolithiatic treated astaxanthin 50mg/kg b.wt. b.wt..	7.63±0.92 ^d	3.14±0.40	1.40±0.19 ^d	0.70±0.09	1.73±0.10	0.81±0.01

Values are provided as mean ± standard deviation for six animals of each group. a, b ,c, d Indicate Significant differences from the Control, Astaxanthin 25mg/kg b.wt.- control, Astaxanthin 50mg/kg b.wt.- control and Nephrolithiatic groups respectively. p<0.05 was considered significant.

The increase in absolute and relative kidney and liver weights could be due to the deposition of calcium oxy monohydrate crystals in the kidneys of stone forming animals and inflammation in the liver. Both the astaxanthin treated groups show liver and kidney weights similar to controls. The relative kidney and liver weights also follow the same pattern with a significant lowering of the values for both the astaxanthin treated groups. The decrease in the extent of calcium oxalate deposition in astaxanthin administered nephrolithiatic rats was also observed in a previous study by SEM EDX analysis which confirms our observations on the organ weights [12]. However the absence of any significant variation in the brain weights of treated and nephrolithiatic animals indicates that calcium oxalate nephrolithiasis does not significantly affect the brain tissue of the experimental animals.

IV. CONCLUSION

Astaxanthin at both the administered doses was found to be effective in restoring the fluid balance, food intake and body weight of the nephrolithiatic animals. There is also a reduction in relative organ weights to normal except in brain which seems unaffected, indicating an organ specific protective effect of astaxanthin. Of the two doses the 50mg/kg b.wt was found to exert more diuretic effect than the 25mg/Kg b.wt. It can hence be concluded that astaxanthin has a protective effect on calcium oxalate nephrolithiasis possibly by its diuretic action and effect on body composition. Further studies are required to confirm our observations.

V. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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