β–Carotene, A Potent provitamin A Carotenoids in the Amelioration of the Effects of Heroin

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Abstract
β-Carotene, a potent provitamin A carotenoid having multifaceted physiological activities, could ameliorate the different types of abnormal behaviour in rats after the administration of heroin. The administration of different doses of heroin, viz LD_{25} and LD_{50} showed various degrees of abnormal conditions and behaviours such as depletion of body weight, retinol reserve, decrease of water intake, piloerection, drowsiness, wet shakes, restlessness and jumping etc. β-carotene accounts for the amelioration of the above conditions and abnormal behaviour to a certain extent along with restoration of the retinol level in the liver of albino rats receiving heroin of different doses.

Keywords: Narcotic drug; Carotenoids; Retinoids; Drug abuse; Vitamin A

Introduction
The manifold effects of narcotic drugs on various metabolic processes have been known for a long time. Heroin is a narcotic drug which causes serious problems in the human health as well as those pertaining to a series of social problems. Weight loss, reduced food, water intake, vitamin deficiencies, anaemia, general malnutrition, gastrointestinal disorders as well as effect [1-3]. Heroin addicted rats showed many withdrawal syndromes that can be divided into two phases as- (i) early or primary abstinence and (ii) protracted abstinence [4]. Besides that, heroin affects withering apoptosis, piloerection, continuation of wet shake and aggression [5-7]. During the protracted phase, food, water intake, body weight, temperature and locomotion activities increase and persist for a long period [8,9]. β-Carotene, a provitamin A carotenoids, potent antioxidant, immunomodulator and growth promoter, has manifold properties to ameliorate the effects of various harmful agents, xenobiotics has been tested. We report here the β-carotene can ameliorate several effects of heroin after feeding both LD_{25} and LD_{50} doses.

In the present study an attempt has been made to study the effects of LD_{25} and LD_{50} doses of heroin on the growth and retinol reserve in liver of albino rats along with the amelioration of the effects of heroin in regulation of growth, water intake, piloerection, drowsiness, itching, jumping, wet shakes and weight loss.

Materials and Method
Maintenance of rats
Young male albino rats (Rattus albino, Sprague dawley) of weight about 100-110 g were taken and were maintained at 27-30 °C in plastic cages (18 cm X 12 cm X 6 cm, 10 animals/cage) in the animal house of the Zoology Department, Gauhati University, India. The rats were maintained on the diets (10 g / 100 g body weight) as described earlier [10] taking the reference diet referred by [11] and termed as control diet. 0.5 ml of ground nut oil containing 50 µg β –carotene was fed, (0.0001863 M) to each rat daily studying its effects in combating the effects of various doses of heroin.

The rats were properly maintained with different feeds, cleaned every day, provided clean filtered water supplied in graduated plastic bottles. The rats maintained in these conditions [10] showed normal and satisfactory growth without any abnormalities. The rats were acclimatized to the control and β–carotene added diets for 2 weeks and the effects of LD_{25} and LD_{50} doses of heroin were tested in rats maintained in the above mentioned diets containing either β-carotene or in absence of β-carotene for another 7 weeks. The effects of different diets and heroin administration were repeated in triplicate and its metabolism on the regulation of growth and retinol reserves were recorded.

Administration of heroin
LD_{25} and LD_{50} dose of heroin was calculated following the method developed by us [10,12] and found to be 0.0833 mg and 0.167mg/100 g body weight respectively.

In order to study the effects of heroin on various behaviours and its possible amelioration with β-carotene, the intramuscular injection of heroin were administered in the morning (7 am) and various behaviours were observed. Different withdrawal symptoms were measured from the earlier works as shown piloerection [13], itching, drowsing, wet shakes [14], and jumping [15]. The intensity of all symptoms was measured through various degrees of behaviour showing doubtful (-), low (+), moderate (+ +) to high (+ + +) or highest (+ + + +) along with the % of animals affected or showed abnormal behaviours from 0 to 6 hours.

Chemicals
Crystalline β-carotene, retinol, Retinyl acetate, and retinoic acid were obtained from F. Hoffman La-Roche, Basel, Switzerland, BASF – Germany. Other chemicals of analytical grade were purchased from British Drug House (B.D.H.), Laboratory Chemicals Division, Glaxo Laboratories (India) Ltd., Bombay and from Sigma-Aldrich India. Heroin was obtained from Assam Forensic Science Laboratory.

Estimation of vitamin-A (Retinol) in rat Liver
The animals were sacrificed under anesthesia and the livers were dissected. Extraction of retinol was done in diffused light following the method of [16]. Liver tissue (5 g) was grounded with five times anhydrous Na_2SO_4 and made into a dry powder. All retinoids and

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carotenoids were then extracted with light petroleum ether (40°C-60°C) in darkness and evaporated it to dryness. Alkaline hydrolysis was made by alcoholic KOH solution by keeping it overnight in darkness at low temperature. It was then extracted with diethyl ether and dried over anhydrous Na₂SO₄. Diethyl ether was evaporated and retinoids were stored in methanol at low temperature (below -10°C).

**HPLC**

The HPLC were conducted using reversed phase system. (Column- μ Bondapak C₁₈, Water, 300 mm x 4.6 mm, 5 μ; mobile phase: Acetonitrile (50 mM); Ammonium acetate (pH 6.9), Detector: 135-diode array detector (Perkin Elmer); Integrator: LCI 100 (Perkin Elmer); Flow rate of the eluent: 1.2 ml/ min.

The HPLC system consists of Perkin Elmer 125 binary pump. The Perkin Elmer 135-diode array detector was set at 315 nm. The instrument was fitted with Rhyodine injector with a loop of 20µl. The lowest limit of detection for each assay was 0.2 pmol. The amount of retinoids in the sample was assessed on the basis of the calibration curve. The Perkin Elmer 135-diode array detector was set at 315 nm. The mobile phase: Acetonitrile (50 mM): Ammonium acetate (pH 6.9), Detector: 135-diode array detector (Perkin Elmer); Flow rate of the eluent: 1.2 ml/ min.

**Results**

The effects of the different diets containing β-carotene with heroin of different doses and in absence of β-carotene were compared with the animals reared with normal or control diets from its initial start, viz. 0 day to 7 weeks. β-carotene showed better growth along with high retinol reserve in the livers of rats treated with the β-carotene added diets. β-carotene helped in maintaining a normal retinol reserve and reserve poor growth in LD₅₀ doses of heroin respectively. In the absence of β-carotene, heroin treated groups of both LD₂₅ and LD₅₀ dose showed poor growth to some extent in rats administered with LD₂₅ dose and reserve and poor growth in LD₅₀ doses of heroin respectively. In the absence of β-carotene, heroin treated groups of both LD₂₅ and LD₅₀ dose showed poor growth and loss of retinol from their livers (Table 1). Weekly examination of body weight and retinol reserves was conducted. Retinol reserve was estimated from its HPLC profiles as described earlier. It may be mentioned that β-carotene or β-carotene added diet did not show any toxic effects on the experimental rats.

Several behavioural changes such as water intake, piloerection, itching, drowsiness, jumping and wet shake etc. were recorded after 6 hours. The details are shown in (Table 2-6).

**Discussion**

Drug-nutrient interactions is a significant issue, where such interactions may interfere with the pharmacokinetics process and the nutrition status plays an important role in a drug's pharmaco logic response which may interfere with the pharmacokinetic process [17-19]. Increasing attention has been focused on nutrient-drug interaction because many drugs have become more potent and have greater specificity and with extended duration have increased the incidence of nutrient-drug interactions. Drug abuse has become a great social menace where it involved a series of social, health and other economic situation of the involved persons as well as society. Further it has been seen that vitamin A deficiency is also a great problem in South East Asian countries. Considering all these collective issues and problems we have shown an interaction of heroin and β-carotene, where β can ameliorate certain effects of heroin administration in animals taking rat as a model.

β-carotene supplementation has no apparent effects either in weight loss or depletion of retinol reserves as well as on expression of unusual behaviours. Increased dietary β-carotene or other provitamin A carotenoids may effects the steady-state concentration in body fluid or tissues and serve as localised substrates for retinoids formation. Retinol which exerts striking effects on diverse functions such as growth, development, and differentiation can be exerted from β-carotene. The present studies have been designed to determine whether an interaction exists between heroin and β-carotene or its metabolites to affect the growth and expression of regulatory activities in amelioration of unusual or abnormal activities that has been encountered after the administration of different doses of heroin.. The decrease of weight after administration of heroin (LD₂₅ and LD₅₀) in the animal is found to be consistent with earlier findings [9,14,20]. Two possible mechanisms may act (i) altered level of anabolic hormones [21] or (ii) decrease of food intake [22]. Further, the increased physical activity or other undefined factors in heroin treated rats might of some importance in causing body weight loss. The support of β-carotene in the increase of body weight and in the prevention of loss of body weight by heroin administered with different doses of heroin added with β-carotene, or in absence of β-carotene. It has been found that β-carotene can ameliorate the above mentioned drug-induced behaviours. No abnormal behaviours have been recorded after 6 hours. The details are shown in (Table 2-6).

**Table 1**: Change of body weight and retinol reserve of control and treated animal of different diets.(10 rats in each cage, repeated in three sets of experiments for each diet) The results are the mean and ± SD 3 sets of experiments of each diets; * significant at P<0.05%.

| Week | Control diet (CD) | Control diet + Oil | Control diet + β-carotene | Control diet + β-carotene + Heroin (LD₂₅) | Control diet + β-carotene + HLD(D₂₀) | Control diet + Heroin (LD₂₅) | Control diet + Heroin (LD₅₀) |
|------|------------------|--------------------|--------------------------|-----------------------------------------------|----------------------------------|--------------------------|--------------------------|
|      | Wt(g) Retinol (µg) | Wt(g) Retinol (µg) | Wt(g) Retinol (µg) | Wt(g) Retinol (µg) | Wt(g) Retinol (µg) | Wt(g) Retinol (µg) | Wt(g) Retinol (µg) |
| 0    | 110.5 ±2.5 | 23.2 ±0.5 | 114 ±3.5 | 23.5 ±1.5 | 113 ±3.5 | 23 ±0.5 | 109 ±2.5 | 23.5 ±0.5 | 112 ±3.5 | 24 ±0.5 | 114 ±2.0 | 23.5 ±0.5 | 113 ±3.2 | 24.2 ±1.0 |
| 2    | 122* ±2.5 | 29* ±1.0 | 125* ±3.5 | 28.5* ±0.5 | 131* ±3.5 | 117* ±4.0 | 135* ±3.5 | 119* ±5.5 | 138* ±2.5 | 116* ±7.0 | 136* ±1.5 | 116* ±1.0 | 134* ±3.0 | 30* ±1.5 |
| 3    | 130* ±3.0 | 31* ±1.0 | 134* ±3.5 | 32 ±1.5 | 143* ±4.5 | 123 ±5.0 | 138 ±7.0 | 124 ±6.5 | 132 ±2.5 | 91* ±3.5 | 126* ±2.5 | 24* ±0.5 | 124* ±5.5 | 21* ±0.5 |
| 4    | 138* ±3.0 | 34* ±1.5 | 142* ±4.5 | 35 ±2.5 | 158* ±4.5 | 130 ±7.5 | 142 ±5.5 | 130 ±5.5 | 127 ±3.5 | 79* ±3.5 | 118* ±5.0 | 15* ±3.5 | 119* ±12* | ±3.5 | 0.5 |
| 5    | 145* ±3.5 | 37* ±1.5 | 148* ±4.5 | 36 ±1.0 | 136* ±3.5 | 136* ±6.5 | 146* ±3.5 | 137* ±4.5 | 124 ±7.0 | 74* ±5.5 | 116 ±3.5 | 13* ±5.0 | 115* ±10* | ±5.5 |
| 6    | 155* ±3.5 | 38* ±1.0 | 155* ±5.5 | 39 ±1.0 | 170* ±5.5 | 141* ±4.5 | 150* ±7.5 | 130* ±4.5 | 121* ±0.5 | 65* ±3.5 | 113 ±2.5 | 10* ±5.5 | 112* ±8 | ±0.5 |
| 7    | 160* ±4.5 | 39* ±1.5 | 163* ±4.5 | 41* ±1.0 | 175* ±2.5 | 148* ±3.5 | 156* ±1.5 | 112* ±0.5 | 119* ±6.0 | 60* ±1.0 | 112* ±8.25 | ±5.0 | 108* ±6.25* | ±0.5 |

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Table 2: Amount of water intake (ml) measured in rats fed with different diets containing different doses of heroin with β-carotene or without β-carotene. (10 rats in each cage, repeated in three sets of experiments for each diets, n=14 observation).

| Days of Heroin Injection | CD + Oil | CD + β-Carotene + Heroin (LD<sub>25</sub>) | CD + β-Carotene + Heroin (LD<sub>50</sub>) | CD + Heroin (LD<sub>25</sub>) | CD + Heroin (LD<sub>50</sub>) |
|-------------------------|---------|---------------------------------|---------------------------------|-----------------|-----------------|
|                         | 0-6 h   | 0-6 h                           | 0-1 h                          | 1-2 h           | 3-4 h           | 4-6 h           |
| 1<sup>st</sup>          | N       | N                               | N                              | N               | N               | N               |
| 2<sup>nd</sup>          | N       | N                               | N                              | N               | N               | N               |
| 3<sup>rd</sup>          | N       | N                               | N                              | N               | N               | N               |
| 4<sup>th</sup>          | N       | N                               | N                              | N               | N               | N               |
| 5<sup>th</sup>          | N       | N                               | N                              | N               | N               | N               |
| 6<sup>th</sup>          | N       | N                               | N                              | N               | N               | N               |
| 7<sup>th</sup>          | N       | N                               | N                              | N               | N               | N               |
| 8<sup>th</sup>          | N       | N                               | N                              | N               | N               | N               |
| 9<sup>th</sup>          | N       | N                               | N                              | N               | N               | N               |
| 10<sup>th</sup>         | N       | N                               | N                              | N               | N               | N               |
| 11<sup>th</sup>         | N       | N                               | N                              | N               | N               | N               |
| 12<sup>th</sup>         | N       | N                               | N                              | N               | N               | N               |
| 13<sup>th</sup>         | N       | N                               | N                              | N               | N               | N               |
| 14<sup>th</sup>         | N       | N                               | N                              | N               | N               | N               |

Table 3: Observation of Piloerection FROM 0-6 h in rats fed with different diets containing different doses of heroin with β-carotene or without β-carotene. (Abbreviation: N no piloerection, -- Doubtful piloerection, + Moderate piloerection, ++ Highest Piloerection more intense, +++ Highest intensity of piloerection, CD=Control diet). The intensity is expressed in % from the number of animal affected.

| Days of Heroin Injection | CD + Oil | CD + β-Carotene Heroin (LD<sub>25</sub>) | CD + Heroin (LD<sub>25</sub>) | CD + Heroin (LD<sub>50</sub>) |
|-------------------------|---------|---------------------------------|-----------------|-----------------|
|                         | 0-6 h   | 0-6 h                           | 0-1 h           | 1-2 h           |
| 1<sup>st</sup>          | N       | N                               | N               | N               |
| 2<sup>nd</sup>          | N       | N                               | N               | N               |
| 3<sup>rd</sup>          | N       | N                               | N               | N               |
| 4<sup>th</sup>          | N       | N                               | N               | N               |
| 5<sup>th</sup>          | N       | N                               | N               | N               |
| 6<sup>th</sup>          | N       | N                               | N               | N               |
| 7<sup>th</sup>          | N       | N                               | N               | N               |
| 8<sup>th</sup>          | N       | N                               | N               | N               |
| 9<sup>th</sup>          | N       | N                               | N               | N               |
| 10<sup>th</sup>         | N       | N                               | N               | N               |
| 11<sup>th</sup>         | N       | N                               | N               | N               |
| 12<sup>th</sup>         | N       | N                               | N               | N               |
| 13<sup>th</sup>         | N       | N                               | N               | N               |
| 14<sup>th</sup>         | N       | N                               | N               | N               |

Table 4: Etching Behavior observed within 4-6 h in rats fed with different diets containing different doses of heroin with β-carotene or without β-carotene. (Abbreviation: N = No etching, - A few cases of doubtful etching, + Frequent etching, ** Higher No. of etchings, *** Highest cases of etchings, CD=Control diet). The intensity is expressed in % from the number of animal affected.
The present diet used for feeding the rats was followed after [11], and also from our earlier reports [10]. The diet amount, 10g/100g body weight has been accepted as normal consumption rather than the acceptance of the diet has been tested by administrating 8, 10, 12, 15 g per 100g body weight of the animal. It has been found that 10g/100g body weight has been associated with various withdrawal symptoms. The drug has affected growth as well as triggering various abnormal behaviours of the rats.

The various withdrawal symptoms were noticed after the administration of β-carotene and heroin added diets of different concentrations. The reduction of water intake, is significantly reduced in the diets added with heroin LD25 and LD50 doses. β-carotene added diets showed better water intake than the heroin added diets. In (Tables 2-6) the various effects are shown. Several workers have reported the loss of weight in the heroin treated experimental animals [2,9,14,20-23] have opined that there may be two possible mechanisms, where there are altered levels of anabolic hormones with decrease of food intake. In the present investigation also, it has been found that there is a decrease of food intake in batches of the animals fed with heroin LD25 and LD50. However β-carotene + heroin fed animals with varying doses of heroin did not show any significant low intake of food. The support of β-carotene in maintaining normal growth or increase of body weight is due to its multifaceted nutritional role and its capacity of being able to convert it into retinol, as well as its other manifold functions.

### Table 5: Jumping behavious: within 0-6 h in rats fed with different diets containing different doses of heroin with β-carotene or without β-carotene. (Abbreviation: N= No jump., ++Double Jumpings, +++Frequent jumping. +++=Higher No. of jumping; CD=Control diet). The intensity is expressed in % from the number of animals affected.

| Day of Heroin Injection | CD + Oil feeding | CD + β-Carotene Heroin (LD25) | CD + β-Carotene-Heroin (LD50) |
|-------------------------|------------------|-------------------------------|-----------------------------|
| 0-4h                    | 0-4h             | 0-4h                          | 0-4h                        |
| 0-1h                    | 1-2h             | 2-3h                          | 4-6h                        |
| 1st                     | N N N N         | N N N N                       | N N N N                     |
| 2nd                     | N N N N         | N N N N                       | N N N N                     |
| 3rd                     | N N N N         | N N N N                       | N N N N                     |
| 4th                     | N N N N         | N N N N                       | N N N N                     |
| 5th                     | N N N N         | N N N N                       | N N N N                     |
| 6th                     | N N N N         | N N N N                       | N N N N                     |
| 7th                     | N N N N         | N N N N                       | N N N N                     |
| 8th                     | N N N N         | N N N N                       | N N N N                     |
| 9th                     | N N N N         | N N N N                       | N N N N                     |
| 10th                    | N N N N         | N N N N                       | N N N N                     |
| 11th                    | N N N N         | N N N N                       | N N N N                     |
| 12th                    | N N N N         | N N N N                       | N N N N                     |
| 13th                    | N N N N         | N N N N                       | N N N N                     |
| 14th                    | N N N N         | N N N N                       | N N N N                     |

### Table 6: Variation of wet shakes within 4-6 h in rats fed with different diets containing different doses of heroin with β-carotene or without β-carotene. (Abbreviation: N= No wet shake, ++Double wet shake; +++Frequent wet shakes; +++=Highest wet shakes; CD=Control diet). The intensity is expressed in % from the number of animal affected.

| Day of Heroin Injection | CD + Oil feeding | CD + β-Carotene Heroin (LD25) | CD + β-Carotene-Heroin (LD50) |
|-------------------------|------------------|-------------------------------|-----------------------------|
| 0-4h                    | 0-4h             | 0-4h                          | 0-4h                        |
| 0-1h                    | 1-2h             | 2-3h                          | 4-6h                        |
| 1st                     | N N N N         | N N N N                       | N N N N                     |
| 2nd                     | N N N N         | N N N N                       | N N N N                     |
| 3rd                     | N N N N         | N N N N                       | N N N N                     |
| 4th                     | N N N N         | N N N N                       | N N N N                     |
| 5th                     | N N N N         | N N N N                       | N N N N                     |
| 6th                     | N N N N         | N N N N                       | N N N N                     |
| 7th                     | N N N N         | N N N N                       | N N N N                     |
| 8th                     | N N N N         | N N N N                       | N N N N                     |
| 9th                     | N N N N         | N N N N                       | N N N N                     |
| 10th                    | N N N N         | N N N N                       | N N N N                     |
| 11th                    | N N N N         | N N N N                       | N N N N                     |
| 12th                    | N N N N         | N N N N                       | N N N N                     |
| 13th                    | N N N N         | N N N N                       | N N N N                     |
| 14th                    | N N N N         | N N N N                       | N N N N                     |

probably due to nutritional effects of β-carotene and its function of converting into retinol, as well as its other manifold functions.
few cases, the β-carotene + heroin (LD₅₀) did not show any jumping. β-carotene could play a positive role in preventing the withdrawal symptoms such as jumping in the heroin administered rats.

After injecting doses of heroin on alternate days, there was no further increase in the number of jumps after six days. The same results were obtained in morphine injection [24]. The number of jumps correlates with other withdrawal syndromes. These results suggest that in later type of experiment a study has been achieved where withdrawal between doses balances the continued administration of the same dose of morphine [15] and hence heroin also.

Wet shakes (or body shakes) constitute a widely used and reliable criterion for narcotic withdrawal. The type of wet shakes observed was supported by the findings of [14].

The decrease of water intake after heroin treatment has been shown by several workers [25-27]. Naloxone, a narcotic drug suppressant affects the food and water intake. Some of the hypothesis of above findings stated that after each daily heroin injection, the rats demonstrated relatively little intracranial self stimulation on food and water. The several withdrawal symptoms shown in the present experiments are consistent with earlier findings [13-14]. Since β-carotene is effective in the therapy of heroin addicts and since β-carotene has been shown to partially prevent the loss of body weight by heroin, it could not partially block the abrupt withdrawal reaction after heroin addiction. However, it is not clear whether the weight loss can be attributed primarily due to the decrease in food and water consumption during withdrawal or due to the loss of bulk and fluids through increased defecation and urination during withdrawal.

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