

## Alterations In Some Haematological Parameters And serum Proteins During Addiction To Opioid Drug Contramal

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*The male albino mice were made addicted to opium by administering (im) smaller doses of opioid drug "Contramal" daily for 60 days. After 60 days the mice were sacrificed for investigations pertaining to serum protein profile and haematological alterations. Significant increase in  $\alpha_2$  and  $\gamma$  globulins was recorded indicating nephrotoxic and hepatotoxic effect of opium after long term addiction*

### Introduction

Recent speculations have suggested that haematological tests are important diagnostic tools which may be equally valuable as indicators of disease or stress due to drug reactions or toxicants or pollutants. The blood plays an integrated and inevitable part in all immune systems (Lachmann and Peters. 1986; Sadhana *et al.* , 1987; Haedikarin, 2000) Haematological parameters are related to the responses of the organisms to the changing physiological conditions and hence can be used to know the healthy state and tolerance capacity of the animals (Briton, 1963 and Johri *et al.* , 1990). Further, haematological changes obtained during toxicological studies.

Anaemia due to decreased level of hemoglobin content or decreased number of erythrocytes, increase on lymphocytes and neutrophils in nephrosis and liver necrosis are well known haematological alterations (Dacie and Lewis, 1977; Singh *et al.* ,1984 Gupta *et al.*,1986)

An opioid analgesic drugs which interferes with functioning of cells involved in immune response as this drug is known to cause injury to bone marrow cells. Anaemia associated with decreased erythrocytes, hemoglobin percentage and haematocrit value has also been recorded to occur in response to drug addiction (Mello,1987)

Because of the above rather contradictory results in the haematological literature and histo-pathological lesions obtained it was thought to study the chronic effects of contramal with special reference to serum protein profile and haematological alterations in the albino mice.

### Materials and Methods

The present investigations were carried out on the male Swiss albino mice weighing about  $30 \pm 2$  g. The mice were obtained from the animal house of P.D. Medical college, Amravati. They were maintained under standard laboratory conditions and fed with pellet diet. (Lipton, India) and water *ad libitum*. Proper care was taken to avoid any infection and only healthy mice were used for experimental purpose.

The mice divided into two groups. Group I was treated as control and it comprised of 10 male and 10 female mice, administered (im) with 0.2ml distilled water, as a vehicle daily once up to 60 days. The Group II was an experimental group, comprised of 10 males and 10 females mice. Only male mice were administered (Im) with 0.05 mg/kg dose of contramal daily once up to 60 days.

Contramal (tramadol Hydrochloride, 100 mg) was purchased from S G Pharma, Baroda , India. The chemical name of the drug is (I RS: 2RS)-2(dimethylamino methyl)-I-(M-methoxyphenyl)cyclohexanol-hydrochloride.

After 30 And 60 days of the drug administration the male mice of both the groups i.e. control as well as experimental, venous blood from the orbital sinus of each mice was drawn with the help of a corning thin glass capillary. 0.5 ml blood was collected in heparinized glass was taken into eppendorf tube to separate the serum. After blood collection the male mice was sacrificed with cervical dislocation.

The haemoglobin (g %) was recorded by the method of sahil' s acid haematin and RBC count, total leucocytes count, differential leucocytes count was calculated by the method of Neubauer's chamber (Dacie and Lewis, 1977) The serum protein profile was recorded by using PAGE and scanned on Systronic Densitometer (Type 201)

The results are reported as mean  $\pm$  SE (n =6) and statistical analysis was done by student ' t ' test (Fischer, 1950)

### Observations and results

No mortality was observed during the experimental period in all the mice administered (im) with the vehicle and contramal separately. The data showing the changes in some blood parameters of male albino mice after intramuscular administration of contramal for 60 days has been summarized in table (I). The intramuscular

administration of 0.05 mg/kg dose of contramal resulted into decreased haemoglobin content in male albino mice. The present decrease was 12.94 % and 34.49 %. After 30 and 60 days of treatment (Table 1). After 60 days the decrease was significant at  $P < 0.01$ , similar decrease in total erythrocytes count was recorded. The decreased erythrocytes count was recorded. The decreased erythrocytes count was moderately significant after 30 days of treatment ( $p < 0.05$ ) and it was highly significant ( $P < 0.01$ ) after 60 days of treatment. The percent decrease was 6.55 and 25.68 in male mice after 60 days of treatment. Table 1 represent the effects of opioid drug i.e. contramal on the total leucocyte count (TLC) of the male mice, an increase was recorded in TLC after 30 and 60 days of contramal administration. The rise in total leucocyte count was 76.43 % and 125.83 % however, differential leucocyte count was undertaken to know the alterations in lymphocytes. Monocytes, neutrophils, eosinophils and basophils number in control as well as experimental mice. A duration dependent increase in lymphocyte number was observed in contramal treated mice with simultaneous decreases in neutrophils. However, no significant change was recorded in eosinophils, basophils and monocytes.

The serum proteins like albumin and globulins were studied in control as well as drug treated mice. The results are summarized in table 1. The present data indicate that the amount of  $\alpha$  globulin and  $\beta$  globulin decreases along with decreases in albumin. The A:G ratio was decreased in experimental mice over that control group.

### Discussion

Administration of opioid drug, i.e. contramal in male albino mice resulted into decreased haemoglobin content and decrease in the number of total erythrocytes (Table 1) The present findings are in agreement with the findings of the earlier workers ( Briton. 1963 Lachmann and Peters. 1986: sadhana *et al* , 1987; Young and Liu, 1988; Johri *et al*, 1990; Hardikarin 2000).

The decreased haemoglobin content observed in the present investigations might be due to (1) reduced erythrocytes count recorded in all the experimental mice interference of this drug (Table -1) (2) Glucoses-6-Phosphate dehydrogenase deficiency induces massive level when treated with opioid drugs (Rodwell, 1993) Normal functioning of glucose-6-phosphate dehydrogenase is necessary for maintaining glutathione in its reduced or which in turn protects the erythrocytes from damage, The decreased haemoglobin content observed in the present investigation could also be due to deficiency of glucose-6-phosphate dehydrogenase (Hartl, 1993 and kumar *et al.*, 1985). (3) The decreased erythrocytes count and haemoglobin content in the present investigation might be due to the interference of contramal with the development of erythrocytes in the bone marrow. According to Nieforth and Cohen (1989) opioid drugs are known the cause injury to bone marrow cells depleting the haemoglobin and erythrocyte count. In the present investigation depleted neutrophil count has been observed which might have resulted due to immune mechanism when the excess to antibodies produced lyse the granulocytes in the presence of drugs (Jain *et al.*, 1988). However, the increased lymphocyte count was noted after 60 days of contramal treatment which might be preventing the damage to the immune mechanism during the course of treatment.

Serum protein studies resulted into increased  $\beta$  globulin in the experimental mice administered with the contramal after 60 days treatment (Table 1). This is due to the accelerated production of Ig-G as an immune response to drug, Increased  $\beta$  globulin during histopathological lesions is recorded by several workers (Lachmannans Peters, 1986; Sadhana *et al.*, 1990; Dasgupta, 1992; Hardikarin, 2000), Thus the present results are well in agreement with those of above workers, Similarly .the increased level of  $\alpha_2$  globulin in contramal treated mice indicate hepatotoxic nature of drug and increased level of  $\beta$  globulin during same treatment indicate nephrotoxic nature of contramal. During hepatic lesions there is particularly rise in  $\alpha_2$  globulin and during nephrosis,  $\beta$  globulin increases (Varley, 1969, Neiforth and Cohen, 1989; Rodwell, 1993). In the present investigation, serum albumin was found to be depleted significantly which could be due to increased denaturation and increased production of globulin seen during course of treatment. This has resulted into decreased A:G ratio. Our results are well in agreement with that of Bhaskara Rao *et al* (1985), who reported depleted serum albumin in human beings treated with the analgesic drug, acetic salicylic acid and decreased A:G ratio in the opioid drug like morphine treated mice.

Thus, the present results indicate that the long term use of the drug, contramal produce secondary haematological alterations indicative of hepatotoxicity and nephrotoxicity.

**Table 1, Serum proteins and haematological alterations in albino mice (male) administered (im) with contramal for 60 days.**

Parameters	Control (With vehicle)	Experimental (After 60 days treatment)	
		30 days	60 days
Haemoglobingm%	11.6 $\pm$ 0.66	10 10.1 $\pm$ 0.12**	07.6 $\pm$ 0.05**
R.B.C Count/mm	7.79 $\pm$ 0.05	7.28 $\pm$ 0.08**	5.79 $\pm$ 0.02**

W.B.C Count/mm.	8233.00 ± 168	14526.15 ± 331**	18593.03 ± 265**
Neutrophils%	49	25.5	23.9
Lymphocytes%	34	59	63.0
Eosinophils%	06	02	03.0
Monocytes%	10	12	09.0
Basophils%	01	1.1	1.5
Serum Proteins (Total)	4.87 ± 0.15	6.68 ± 0.23**	6.99 ± 0.35**
Albumin mg/100ml	02.26	01.61	00.55**
α <sub>1</sub> Globulin	00.26	00.20	0.40
α <sub>2</sub> Globulin	00.40	00.52	1.09
β Globulin	1.62	03.98	4.70*
γ Globulin	00.46	00.68	00.24
A:G Ratio	0.44	0.30	0.17

Values are ± SE of 5 replicates from each group \*p<0.05. \*\*p<0.01, NS- Not Significant  
Serum protein values denote densitometer reading in percent fraction

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