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SOME TOXICOLOGICAL STUDIES ON CROTON OIL IN WHITE RATS

By

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INTRODUCTION

Recently the medicinal plants occuapy a very important place in the pharmacological field in many countries. They are taken as the main source of pharmaceutical medicants such as cortisone, sex hormones and plasma substitute. The oil extracted from croton tiglium plant is widly used in veterinary practice. Unfortuenatly it has hazard effect. Baggi and Favilli (1953), and Mcmahon et al. (1987) recorded symptoms of croton oil toxicity as diarrhoea, dispnea, hyperexcitability, convulsion and death.

Hellman (1985), Tubaro et al. (1985), Katoik et al. (1987), Kolde and Knop (1987) and Tarinuki and Tagami (1988) observed the post-Mortem finding due to croton oil toxicity.

The effect of croton oil on blood picture was recorded by Kupchan et al. (1976), Benjamin (1985) and Barton et al. (1989).

The variation of serum transaminases as well as the other biochemical constituents were discussed by Van Durren and Sivak (1968), Giagnoni et al. (1976) and Benjamin (1985).

Due to scarety information on croton oil the present study was directed to clarify the biological and side effects of it on blood picture, biochemical constituents as liver and kidney functions.

MATERIAL AND METHODS

Materials:

A. Croton oil:

It extracted by soxholet apparatus and it represented 48% of croton tiglium seeds. Esters tetracyclic diterpene is the active principle of this oil and known as phorbol. The oil is water insoluble but soluble in tween 80.

B. Animals:

80 albino rats of both sexs, 90 days old weighing from 140-180 gm, were kept under standard measure in hygienic food and water supplies. After 2 weeks of accomodation they were divided into

four equal groups 1, 2, 3 and 4 (20 rats each).

Methods:

Groups 1, 2 and 3 were daily injected I/p with doses of 0.25 mg, 0.5 mg and 1 mg. Croton oil dissolved in 0.5 ml tween 80/kg. B.wt. respectively for 8 successive weeks. While group 4 was served as control injected with 0.5 ml Tween 80 I/p according to Hellman (1985). Animals were under close observation for any abnormalities during the experimentatal period. At the end of experiment animals were sacrificed, blood were collected, for haematological and serum were separated for biochemical examinations. Post-Mortem examination was carried out.

Haematological examination:

Haemoglobin content, Packed Cell Volume (P.C.V.), R.B.Cs., W.B.Cs. and differential leucocytic counts were performed according to Schalm (1975).

Biochemical examination:

GOT and GPT, AP, T.P., albumin, globulin, Urea, Creatinine, Calcium, inorganic Phosphorus, Sodium and Potasium were measured according to Reitman and Frankel (1957), Roy (1970), Wootton (1964), Bartholonew and Delancy (1966), Colos (1974), Patton and Grouch (1977), Husdan (1968), Glindler and King (1972), Fiske and Subbarow (1925) and Oser (1979) respectively.

Histopathological examination:

Liver, kidney, heart, lung, brain and testes were collected from each rat and preserved in 10% formol saline processed routinely to paraffin 5 M thin sections were prepared and stained by H & E.

The obtained data were statistically analysed according to method of Snedecor (1971).

RESULTS

Symptoms:

There were general depression, loss of appetite, decreased B.wt., frequent micturation and softy faeces in a dose dependant manner.

Haematological examination:

The examined blood parameters were tabulated in table (1) which showed significant decrease in HB content and PCV values while there was in-signifcant decrease of R.B.Cs. and significant increase of W.B.Cs. in all treated gorups. Concerning the differential leucocytic count there were also highly significant decrease of lymphocytes and significant increase of neutrophils.

Biochemical examination:

The examined serum parameters

Table (1): Effect of croton oil intoxication on haemoglobin concentrations (Hb), haematocrit values (P.C.V.), erythrocytic count (R.B.Cs.), leukocytic count (W.B.Cs.) and differential leukocytic count of albino rats received daily intraperitoneal injection of croton oil for 8 weeks (Mean ± S.E.).

Criteria \ Group	Control	Group I 0.25 mg/kg body weight	Group II 0.50 mg/kg body weight	Group III 1.00 mg/kg body weight
Hb (g%)	12.75±0.74	11.04±1.22	10.70±1.26	9.22±1.19
P.C.V. (%)	37.80±1.14	31.60±1.84*	32.10±1.75	31.66±0.63**
R.B.Cs. ($X10^6/mm^3$)	7.02±0.86	6.78±0.97	6.29±1.09	6.35±0.98
W.B.Cs. ($x10^3/mm^3$)	10.76±0.79	15.48±2.11	15.65±1.90	16.11±2.31*
Lymphocytes(%)	86.10±1.69	73.5±2.2.97**	76.10±2.23**	71.40±2.88**
Neutrophils (%)	11.20±1.41	21.60±2.71**	18.30±2.07**	23.40±2.97**
Monocytes (%)	1.90±0.55	3.40±0.91*	3.40±0.37*	2.20±0.36
Eosinophils (%)	0.90±0.23	2.50±0.65	2.30±0.50*	2.80±0.42*
Basophils (%)	0.00	0.00	0.00	0.20±0.13

± S.E. :Standar error.
* : Significant at $p<0.05$.
** :Significant at $p<0.01$.

were tabulated in tables (2) and (3) which showed significant increase in GOT, GPT, AP, Urea, Creatinine and Potasium in the serum of all treated groups. Total protein, albumin and globulin were significantly decreased ina ll treated groups. Concerning the serum sodium level there was insignificant decrease in the group 1, while highly significant decrease in the 2 and 3 groups was observed. While serum levels of Calcium and phosphorus showed non-significant decrease and increase values respectively.

Gross pathology:

The macroscopical examination revealed yellowish fluid in serous cavities. The liver appeared softy, friable and tinged with pale yellowish brown colour. The kidneys showed enlargment blackish in colour, while lungs revealed dark red area.

Histopathological findings:

The liver showed congestion with presence of focal mononucle-

Table (2) : Effect of croton oil intoxication on some liver and kidney function tests in albino rats serum.

Criteria \ Group of rats	Control	Group I 0.25 mg/kg body weight	Group II 0.50 mg/kg body weight	Group III 1.00 mg/kg body weight
GOT U/l	79..56±2.48	114.27±3.72**	116.39±3.38	114.12±3.29**
GPT U/l	78.72±2.17	112.96±1.71**	116.14±2.67**	117.36±2.68**
Alkaline phosphatase U/l	29.76±2.13	44.41±2.42**	45.75±3.16**	51.82±3.81**
Total protein (g%)	4.93±0.47	3.16±0.26*	3.01±0.21*	2.51±0.36**
Albumin (g%)	3.68±0.24	2.96±0.2777777	2.75±0.21*	2.38±0.34*
Globulin (g%)	1.30±0.44	0.20±0.06	0.23±0.05**	0.18±0.6
Urea (mg%)	249.88±9.05	406.17±7.00	413.88±6.57**	491.35±8.47**
Creatinine (mg%)	0.21±0.04	0.77±0.04**	0.76±0.04**	0.93±0.03**

± S.E. :Standar error.
* : Significant at $p<0.05$.
** :Significant at $p<0.01$.

Table (3): Effect of croton oil intoxication of some electrolyte levels in serum of albino rats.

Criteria \ Group of rats	Control	Group I 0.25 mg/kg body weight	Group II 0.50 mg/kg body weight	Group III 1.00 mg/kg body weight
Sodium (mg%)	25,40±1.40	22.40±1.08	19.39±0.98**	17.98±1.44**
Potassium (mg%)	3.60±0.13	3.38±0.16	4.73±0.23*	4.60±0.43*
Calcium (mg%)	4.38±0.95	3.76±0.65	3.62±0.91	3.39±0.56
Phosphrous (mg%)	3.48±0.91	3.95±0.58	4.46±0.84	4.90±0.88

± S.E. :Standar error.
* : Significant at $p<0.05$.
** :Significant at $p<0.01$.

ar aggregation. The hepatocytes showed vacular degeneration,

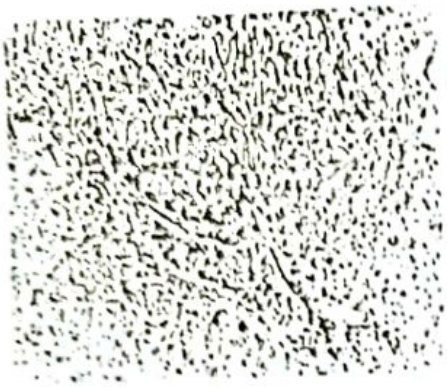

Fig. (1): Liver showing focal mononuclear aggregation with congestion of portal vein, and vacuolation of hepatocytes. (H & E x 200)

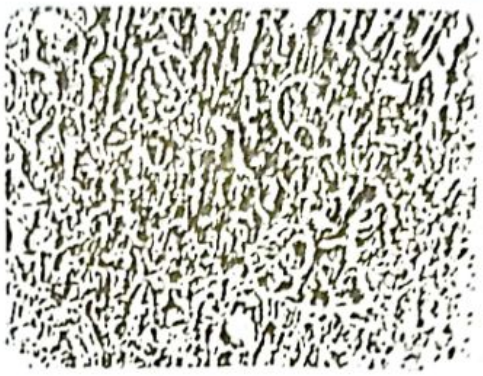

Fig. (2): Kidney Notice the focal interstitial mononuclear cell infiltration with hypercellularity of glomeruli and some haemosiderine pigment. (H & E x 200).

Fig. (3): Lung showing hyperplasia of the bronchial epithelium with leucocytic infiltration (H & E x 200)

Fig. (4): Heart showing intermuscular haemorrahge (H & E x 100)

moreover there was activation of kupffer cells. Fig. (1).

The kidneys showed focal areas of haemorrhages with the presence of hemosiderine pigment in the in-

tertubular spaces. Focal interstitial mononuclear infilteration particularly lymphocytes were also seen. Blood vessels showed congestion with the presence of fibrinoid necrosis of their walls. The renal tubules showed granular casts in their lamina with slight degenerative changes of their epithelial cell lining. The glomeruli showed hypercellularity Fig. (2).

The lungs suffered from congestion with perivascular haemorrhages Fig. (3) and emphysema while hyperplasia of epithelial lining was noticed in bronchioles.

Congestion in blood vessels of the heart accompanied with few hemorrhagic areas Fig (4) also hyalinization of cardiac muscle was noticed in some cases.

The brain showed few extravasated R.B.Cs. in the meninges.

The testicles suffered from few oedema in the intertubular spaces.

DISCUSSION

The observed symptoms due to croton oil toxicity as diarrhea, general depression, loss of appetite and softy faeces was also noticed by **Baggi and Favilli (1953) and katoik et al. (1987)**. The frequent micturation observed in symptoms was attributed to the hyperexcitable bladder reflexes which was noted by **Mcmahon and Abel (1987)** in case of croton oil toxicity.

The effect of croton oil on blood picture revealed decrease of haemoglobin concentration, haematocrit values as well as decrease in erythrocytic counts even non-significant and significant increase in leukocyte These effects was in agreement with **Kupchan et al. (1976) and Barton et al. (1989)**.

The observed reduction of haemoglobin concentration, haematocrit values and erythrocytic count was refered by **Van Durren and Sivak (1968)** due to the influences of croton oil on cellular metabolism by feed back inhibition and/or depression, in addition to change of cellular permeability. More recently, **Benjamin (1985)** attributed such effect to the cytotoxic effect of croton oil on red cells which are sensitive to oxidative agent. A point of consideration was observed in the histopathology, where kidney, lung, heart and brain showed extravassated R. B.Cs. That could sure in the decrease of erythrocytic count. Some remarks was observed by **Finkel and Siegmund (1960)**.

Concerning the observed significant increase in leukocytic count was clarified by **Baggi and Favilli (1953), Finkel and Siegmund (1960), Levey-Appert-Collin and Levey (1976), Swingle et al. (1981), Hellman (1985), Katoik et al. (1987) and Tarinuki and Tarinuki and Tagami (1988)** can be explaned by the direct reflect of the inflammatory process occuring in the croton oil toxicity syndrom, it is known that croton oil is an in-

flammatory agent.

In the differential leukocytic count, there was significant decrease in lymphocytes, this correlated to the inflammatory processes observed histopathologicaly as accumualation of lymphocytes in liver and kidneys. In contrast neutrophils, monocytes and eosinophils percentages in blood increased significantly, which reflected the inflammatory process created by the croton oil syndrom (Tarinuki and Tagami, 1988).

Increases in serum transaminases activities, and A.P. levels are going well with the result of Van Durren and Sivak (1968), Giagnoni et al. (1976). Generally Harper (1975) attributed these increase to the effect of croton oil on liver, kidney and heart muscle and consiquantely liberating their intracellular enzymes in blood stream, more over Benjamin (1985) correlate the increased GOT to heart muscle damage. This is noticed in this study histopathologicaly by hyalinization of cardiac muscle. Also the increased GPT level was correlated to liver damage which was proved in our result by the presence of degenerative change in hepatocytes. More over the increased AP is reflected to the liver damage as gastrointestinal mucosa damage effect.

The significant decrease of total protein, and albumin observed in our result was in agree with Benjamin (1985) who correlated it to liver damage. While Van Durren and Sivak (1968) attributed such decrease to the phorbol interact with cell membrane lead to changes in permeability of cell membrane with respect to entry or depature of small molecules (amino acids and nucleotide).

As a conclusion croton oil could be used but with precaustion due to its hazard effect.

SUMMARY

Eighty albino rats of both sexs were used in this study and divided into 4 equal gorups. The first 3 groups were injected I/p with croton oil in a dose of 0.50 and 1.00 mg/k gm B.Wt. respectively while the 4th group kept as control and injected 0.5 ml tween-80 I/p. Results revealed that R.B.Cs. count, HB% and PCV were decreased while W.B.Cs count were increased significantly. Differential leucocytic coutns were significantly decrease in lymphocytes and significantly increease in neutrophils and monocytes while eosinophil was slightly increased but basophils was not affected. Levels of GOT, GPT and AP were significantly increased, while T.P., albumin and globulin were significantly decreased in serum of toxicated rats. Urea and creatinine were decreased but potassium was increased in camparison to control group.

Calcium and phosphorous levels were slightly changes in serum of treated rats. Microscopically the liver was congested with mononuclear aggregation and the hepatocyte suffered from dgenerative changes. The kidney showed haemorrahages with haemosidrosis, mononculear cell in-

filteration, congestive blood vessels with fibrinoid necrosis of their wall, the tubules showed degenerative changes and hyaline cast. The lung suffered from congetion with emphysema. The heart revealed haemorrahages with some hyalinization. The brain lesion was extravasated T.B.Cs. in meninges. Testicls showed few oedema in the intertubular spaces.

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