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Effects of corn oil as a vehicle on liver of female Swiss albino mice *Mus musculus*

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Abstract : *The study was conducted to evaluate corn oil as a vehicle for delivery of toxins and to observe its effect on liver of female Swiss albino mice. Mice were randomly divided into five groups of six mice each and were administered corn oil at a dose of 10 ml /kg body weight orally for a period of six weeks. Mice of control Gr-1 were kept on normal diet and tap water ad libitum. Histopathological examination of the liver of female albino mice revealed mild alteration in Gr.-4 and mild fatty degeneration was observed in Gr.-5. Mice in Gr.-2 and Gr.-3 showed neither abnormal conditions nor any change in the liver architecture. The biochemical parameters like SGPT, ALP, total protein, and albumin (LFT) were estimated for all the treatment groups and compared with their normal values of the control Gr.-1. Statistical analysis was done using one way analysis of variance (ANOVA). The values obtained were not significant (P>0.05) in all the treated groups for all the biochemical parameters. However the levels of ALP and Albumin in Gr.-5 were higher as compared to the control Gr-1. These data indicate that corn oil at the dose of 10 ml/kg body weight can be used as a vehicle for toxicological studies but may cause adverse effects on the hepatic tissue if used for prolonged periods, suggesting that corn oil as a vehicle can be a confounding factor in the toxicity study depending on time and dose.*

Key Words: Corn oil, Vehicle, Liver, Biochemical, Histopathological.

Introduction

The response of animals in toxicity studies depends upon a number of intrinsic variables, study design, dosing vehicle and the treatment itself. Studies have shown that the choice of vehicle can affect uptake, distribution, pharmacokinetics, and toxicity of chemicals (Condie *et al.*, 1986; Eaton and Klaassen, 1995; Farooqui *et al.*, 1995; Withey *et al.*, 1983). Corn oil has been used as one of the most common vehicles to administer lipophilic chemicals to rodents in toxicity studies. Corn oil has about 60% polyunsaturated fatty acid; therefore, it is one of the oils that have been recommended as a replacement for saturated fat (Dupont *et al.*, 1990). However, corn oil itself is a nutrient and may have some biological effects on animals. In addition to the dose, the time period for which the vehicle is administered to the animals is an important factor in toxicological studies.

Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Balistreri and Shaw, 1987). In light microscopy observations the hepatic tissue showed normal polygonal cells with prominent round nuclei and eosinophilic cytoplasm and few spaced sinusoids arranged in between the hepatic cells with fine arrangement of Kupffer cells. In liver different cells have different enzymes inside them, depending on the function of the cell. When cells die or are damaged, the enzymes leak out causing an increase or decrease in their level in the blood. Serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase are some liver-function test

enzymes, the elevation of which, in serum, reflects some hepatic disorders .Bilirubin, albumin and total protein levels, as well as the tissue histological study are known to be useful in assessing the functional integrity of the liver. Therefore, in order to evaluate the liver damage, estimation of these enzymes in serum and histopathological study of the tissue is essential. In our study we evaluated whether the use of corn oil as a vehicle for toxicants on female Swiss albino mice at a dose of 10 ml/ kg body weight for a period of six weeks, can affect the histological and biochemical parameters.

Materials and Methods

Animals-Female Swiss albino mice, reared in the animal house of Mahavir Cancer Sansthan, Patna, and weighing 25-30g were randomly divided into five groups of six mice each. Mice were individually housed in stainless-steel wire cages in a temperature- (24 ±1°C), humidity- (55 ± 5%), and lighting- (12-h light/dark cycle) control room. Food and tap water were given *ad libitum* throughout the study. All animal experiments were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA)

Group 1 - Control group

Group 2 - Corn oil treatment for one week only

Group 3 - Corn oil treatment for two weeks

Group 4 - Corn oil treatment for four weeks

Group-5- Corn oil treatment for seven weeks

Study design

After acclimatization, mice were administered orally 10 ml corn oil/kg body weight daily during the experimental period. Treatment volume of corn oil was based on body weights, which were measured daily before giving the dose. Corn oil was obtained from Nieshiel Chemical Pvt. Ltd., India. All animals were examined twice a day for general physical condition. Visible physical abnormalities or abnormal demeanor of the mice was recorded during the experimental period .All the procedures involving animals in this study were approved by the Institutional Animal Ethics Committee (IAEC).

Statistical analysis

Mean and Standard Error of Mean (SEM) were calculated from the six replicates of each group. Each experimental value was expressed as the mean ± SEM. Statistical calculations of the data were performed using one way analysis of variance (ANOVA).

$P<0.05$ was considered statistically significant.

Physical parameter

Physical parameters (body weight, food and water intake), and local injury were studied during the treatment period. Mortality in all the groups, during the course of treatment was also recorded. After the treatment biochemical (liver function tests) and histological parameters were studied. The dissected organs were quickly blotted, weighed on digital balance and processed for histological studies.

Biochemical study

Blood samples were obtained by orbital sinus puncture from control and corn oil treated mice were taken. Serum was obtained from the blood by centrifugation (3000 rpm for 15 minutes) for the estimation of various biochemical parameters. After separation of the serum, it was collected in separate small labeled vials at 4°C for biochemical Liver function test (LFT) parameters- SGOPT, ALP, total protein, albumin and bilirubin. The serum biochemical liver function test parameters were estimated by Alkaline phosphatase kit (Kind and King, 1954), SGPT Kit (Reitman and Frankel, 1957), total protein by Biuret method, albumin by BCG method (Doumas. *et al.*, 1971) and bilirubin by bilirubin kit, using fully Automated Biochemistry

Analyzer (Model No- SELECTRA-"E",VITALAB BY MERCK) at Mahavir Cancer Sansthan and Research Centre ,Patna, for all the treated groups. Comparative study with control was done.

Histological examination

Animals were sacrificed and liver was dissected out. It was washed thoroughly in normal saline (0.85 %) and was collected for histological examinations. The tissue was examined grossly, any lesions observed were recorded. The liver was trimmed, processed, embedded in paraffin, sectioned at a thickness of 4- 5 µm, stained with hematoxylin and eosin, and examined microscopically. The pathological findings from the prepared slides were observed and recorded.

Results and Discussion

The present study was carried out on female Swiss albino mice to evaluate the effect of corn oil on physical, biochemical and histopathological parameters. In lieu of that, the morbidity after corn oil administration was more or less negligible and no physical abnormality was recorded. Only few deaths were recorded during the experimental period. The changes in body weight and liver weight (Table 1) were statistically not significant (P> 0.05) in all the groups. The amount of food consumed by animals greatly influence growth response (Fashakin and Unokiwed, 1993). Since we used a very moderate dose of corn oil for treatment (at 10 ml / Kg / b. wt.), therefore, no significant changes in body weight and organ weight were observed (Table 1 and Fig.1 and 2).

LFT are helpful screening tools to detect hepatic dysfunction (Thapa and Walia 2007) and the obtained data can be used as an Indicator of the possibility of using corn oil as a vehicle for toxicological studies by oral method. Levels of SGPT, ALP, total protein, bilirubin and albumin (Table 2) in all the treated groups as compared to the control group were also not significant (P>0.05). ALP (Alkaline Phosphatase) is found histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes (Rosalki and Mcintyre, 1999). Highest level of alkaline phosphate occur in cholestatic disorders and that elevation occurs as a result of both intra hepatic and extra hepatic obstruction to bile flow (Friedman *et al*, 2003). The mechanism by which alkaline phosphatase reaches the circulation is uncertain. Leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions (Kaplan 1986, and Rosalki and Mcintyre, 1999). The ALP level recorded in Gr-4 and Gr-5 were slightly higher as compared to control Gr-1 (Fig 4) but it was statistically not significant (P>0.05). Therefore, it can be inferred that corn oil does not cause intrahepatic and extrahepatic obstruction to bile flow. Bilirubin in body is a careful balance between production and removal of the pigments. The observed values in all the treated groups and in control Gr-1 were more or less similar (Fig 7). Our result was in agreement with the similar study conducted in Wistar rats using a diet containing red palm oil (Edem, 2009). Hence, the result of this investigation indicates no severe hepatic damage by corn oil. Serum Glutamic Pyruvate Transminase (SGPT) is specific indicator of hepatocellular necrosis. SGPT is primarily localized in cytosol of hepatocytes (Sherlock, 1997 and Rosen and Keefe, 2000). In our investigation the level of SGPT is more or less similar (Fig 3) and statistically non significant in all the treated groups, indicating corn oil is not causing hepatocellular necrosis. The total protein represents the sum of albumin and globulin. Albumin is a plasma protein, synthesized only by the liver (Rosalki and Mcintyre, 1999) and its serum level is affected by liver diseases, nutritional status and also by hormonal imbalances. Albumin maintains the fluid volume within the vascular space and its

Table 1. Body weight and organ weight of control and corn oil treated female mice

Sl. No	Weight of body and organs (gram)	Control (Gr 1)	Corn oil treated mice (Female)			
			1 week (Gr 2)	2 weeks (Gr 3)	4 weeks (Gr 4)	6 weeks (Gr 5)
1.	Body weight	30.1±0.247	29.9±0.330	30.4±0.217	30.9±0.156	31.5±0.264
2	Weight of Liver	1.829±0.035	1.828±0.018	1.802±0.038	1.840±0.035	1.873± 0.017

Values expressed as mean ±SEM of 6 mice. P<0.05 was considered statistically significant

Table 2 Liver function test parameters

SL. No.	Liver function test	Control (Gr-1)	Corn oil treated mice(female)			
			1 week (Gr-2)	2 weeks (Gr-3)	4 weeks (Gr-4)	6 weeks (Gr-5)
1.	SGPT(IU/L)	30.6±1.123	30.3±0.944	30.6±0.792	30.9±0.858	31.4±0.855
2.	ALP(IU/L)	219.8±34.35	220.8±32.2	226.8±30.45	278±7.87	296±5.54
3.	Total Protein (gm/dl)	6.04±0.26	6.12±0.23	6.12±0.23	6.14±0.15	6.2±0.11
4.	Albumin (gm/dl)	3.0±0.128	3.14±0.126	3.4±0.074	3.14±0.103	3.36±0.120
5	Bilirubin(mg/dl)	0.46±0.02	0.44±0.021	0.46±0.021	0.46±0.02	.47±0.019

Values expressed as mean ±SEM of 6 mice. P<0.05 was considered statistically significant

low value is a sign of poor health. In present observation there were no significant changes in Gr-2, Gr-3 and Gr-4 (P>0.05) while a slight increase in the level of albumin which was statistically insignificant in Gr-5 (Fig. 5) indicates that the nutritive value of corn oil may be responsible for this factor. The plasma protein is a function of the nutritional status which is one of the factors affecting the state of health of the animal (Igwebuike *et al.*, 2008). The normal values (Fig. 6) and the non significant variations (P>0.05) in our experiment indicate nutritional adequacy of corn oil. The results of the present study are in corroboration with the total protein concentrations of the plasma that were close to the reference value of 7.52 ± 0.27 g/dL for rats (Kaneko *et al.*, 1997).

Control Gr-1 of female mice (Fig. 8) showed that the cytoplasm of hepatocytes was not vacuolated and had well demarcated sinusoids. Also, no area of infiltration by inflammatory cells and fatty degenerative changes were observed in the tissue sections. The histopathological studies revealed no significant abnormalities in the liver architecture of corn oil treated female mice in Gr-2 (Fig 9) and Gr-3 (Fig. 10) as compared to control Gr-1 (Fig. 8). The results are consistent with the reports of Manorama and Rukmini (1991) who observed no abnormalities in the liver of Wistar strain of albino rats fed 10% RPO (Refined palm oil) and 10% REFPO (Refined palm olein oil) containing diets for 90 days. However, histopathological alterations were observed in Gr-4 (Fig. 11) and Gr-5 (Fig. 12), and mild fatty degeneration was observed in Gr-5 and some areas of infiltration of inflammatory cells with mild hemorrhage (Fig. 12) were also seen. Therefore, these data indicate that corn oil at the dose of 10 ml/kg body weight can be used as a vehicle for toxicological studies but may cause adverse effects on the hepatic tissue if used for prolonged periods, suggesting that corn oil as a vehicle can be a confounding factor in the toxicity study depending on time and dose.

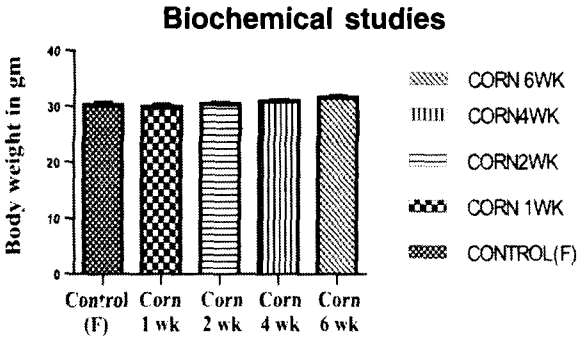

Fig. 1 Body weight of corn oil treated female mice

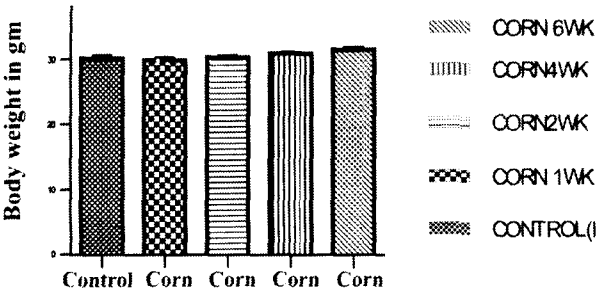

Fig. 2. Liver weight of corn oil treated female mice

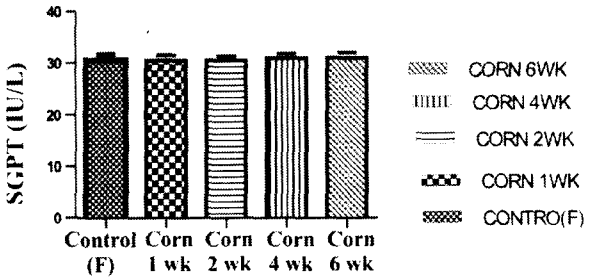

Fig. 3 Levels of SGPT in corn oil treated female mice

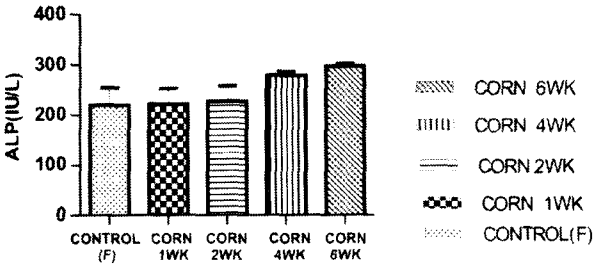

Fig. 4 Levels of ALP in corn oil treated female mice

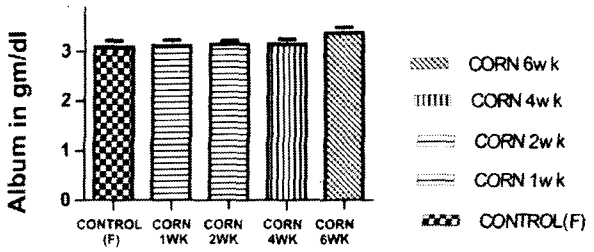

Fig. 5 Levels of albumin in corn oil treated female mice

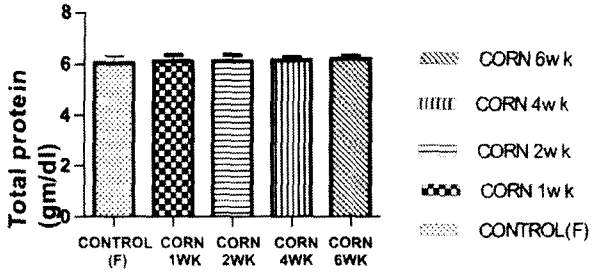

Fig. 6 Levels of total protein in corn oil female mice

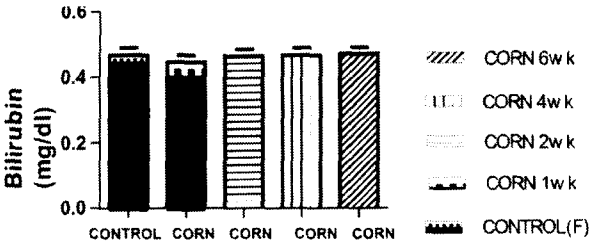

Fig. 7 Levels of bilirubin in corn oil female mice

Histopathological studies

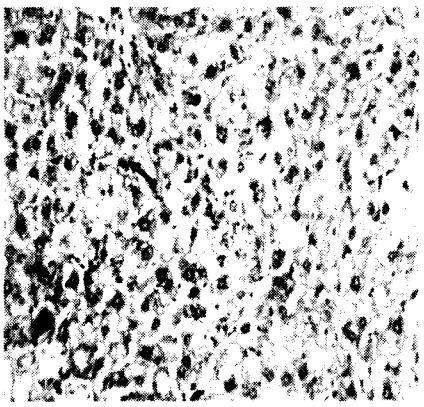

Fig. 8 Microphotograph of liver of Group-1 (Control)showing polygonal cells with centralized nucleus (arrow). H and E. X 400

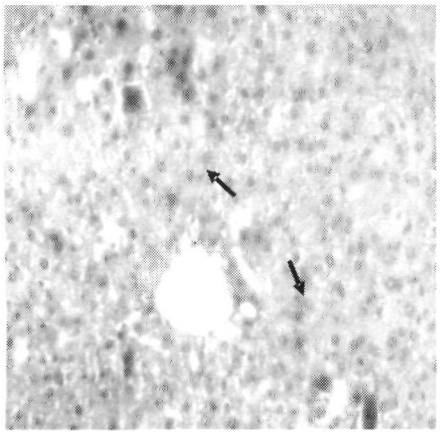

Fig. 9 Microphotograph of liver of Group-2 (corn oil 1 wk) showing similar cellular arrangement as in Group-1 (arrow). H and E. X 400

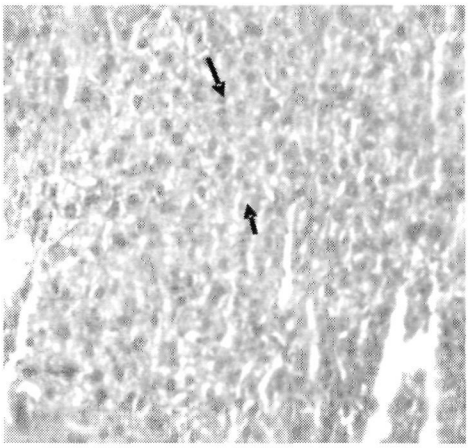

Fig. 10 Microphotograph of liver of Group-3 (Corn oil 2 wk.) Showing mild changes in cellular structure (arrow). H and E. X400

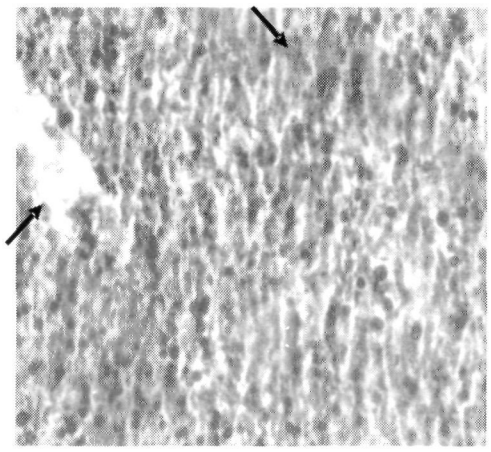

Fig. 11 Microphotograph of liver of Group-4 (Corn oil 4 wk) showing mild changes in cellular structure with few foci of hemorrhage(arrow). H and E. X400

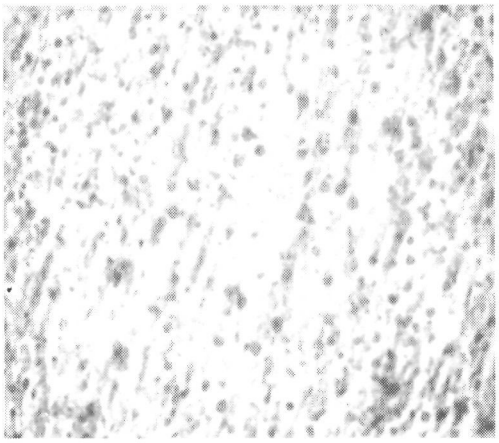

Fig. 12 Microphotograph of liver of Group-5 (Corn oil 6 wk.) showing Mild degenerative changes with pleiomorphic nuclei (arrow). H and E.X400

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