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PROTECTIVE EFFECT OF FRACTION OF *AZADIRACHTA INDICA* LEAF EXTRACT ON CARBON TETRACHLORIDE INDUCED HEOATOTXICITY

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ABSTRACT: *The hepatoprotective activity of a fraction of the leaf extract of A.indica against carbon tetrachloride : liquid paraffin (1:1) induced liver damage in rats at doses of 100 mg/kg and 200 mg/kg was evaluated. A significant dose dependent hepatoprotective activity was evidenced by lowering of the elevated levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), acid phosphatase (ACP) and alkaline phosphatase (ALP) in the serum of CCl_4 : liquid paraffin (1:1) treated rats.*

Azadirachta indica (Family : Meliaceae) is well known for its various medicinal properties. Various parts of the plant like bark, young fruit, seeds and flowers have been studied for their pharmacological actions and the leaves of this plant have been found to possess various pharmacological properties[1]. The leaves are insecticidal, good in ophthalmia, biliousness, skin diseases. Leaves are applied to boils in the form of poultice and a decoction is recommended in ulcer and eczema[2]. In view of its reputed use in various ailments the leaf extract of *A.indica* was taken up for investigation as hepatoprotective against carbon tetrachloride : liquid paraffin induced toxicity.

MATERIALS AND METHODS

Plant Material :

Fresh leaves were collected locally during the months of June and July and identified as Botanical Sruvey of India, Shibpur, Howrah. The leaves were dried, pulverized and passed through 60 mesh sieve.

Diagnostic reagent kits supplied by Span Diagnostic Private Ltd., 173 – b, New Industrial Estate, Udhna – 394 210 (Surat) India (Packaging slip No. 63857) were used for estimation of SGOT, SGPT, acid phosphatase and alkaline phosphatase.

Preparation of the extract:

The dried powdered leaves (430 g) were extracted in a soxhlet extraction apparatus with benzene. The extract was discarded and the marc was again extracted with 90% methanol. The marc after removing the solvent was digested with water on water-bath for 4 hours and filtered. The filtrate was precipitated by adding ethyl alcohol until precipitation was completed. After centrifugation the precipitate was dissolved in small amount of water and purified with activated charcoal. Finally, the precipitate was treated with ether for drying and preserved in the vacuum deccicator for the study.

Induction of hepatotoxicity in albino rats:

Male albino rats (150 – 200 g) were taken. They were housed in standard metal cages, provided with food and water *ad libitum.* Animals were divided into 4 groups containing 10 in each group. Group – I was considered as control which received only water for injection at a dose 10ml/kg. Group – II, III and IV received CCl_4 : liquid paraffin (1:1) at a dose of 10 ml/kg at every alternate days upto 5^{th} day. On the 6^{th} day 3^{rd} and 4^{th} group of animals get the extract of *A.indica* leaf at a dose of 100 mg/kg and 200 mg/kg via i.p. route by dissolving in water for injection. On the 7^{th} day blood sample was collected from heart and analysed biochemical. Reitman for Frankel method[4] was followed for SGOT and SGPT measurement. For this estimation buffered aspartate – ketoglutarate substrate, pH 7.4 and buffered alanine-ketoglutarate substrate, pH 7.4 were used for SGOT and SGPT respectively. For acid phosphatase and alkaline phosphatase Kind and King's method[5] were followed.

RESULTS

A significant increase in the levels of serum GOT, GPT, ACP and ALP was found due to CCl_4 : liquid paraffin (1:1) treatment (Table I).

Animals receiving *A. indica* leaf extract at a dose of 100 mg/kg exhibited significant recovery as evidenced by GOT, GPT and ALP estimation ($P<0.001$) but in case of ACP it was not significant (Table I, Fig. I and II).

Animals receiving *A. indica* leaf extract at dose of 200 mg/kg exhibited highly significant recovery ($P < 0.001$) as evidence by GPT and ALP estimation and significant recovery ($P<0.01$) evidenced by GOT and ACP estimation (Table I, Fig.I and II).

TABLE NO. I

EFFECT OF DIFFERENT DOSES OF *A. indica* EXTRACT ON LIQUID PARAFFIN : CARBON TETRA CHLORIDE (1:1) INDUCED HEPATIC DAMAGE IN RATS

(values expressed as mean ± S.E Groups of 10 animals each)

Parameters ***Serum* (U/ml)**	**Group – I** ***Control***	**Group – II** ***CCl_4 : Liq. Paraffin***	**Group – III** ***100 mg / kg***	**Group – IV** ***200 mg / kg***
Glutamate oxalacetate Transaminase (GOT)	52.3 ± 1.79	123.6 ± 1.56[a]	62.3 ± 1.42[a]	57.4 ± 1.80[b]
Glutamate Pyruvate Transaminase (GPT)	58.1 ± 3.36	112.4 ± 3.32[a]	68.2 ± 4.47[a]	65.0 ± 4.88[a]
Acid phosphatase	18.1 ± 2.12	34.5 ± 1.97[a]	27.1 ± 3.36[c]	21.9 ± 2.99[b]
Alkaline phosphatase	80.1 ± 1.37	159.8 ± 3.28[a]	134. ± 3.69[a]	123.2 ± 5.29[a]

Group – II was compared with Group – I and all values were significantly different ($P < 0.001$)

Group – III and Group – IV was compared with Group – II, values marked with ‘a’ indicate $P<0.001$, ‘b’ indicate $P<0.01$ and ‘c’ indicate not significant, reversal of liquid paraffin : CCl_4 (1:1) induced changes.

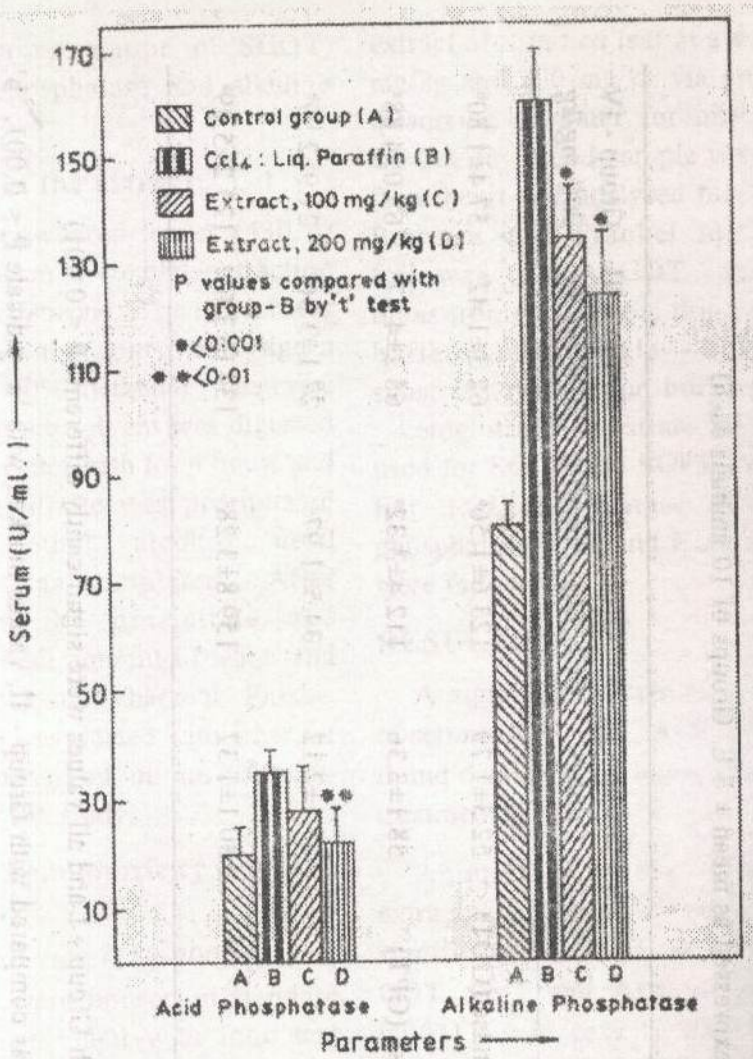

FIG.1 EFFECT OF DIFFERENT DOSES OF A. indica LEAF EXTRACT ON LIQUID PARAFFIN:CARBON TETRACHLORIDE (1:1) INDUCED HEPATIC DAMAGE IN RATS. (values expressed as mean± S.E. groups of 10 animals each).

Fig. I : EFFECT OF DIFFERENT DOSES OF *A. Indica* LEAF EXTRACT ON LIQUID PARAFFIN : CARBON TETRACHLORIDE (1:1) INDUCED HEPATIC DAMAGE IN RATS : (values expressed as mean ± S.E. groups of 10 animals each)

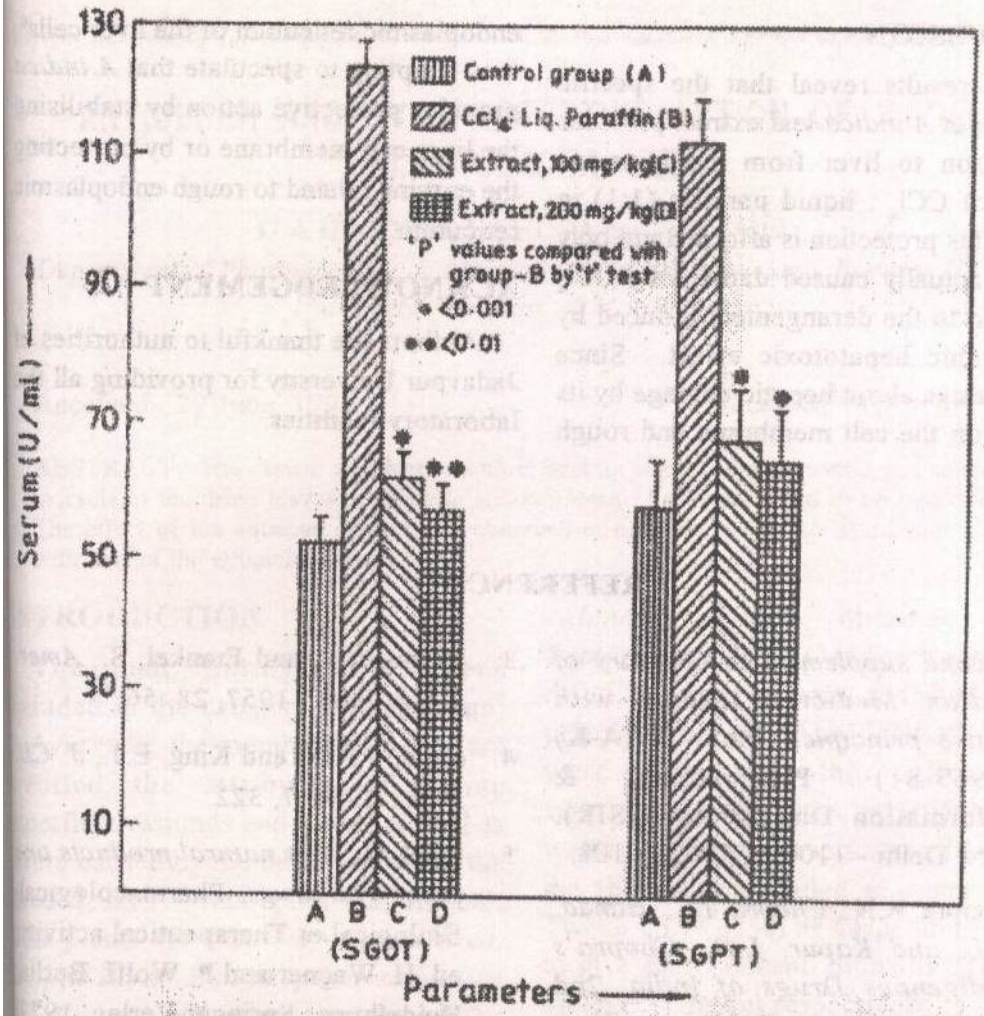

FIG. II. EFFECT OF DIFFERENT DOSES OF A. indica LEAF EXTRACT ON LIQUID PARAFFIN : CARBON TETRA-CHLORIDE (1:1) INDUCED HEPATIC DAMAGE IN RATS. (values expressed) as mean ± S. E. groups of 10 animals each).

Fig. II : EFFECT OF DIFFERENT DOSES OF *A. Indica* LEAF EXTRACT ON LIQUID PARAFFIN : CARBON TETRACHLORIDE (1:1) INDUCED HEPATIC DAMAGE IN RATS : (values expressed as mean ± S.E. groups of 10 animals each)

DISCUSSION

The results reveal that the specific fraction of *A. indica* leaf extract provides protection to liver from the injurious effect of CCl_4 : liquid paraffin (1:1) in rats. This protection is afforded not only to the actually caused damage by CCl_4 but also to the derangement induced by its chronic hepatotoxic effect. Since CCl_4 brings about hepatic damage by its action on the cell membrane and rough endoplasmic reticulum of the liver cells[6]. It is tempting to speculate that *A. indica* provides protective action by stabilizing the liver cell membrane or by protecting the enzyme related to rough endoplasmic reticulum.

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