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ACUTE AND SUB-CHRONIC TOXICITY OF *TAMARINDUS INDICA* L FRUITS' METHANOLIC EXTRACT

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ABSTRACT

In-vivo experiments were performed to assess the toxicological profiles of an active methanolic extract of *Tamarindus indica* L (Aradaib), which showed positive effect against *Salmonella typhi* and *paratyphi*. Experiments were conducted on 40 Wister Albino rats, using 14 days for acute toxicity, with injecting a single dose of 50 mg / kg body weight, and 28 days for sub-chronic toxicity with 250, 500, 1000 and 2000 mg/kg body weight administrated orally. The obtained results revealed that there is neither acute nor sub-chronic toxicity displayed from the applied different concentrations of the active methanolic extract of *Tamarindus indica* L Fruits'.

KEYWORDS: *Tamarindus indica* L, *Salmonella typhi* and *paratyphi*.

INTRODUCTIOM

Tamarindus indica L Aradaib (Vernacular), Tamarind (English), Family, Leguminosaceae. It is a big tree up to 20 meters high, permanently green; it has alternate par- pinnate leaves, widely distributed in middle and south of the Sudan (Fig 1 & 2). It is used traditionally for malaria, infective hepatitis, fever and constipation (EL Ghazali 1997). Chemically it contains fatty acids (saturated and unsaturated), Beta sitosterols , cyclo-artanol and protein. It also contains copper, cadmium, arsenic, zinc, lead, sodium, potassium, calcium, magnesium, manganese, iron and phosphorus. Khanzada *et al* (2008). Rashid *et. al*. (2014) isolated 1, 2 – Benzene di-carboxylic acid, diisooctyl ester from the methanolic extract of the plant fruits.

Silva *et al* (2009) mentioned that *Tamarindus indica* L has been used in folk medicine as an anti diabetic, a digestive aid, and a carminative among other uses, currently; there is no information in the toxicology literature concerning the safety of *T.indica* L extract. They evaluated the clastogenic and/or genotoxic potential of fruit pulp extract of the plant *in-vivo* using Wistar rats, and they found that the extract was devoid of clastogenic and genotoxic activities in the cells of the rodents when administrated orally at three acute doses, 1000, 1500 and 2000 mg/kg body weight. Ranjan *et al* (2009) examined aqueous extracts of T. *indica* L fruit pulp (100 mg/kg body weight) (50 mg /kg body weight) orally once daily for 90 days, lowered plasma fluoride concentrations in rabbits receiving fluorinated drinking water (200 mg Na F / litter water). Changes in plasma biochemistry suggested less hepatic and renal damages in animals receiving plant extracts along with fluorinated water with comparison to that receiving fluorinated water alone. Many authors stated that the plant have antimicrobial activity Muthu *et al* (2005) , Abukakar *et al* (2008), Shital *et al* (2010) , Abdel Gadir *et al* (2007) and Rashid *et.al* (2013). Also many authors reported that *Tamarindus india* L showed Hepatoprotective, Analgesic activity, Antipyretic activity , Laxative activity , Anticancer activity and Antiemetic activity. Mahesh *et. al* (2010), Khalid *et. al* (2010). Izquierdo *et. al* (2007), Havinga *et. al* (2010), AL-fatimi et. al (2007) and Khan et. al (2005).

(Fig 1): *Tamarindus indica* L fruit.

(Fig 2): *Tamarindus indica* L tree.

MATERIALS AND METHODS

Plant material: The plant sample was purchased from Omdurman local market, Sudan, identified and authenticated by Dr. Haider Abdelgadir Herbarium Curator. Herbarium

voucher was deposited at the herbarium of the Medicinal and Aromatic Plants Research Institute (MAPRI) Khartoum, Sudan.

Preparation of Crude Extract

Extraction was carried out according to the method described by Sukhdev *et. al.* (2008). 50 g of the plant sample were successively extracted with petroleum ether and methanol (S. D. Fine India) using shaker apparatus (Sturart West Germany) for about three days for petroleum ether and five days for methanol. Solvents were evaporated under reduced pressure using rotary evaporator (Buchii, Switzerland). Extracts were placed in clean tubes till complete dryness and stored in a refrigerator till use. Yield percentages were calculated as follows. Weight of extract / weight of sample X100 %. (Table 1)

Toxicity of *Tamarindus indica* Extract

Fourty Swiss Albino rats of either sex weighing 85-120 gm were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR) , Khartoum- Sudan (K.S). All rats which were used to evaluate the toxicity of the active extract, *T.indica* L methanolic extract, were housed individually in a ventilated Animal House before and after surgery, they had accessed to standard diet which has been prepared in National Experimental Animal House (NEAH) in (MAPRI), supplemented with water adlibitum (as much as one likes). The holdingroom was illuminated with 12 hs. Light/ dark cycles. Room temperature was between 30-35 °C with 45% to 55% humidity.

Acute Toxicity

Ten of the above rates were subjected to experimentally for 14 days to evaluate the acute toxicity. Seven rats were injected by extract with a dose = 50 mg/kg Body Wt per day, a durations of 2 weeks, The other three rats were fed with the normal diet and served as a control group. Evaluation values of the acute toxicity were tabulated (Table 2).

Sub – Chronic Toxicity

Thirty of the above rats were subjected to sub-chronic toxicity for 28 days. Twenty four rats were divided into four groups each of 6-7 rats. Groups 1-4 were given the methanolic extract of *T.indica* L at doses of 250, 500, 1000 and 2000 mg/kg/day respectively via oral route, duration of four weeks. The other four rats were fed the normal diet to serve as a control group. Evaluation values of the Sub-chronic toxicity were tabulated (Table 3).

At the end of each of the previous experiments (Acute and Sub-chronic), rats from each group were killed under ethyl anaesthesia to identify gross lesions and specimens of the liver, intestines, kidneys, spleen and heart, were immediately fixed in 10% neutral buffered formalin and processed for histopathology (Fig 3, 4, 5 and 6). Blood samples were collected at slaughter for serum analysis and haematology.

Blood Analysis

Serum samples were analyzed for the activity of aspartate aminotranferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) and for concentration of total protein, albumen, total bilirubin, creatinine, urea, BUN, calcium, phosphours, sodium and potassium (Table 2 and 3).

RESULTS

Table (1): Yield Percentage of Extracts.

Plant + Part used	Petroleum ether		Methanol	
	weight of extract (g)	Yield (%)	weight of extract (g)	Yield (%)
T. indica L. (Fruits)	0.356	0.712	19.564	39.128

The parameters observations in the toxicity studies of the tested rats with respect to control groups indicated that no up-normal change in the body weight, mortality and vital organs such as liver, intestines, kidneys, spleen and the heart, was observed. (Fig 3, 4, 5 and 6).

Also biochemical parameters which includes Creatinine, Urea, Blood Urea Nitrogen (BUN), Asparate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Cholesterol, Total Protein (TP), Albumin and Na^{+}, K^{+}, Ca^{+2}, PO_4^{-3} irons, concentrations tests did not show up normal change. (Tables 2 &3).

So according to the results of the administrated doses of *T. indica* L fruits' methanolic extract, it can be said that there is no acute or sub-chronic toxicity. These results are on line with Silva *et al* (2009), who found that the extract was devoid of carastogenic and gentoxic activities in the cells of the rodents, and confirmed by Ranjan *et al* (2009), who revealed that *T. indica* L extracts have some potential to mitigate fluoride toxicity.

Table (2): Acute Toxicity Results:

Item	Na^+	K^+	Ca^{+2}	PO_4^{-3}	Creat	Urea	BUN	AST	ALT	ALP	TB	Chole	T.P	Albumin
Control group weight of rat = 85g (3rats)	138	5.5	11.0	5.3	0.25	36	16.8	186	55	186	0.04	68	7.7	4.6
	142	6.6	12.2	6.2	0.31	62	29.0	253	57	149	0.04	77	7.4	4.3
	141	5.9	11.4	5.2	0.28	48	22.4	230	57	198	0.05	63	7.9	4.7
Acute Toxicity 50mg/kg Body Wt. By injection 14 days rat = 100g Body Wt.	145	7.1	12.1	8.9	0.27	39	18.2	136	24	100	0.20	77	8.6	4.5
	139	5.9	10.9	6.7	0.42	41	19.2	154	29	91	0.15	60	8.8	3.6
	138	6.5	9.3	8.0	0.27	45	21.0	153	30	67	0.19	70	7.5	4.1
	140	6.0	10.5	7.6	0.25	36	16.8	174	27	91	0.11	66	7.6	3.3
	142	6.8	11.0	7.7	0.23	43	20.1	131	38	118	0.14	53	7.7	3.6
	142	6.1	11.8	7.7	0.25	42	19.6	176	39	120	0.12	59	7.8	3.4
	141	6.8	11.9	7.8	0.26	43	20.6	177	40	121	0.13	60	7.9	3.5

Table (3): Sub-chronic Toxicity Results:

Item		Na^+	K^+	Ca^{+2}	PO_4^{-3}	Creat	Urea	BUN	AST	ALT	ALP	TB	Chole	T.P	Albumin
Sub-chronic Toxicity orally 28/days	Group (1) 250mg/kg	143	6.1	10.4	5.4	0.30	63	29.5	207	54	153	0.10	68	7.4	4.5
	Group (2) 500mg/kg	142	5.9	11.0	6.1	0.32	61	24.6	227	57	172	0.09	63	7.6	5.9
	Group (3) 1000mg/kg	141	6.1	11.0	5.6	0.31	54	25.0	204	57	185	0.10	76	7.5	4.6
	Group (4) 2000mg/kg	130	6.0	10.6	5.4	0.34	52	23.7	200	42	115	0.05	50	7.7	4.5
	Control Group (4 rats)	142	5.6	11.0	5.2	0.26	62	25.0	200	55	186	0.4	68	7.7	4.6
		141	5.9	11.4	6.1	0.27	48	29.0	230	57	150	0.5	77	7.4	4.3
		142	6.6	12.2	5.3	0.25	54	23.0	253	57	190	0.6	63	7.9	4.7
		141	6.2	12.1	5.8	0.25	53	24.0	231	56	188	0.5	64	7.8	4.5

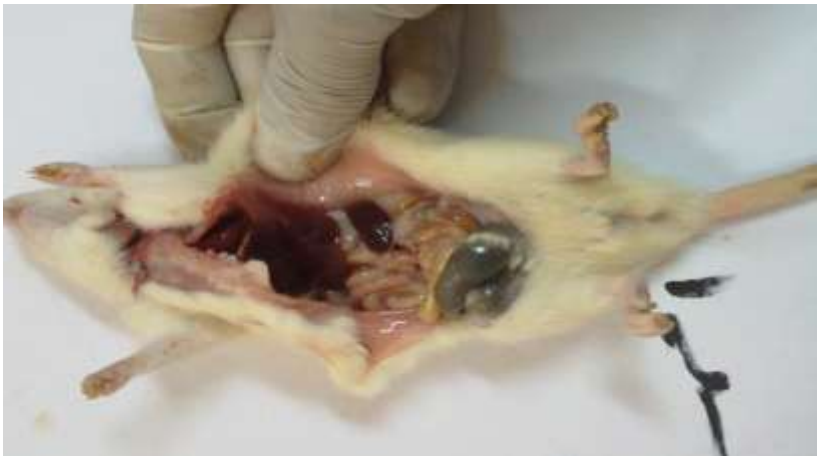

Fig (3): Image showing Gross Lesions and Specimens of the Liver, Intestines, Kidneys, Spleen and the Heart of a rat subjected to Acute Toxicity.

Fig (4) Image of Gross Lesions and Specimens of the Liver, Intestines, Kidneys, Spleen and the Heart of a rat served as a control to Acute Toxicity.

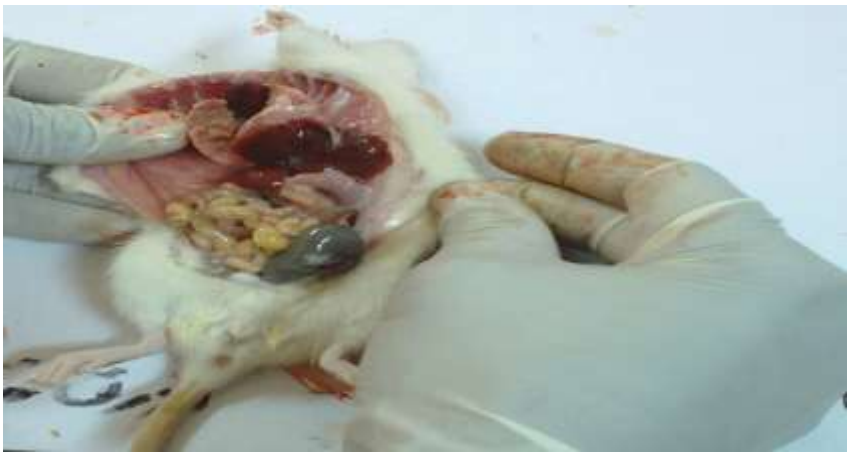

Fig (5): Image showing Gross Lesions and Specimens of the Liver, Intestines, Kidneys, Spleen and the Heart of a rat subjected to Sub - Chronic Toxicity.

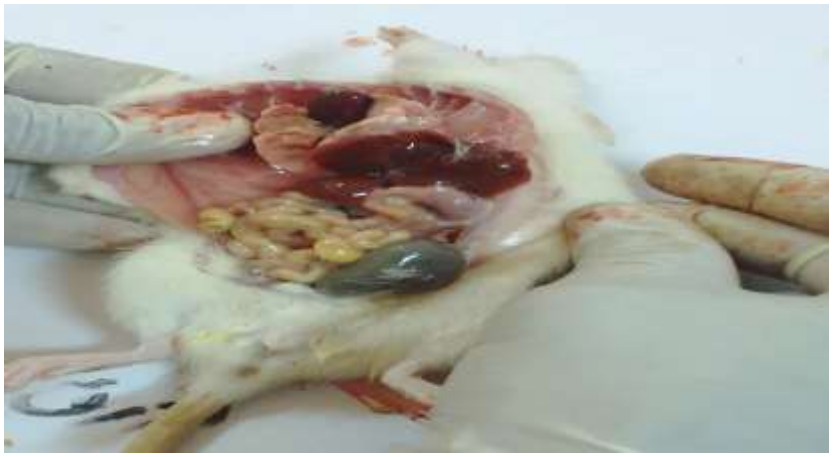

Fig (6): Image showing Gross Lesions and Specimens of the Liver, Intestines, Kidneys, Spleen and the Heart of a rat served as a control to Sub - Chronic Toxicity.

DISCUSSION AND CONCLUSIONS

As the active methanolic extract of *T. indica* L fruits' has no toxicity for humans, and as the bacteria became resistant to antimicrobials, therefore the search for new antimicrobial drugs against Salmonellae strains is a continuous task that faces the scientists and many investigators, and as the trend world wide now is to go to nature, referring to folkloric medicine, many scientists, especially phytochemists and microbiologists embark into the area of medicinal plants, particularly on *T. indica* L to confirm what is reached at, in this study.

As the methanolic extract of *T. indica* L was active against Salmonellae strains, which is supported by many investigators, Abdel Gadir *et. al* (2007), Abukakar *et. al* (2008), and Shital *et.al* (2010), so according to the previous facts collectively, this study can suggest that *T. indica* L plant can be used in treatment of typhoid disease in combination with conventional antibiotics.

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