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Effects of Aqueous Leaf Extract of *Amaranthus tricolor* On the Liver of the Adult Wistar Rat

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ABSTRACT: The aim of this study was to investigate the effects of aqueous leaf extract of *Amaranthus tricolor* on the liver of adult Wistar rats. 24 adult Wistar rats weighing between 250g and 280g were randomly assigned into 4 groups; (A-D) comprising of 6 rats per group. Group A rats were placed on rat food and water only. Group B rats received 100mg/kg body weight / day (BWT/D) of *Amaranthus tricolor* leaf extract. Group C rats received 300mg/kg BWT/D of *Amaranthus tricolor* leaf extract. Group D rats received 600mg/kg BWT/D of *Amaranthus tricolor* leaf extract. The dosages were given for 56 consecutive days via orogastric method. At the end of the 56th day, the animals were sacrificed under chloroform anaesthesia and the liver was harvested and processed for histological examination. The histological sections of the liver of the control group (Group A) showed normal histoarchitecture of hepatocytes radiating from the central vein with intervening sinusoids. There were no observable histological variations in the liver histoarchitecture of the rats treated with 100 mg/kg body weight (Group B), 300 mg/kg body weight (Group C) and 600 mg/kg body weight (Group D) of *Amaranthus tricolor* leaf extract. It was therefore concluded that *Amaranthus tricolor* leaf extract has no histomorphologic effects on the liver tissue of Wistar rats.

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Amaranthus tricolor Linn commonly known as *Lal Chaulai* (in India) or Joseph's coat in English is an important medicinal plant belonging to the family *Amaranthaceae* (Vardharajan, 1985). It is an annual plant known for its brightly colored foliage rather than its flowers. This weed is cultivated throughout India as ornamentals and found in West Bengal, Bihar, and Uttar-Pradesh (Chatterjee and Prakash, 2005). *Amaranthus tricolor* is a very important purple red colour leafy vegetable, consumed in most parts of India mainly in Bihar, Jharkhand and West Bengal. *Amaranthus tricolor* is also available in other tropical countries like South Africa. The Leaves are rich in proteins and micronutrients such as iron, calcium, zinc, vitamin C and vitamin A (Enoch *et al.,* 2014). Its red colour is as a result of its constituent of biologically-active compounds e.g., betalains and anthocyanins which exhibit red or violet (Aneja *et al*., 2013). The leaves of *Amaranthus tricolor* were well recognized by herbalists in Esan Central Local Government Area of Edo State, Nigeria for treating abdominal discomfort in their adult obese clients. The leaves are crushed and the resulting liquid can be used to treat abdominal pains, easy satiety, obesity, diabetes mellitus, inflammations, dysentery, nausea and emesis. Scientists have opined that the active principles which confer medicinally activities on the plant are the flavonoids and terpenoids. The Liver regulates many important metabolic functions, and any injury causes distortion of these metabolic

functions. As per an estimate, about 20,000 deaths occur every year in United States due to liver disorders (Wolf, 1999). Liver-protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavanoids, organic acids, lipids, alkaloids, and xanthones derivatives. Extracts of about 25 different plants have been reported to cure liver disorders (Sharma and Gupta, 2002). Obesity is susceptible to liver disease and if not corrected can lead to liver failure (Mark *et al.,* 2006). Besides obesity, some other conditions can increase susceptibility to the development of liver disease e.g., heavy alcohol use, type 2 diabetes, tattoos, unprotected sex, injecting drugs using shared needles and undiagnosed hepatitis infection (Zahra and Samaneh, 2012). Therefore, a logical long term strategy to avoid or deal with liver disease and its complications is to aim at prevention/treatment of liver disease. This present study investigates the effects of aqueous leaf extract of *Amaranthis tricolor* on the liver of adult Wistar rats.

MATERIALS AND METHODS

Amaranthus tricolor leaves were harvested from the University of Benin Farm project, Benin City. The plant was identified at the herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. The leaves were thoroughly washed to remove sand particles after which they were taken to the Pharmacology Department for the preparation of the extract. *Amaranthus tricolor* leaves were chopped into little bits and allowed to dry at room temperature. The dried leaves were pounded using wooden mortar and pestle and milled into fine powder in an electric blender. Five hundred grams (500g) of the powder was soaked in 2 litres of distilled water for 24 hours. The mixture was filtered with white filter paper and the residue was separated from the filtrate. The filtrate was concentrated using rotary evaporator at the department of Pharmacognosy, University of Benin, Benin City, Nigeria. The crude extract was then preserved in plain specimen bottles. The phytochemical constituents of *Amaranthus tricolor* include amarantin, isoamarantin, betaine, amino acids, sterols, fatty oils, sitosterol, calcium and magnesium (Aneja *et al.,* 2013).Appropriate doses of the extract were made by diluting with distilled water into 100mg/kg body weight, 300mg/kg body weight and 600mg/kg body weight which were administered to the rats orally.

Experimental Animals: Twenty-four (24) adult Wistar rats of either sex weighing between 250g and 280g were used for this study. The animals were allowed to acclimatize for a period of 2 weeks before commencement of the experiment. During this period they were allowed access to standard animal feeds (Vital Growers' Feed, manufactured by Bendel Flour Mll, Ewu) and clean water *ad libitum*. Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

Experimental Design: 24 adult Wistar rats weighing between 250g and 280g were randomly assigned into a control group (Group A) and three treatment groups (B, C and D) comprising of six (6) rats per group. Group A rats which served as control received 1ml of distilled water daily to compensate for stress of administration procured in the test groups. Group B rats were treated daily with oral administration of 100mg/kg body weight of *Amaranthus tricolor* leaf extract. Group C rats were treated with 300mg/kg body weight of*Amaranthus tricolor* leaf extract. Group D rats were treated with 600mg/kg body weight of *Amaranthus tricolor* leaf extract. The dosages were given for 56 consecutive days via orogastric method.

Method of Sacrifice and Sample Collection: At the end of the 8th week, the animals were sacrificed under chloroform anaesthesia; a midline incision was made through the ventral wall of the abdomen of the rats to access the liver. The liver was harvested and immediately fixed in 10% formal saline for 24 hours before the histological analysis.

The tissues were trimmed to about 3-5mm thick sections and processed according to method of Drury and Wallington (1980). And then histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy. Histological sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at various magnifications i.e. x40 and x400.

RESULTS AND DISCUSSION

As shown below in Figure 1, 2, 3, 4, 5, 6, 7, and 8, the histological sections of the liver of control (Group A) showed normal histoarchitecture of hepatocytes radiating from the central vein with intervening sinusoids (Figures 1 and 2). There were no observable histological variations in the liver histoarchitecture of rats treated with 100 mg/kg body weight (Group B) (Figures 3 and 4), 300 mg/kg body weight (Group C) (Figures 5 and 6) and 600 mg/kg body weight (Group D) (Figures 7 and 8) of *Amaranthus tricolor* extract.

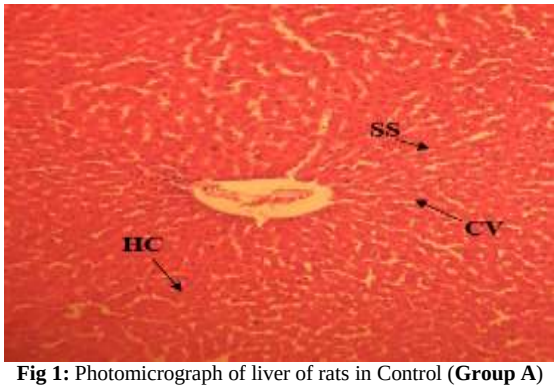

Fig 1: Photomicrograph of liver of rats in Control (**Group A**) showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 40**)

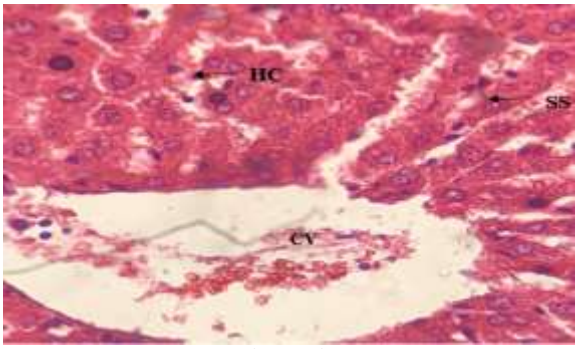

Fig 2: Photomicrograph of liver of rats in Control (**Group A**) showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 400**)

In spite of tremendous efforts made in the field of modern medicine, there is hardly any drug that stimulates liver function, offer protection to the liver from damage or help regeneration of hepatic cells (Chatterjee, 2000). This study was carried out to observe the effects of aqueous leaf extract of *Amaranthus tricolor* on the liver of Adult Wistar rats. The results from this research when observed histologically showed normal histoarchitecture of hepatocytes radiating from the central vein with intervening sinusoids in all the treatment groups (Groups B, C, and D).

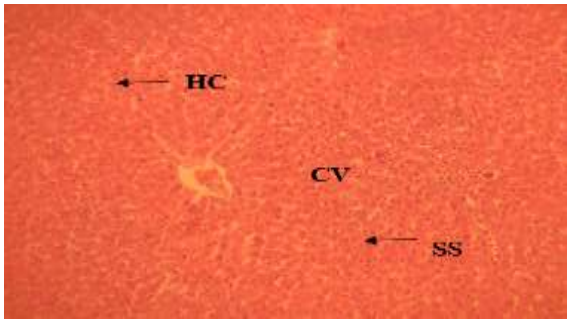

Fig 3: Photomicrograph of liver of rats treated with 100 mg/kg body weight of *Amaranthus tricolor* leaf extract (**Group B**) showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 40**)

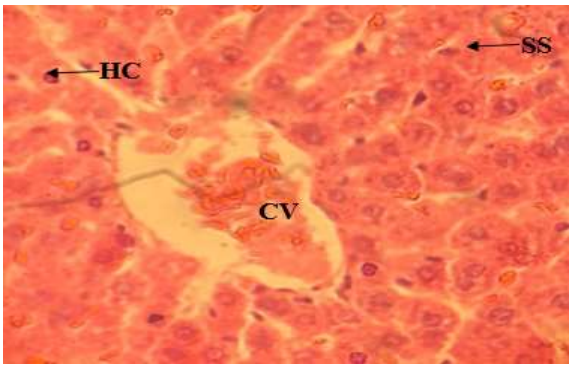

Fig 4: Photomicrograph of liver of rats treated with 100 mg/kg body weight of *Amaranthus tricolor* leaf extract (**Group B**) showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 400**)

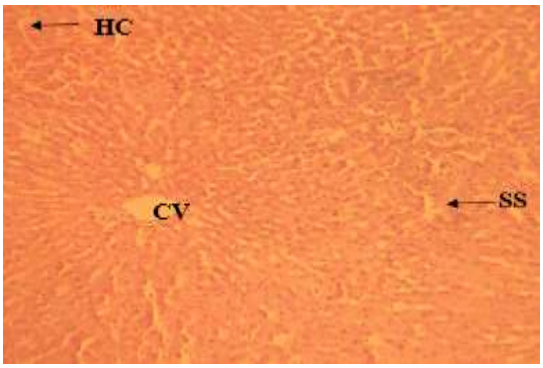

Fig 5: Photomicrograph of liver of rats treated with 300 mg/kg body weight of *Amaranthus tricolor* leaf extract (**Group C**)showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 40**)

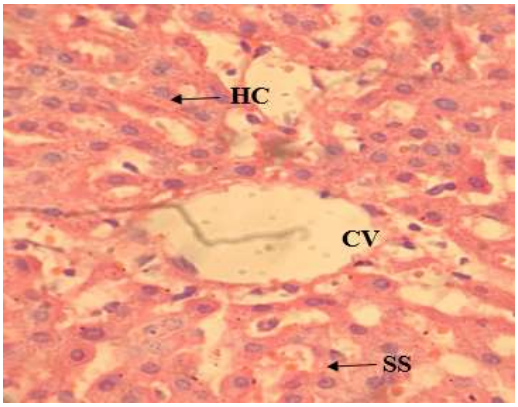

Fig 6: Photomicrograph of liver of rats treated with 300 mg/kg body weight of *Amaranthus tricolor* leaf extract (**Group C**)showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 400**)

There were no observable histological variations in the liver histoarchitecture of rats treated with 100 mg/kg body weight (Group B) (Figures 3 and 4), 300 mg/kg body weight (Group C) (Figures 5 and 6) and 600 mg/kg body weight body weight (Group D) (Figures 7 and 8) of *Amaranthus tricolor* extract when compared with results obtained from the control group (Group

A). Therefore, this study reveals that *Amaranthus tricolor* aqueous leaf extract was not toxic to the liver of the research animals treated with various doses and this could provide explanation for its reported hepatoprotective potential against paracetamol and carbontetrachloride-induced liver damage.

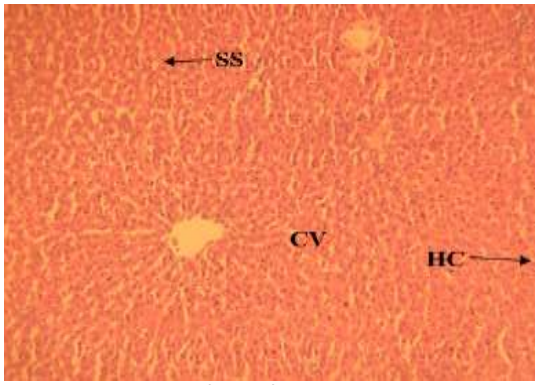

Fig 7: Photomicrograph of liver of rats treated with 600 mg/kg body weight of *Amaranthus tricolor* leaf extract(**Group D**) showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 40**)

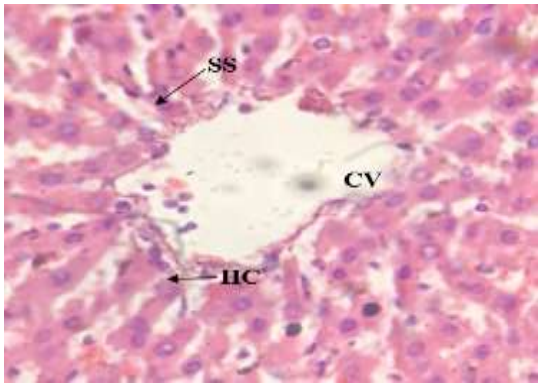

Fig 8: Photomicrograph of liver of rats treated with 600 mg/kg body weight of *Amaranthus tricolor* leaf extract (**Group D**) showing normal cyto-architecture of Hepatocytes (**HC**), Central vein (**CV**) and Sinusoids (**SS**) (**H&E x 400**)

Apart from the use of liver-protective herbal drugs, how else can liver disease be prevented? Over time, condition that damage the liver can lead to scarring (cirrhosis) which may result ultimately in liver failure, a life-threatening condition. But early treatment may give the liver time to heal. Liver disease and its associated complications can be prevented as follows: Drink alcohol in moderation, avoid risky behaviour, use medication wisely, get vaccinated, keep your food safe, take care with aerosol sprays, protect your skin, avoid contact with other people's blood and body fluids and eat foods that support liver health e.g., berries, cruciferous vegetables, beans, whole grains, nuts and fatty fish (Mark *et al.,* 2006).

Conclusion: In conclusion, there were no histomorphological or histopathological changes in the *Amaranthus tricolor* treated groups when compared with the control group. The results from this study proved that *Amaranthus tricolor* has no effect on liver structure and is therefore safe for consumption at these doses.

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