REPORT

THE PROTECTIVE EFFECT OF ALOE VERA JUICE ON LINDANE INDUCED HEPATOTOXICITY AND GENOTOXICITY

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ABSTRACT

The protective effect of fresh aloe vera (AV) leaves extract on lindane (LD) – induced hepatoxicity and genotoxicity was studied. Serum levels of hepatic enzyme markers: glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) were determined after oral administration of aloe vera leaves extract and lindane. The level of polychromatic erythrocytes was also observed. Pretreatment with aloe vera leaves extract at concentration of 1.0 ml/kg body weight significantly decreased ($P<0.05$) the serum levels of GPT (by 41.8%), GOT (by 36.5%), GGT (by 14.3%) and ALP (by 10.7%) induced by 100mg/kg body weight of lindane. The level of polychromatic erythrocytes observed was not statistically significant when compared to control.

Keywords: Hepatoxicity, genotoxicity, polychromatic erythrocytes, enzyme markers.

INTRODUCTION

The fame that aloe vera has acquired over a few years has been due to its pharmacological benefits. Low concentration of bitter aloes, about 0.1g is able to produce laxative properties and increase menstrual flow in female humans. A dose of 0.5g per day could produce a strong purgative and oxytoxic properties provoking uterine contraction in females (George, 1999). Aloe gel or juice has been known for its local actions such as wound healing, burns and skin infections. It is also known to revitalize skin, giving it a better endurance, smoothness and beauty. Aloe vera is known to be a cicatrizant able to regenerate and close its stomas when cut to prevent loss of precious juice. Logically and naturally because of this property, it can heal wounds by regeneration and scar reduction (a key ingredient in health and beautify products). Very little or almost nothing is said in literature about the toxicological implications of aloe vera (George, 1999).

Lindane (benzene hexachloride (BHC) is a monocyclic compound which is synthesized from the chlorination of benzene in the presence of ultraviolet light and gives a mixture of the alpha, beta, gamma and delta isomers. Pure isomers are obtained by selective recrystallization of crude BHC (Sidi *et al.,* 1983). Moreover only the Y - (gamma) isomer better known as lindane and marketed as gammalin 20 or gammazone has powerful insecticidal properties. It shows colourless crystals of melting point 112-119^{o}C. It is the most soluble of the organochlorines in water. Lindane is stable to light and heat and unattacked by strong acid, and dehydrochlorinated alkali. Lindane has a strong stomach poison action, high contact basicity and some fumigant activity in a wide range of insects. (Knight *et al.,* 1981).

Lindane is an organocholorine insecticide and is the gamma isomer of hexachlorocyclohexane (HCH). Of the eight stereoisomers of HCH, only the gamma isomer – lindane is insecticidal. Lindane is a white solid substance (Ferone, 1993), which upon evaporation is colourless with a slightly musty smell.

A critical survey of the available literature failed to reveal the modulatory effect of aloe vera extract on certain biochemical parameters of toxicant-induced hepatic injury including lindane. Since there is an increase and unprecedented exposure of the general populace especially agricultural workers to lindane, certain toxicological consequences have been linked to this exposure. Moreover, in view of the numerous preventive and curative roles of aloe vera, this present investigation is aimed at determining the protective effect of aloe vera on lindane-induced hepatic toxicity and gene toxicity in female wistar rats.

MATERIALS AND METHODS

Plant materials

Fresh aloe vera leaves were bought from Agbowo community opposite the University of Ibadan main gate.

Preparation and extraction

Aloe vera leaves were rinsed in ordinary water. The juices were obtained from the leaves by gently pressing the leaf. It was collected in a sterile container. The yield was calculated based on the weight of the extract compared to the weight of the leaves.

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Experimental design

Sixteen (16) female albino rats of the wistar strain were used for the experiment. They were purchased from the physiology department, University of Ibadan. Nigeria. The animals were maintained and housed in cages kept in the departmental animal house and fed on commercial rat pellets bought from Mokola. The rats were divided into four (4) groups containing four (4) rats in each group.

Group A: Control i.e., rats fed with rat pellets and ordinary water.
Group B: Rats fed orally with aloe vera extract (AV) 1.0ml/kg body weight for four weeks.
Group C: Rats fed orally with aloe vera extract (1.0ml/kg) body weight and Lindane (LD) 100mg/kg body weight for four weeks.
Group D: Rats fed orally with lindane alone 100mg/kg body weight for four weeks.

The animals were sacrificed after the last administration with lindane.

Methods

Glutamate exaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) kits from Randox laboratories U.S.A were used for this assay. The activities of the enzymes GOT and GPT were analyzed according to the method of Reitman and Frankel (1957). Analysis was carried out in serum.

Gamma – Glutamyl transferase (GGT) kit from Biolabo, France was use for this assay.
The enzyme GGT activity was determining as described by Szasz and Persyn (1974).

Alkaline phosphatase (ALP) kit from Lakeview Ave. Anaheim; CA was used for this assay.

Determination of alkaline phosphatase (ALP) activity

The method of King and King (1954) was used. Serum was incubated with disodium phenylphoshate as substrate buffered at pH10 for 15 minutes at 37°C. The hydrolytic products, phenol is condensed with 4-amino antipyrine and then oxidized with alkaline ferricyanide to give a red complex which is measured photometrically at 510nm.

The micronuclei assay

Preparation of slides

Rats were sacrificed by cervical dislocation and femur removed and stripped clean of muscle. A pair of scissors was used to make a small opening in the iliac end of the femur and a pin (safety pin) of approximate size 2½ cm was introduced into the marrow at the epidphyseal end. The pin was slowly pushed up into the canal with a screwing motion. This causes the marrow to exude out of the hole at the iliac end. This method removed most of the marrow from the bone cavity. The marrow was placed onto a slide to which a drop of fetal bovine serum from Pasteur pipette was added. Using the edge of a clean slide, the marrow may be prepared by simply transferring some of the mixed preparation to other clean slides. The procedure gives a homogenous preparation and yet is rapid. The interval from time the femur is cleaned until the smear is prepared is less than a minute.

Procedure for staining slides

After the smears are dried, they are fixed and stained with Geimsa using the following procedure.

I Fixed in absolute methanol for 5minutes
II Air-dried for a few minutes in order to remove the methanol
III Stained in 5% Giemsa (the stain is dissolved in 0.01M phosphate buffer (1.71g Na_2HPO_4, 0.68g, KH_2PO_4, 1 litre distilled water) which is adjusted to pH 6.8.
IV Rinsed in the phosphate buffer for about 30 seconds.
V Rinsed in distilled water.
VI Air-dried.
VII Mount in DPX (BDH) or other neutral mountant.

STATISTICAL ANALYSIS

The results were reported as means (Standard deviations

Table 1 Modulatory influences of Aloe vera (AV) extract and Lindane (LD) on hepatopxicity related parameters in rat.

Group/Treatment	Concentration/U/L			
	GOT	GPT	GGT	ALP
A (Control)	5.44±0.32	9.33±1.19	15.56±1.22	12.03±2.15
B (AV) only	17.47±2.73	18.20±1.93	19.80±2.45	13.79±0.88
C (AV) & (LD)	29.20±1.83	31.33±4.50	29.69±3.68	13.93±1.15
D (LD) only	50.20±2.03	49.32±2.52	34.65±3.24	16.40±1.03

Values are expressed as mean S.D for 4 animals. The results in table 1 revealed that pretreatment with the extract of aloe vera prevented lindane – induced elevation of serum GOT, GPT, GGT and ALP. The decrease is statistically significant ($P<0.05$) when compared with the control and toxicant groups.

(S.D) from individual magnitudes). Statistical differences were analyzed according to student's t-test wherein differences were considered to be significant at $P<0.05$.

RESULT

Table 2: Moldulatory influence of aloe vera (AV) extract and lindane (LD) on rat red cell macronucleus.

Group treatment	PCE1000RBCs
A (Control)	0
B (AV) only	0
C (AV) & (LD)	0
D (LD) only	1

This treatment show that lindane has a tendency of becoming clastogenic with one (1) PCE/1000 RBCs. 4 or more PCE/1000 RBCs is a positive result. Pretreatment with aloe vera extract did not show any PCE.

DISCUSSION

Health and disease are parameters of the effectiveness with which human and animals alike adapt to their environments (Tanaka, 1994). The herbals occupied a distinct place in life right from the primitive period till today. Recent upsurge in identifying non-dietary natural products associated with high degree of safety margin in cancer and hepatoprotective agents has been hailed by many investigators to be practically beneficial when the carcinogenic or hepatotoxic insult is mild to moderate (Morse *et al.,* 1997).

The findings of the present investigation is based on the examination of inducibility of enzymes. GOT, GPT, GGT, ALP and formation of red cell micronucleus. These physiological and drug metabolizing enzymes is one of the reliable markers for evaluating the chemopreventive potential of the test materials in model system. The information regarding the doses of modulators and their toxicity to animals is very crucial. Since chronic treatment with any agent for achieving chemopreventive prophylaxis should be free from undesirable side effect. Liver injuries induced by xenobiotics are commonly used for the screening of hepatoprotective agents (Davis *et al.,* 1974). The rise in serum levels of GOT, GPT, GGT and ALP in this experiment is attributed to the damaged structural integrity of the liver because these enzymes are normally located in the cytoplasm and release into the circulation after cellular damage (Vermuelen *et al.,* 1992). This result provides strong evidence that aloe vera significantly inhibits the acute liver toxicity induced by lindane as shown by reduction of serum liver enzymes activities. The elevation of these enzymes was noticed in lindane-treated rats compared with the control rats.

However, the activities of alkaline phosphatase in the experimental rats did not follow the same pattern. It should be noted that response of the liver to a particular toxin is determined by a variety of factors such as dose, the duration of exposure, route of administration, age of subject, presence or absence of established liver disease and inherent sensitivity of the exposed individual to noxious agent (Farver, 1979).

The micronuclei assay was used in this experiment because it is an *in vivo* test, sensitive, rapid and less expensive (Schmid, 1976). Other advantages of this assay include the short time required to complete and *in vivo* test assay and the fact that chromosomal breakage monitored is an important genetic event. Lindane was tested in the presence and absence of aloe vera. In presence of aloe vera, no micronucleated polychromatic erythrocyte (PCE) was observe indicating a modulatory effect of aloe vera. An average of one (1) PCE/1000 RBCs was noticed when treated with lindance alone indicating a tendency to be clostogenic.

Our environment abound with lots of substances which can induce cancer (Hartman *et al.,* 1990). Examples of such substances include foodstuff, house whole chemicals, industrial and agro-chemicals etc. This has made the screening of such chemicals necessary in order to monitor the degree of human exposure and how their *in vivo* toxic effects may be caused and prevented.

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