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Radioprotective Effect of the Methanolic Leaf Extracts Of Terminalia Catappa (Tropical Almond Leaf) On Albino Wistar Rats

[1]Joseph Abigail Yok, [2]C.N Okwuosa
[1,2]*University of Nigeria Nsuka, Nigeria*

Abstract: *This study was designed to evaluate the radio-protective effect of the crude methanolic leaf extract of Terminalia catappa. Twenty five (25) male albino wistar rats were used for the study, they were placed into five groups (n=5). Group A served as the positive control (radiation only) while group E served as the negative control and received 5 ml/kg of 0.25N of Na_2CO_3 (vehicle).Group B,C and D received 100 mg/kg, 200 mg/kg and 500 mg/kg respectively for Six (6) consecutive days prior to irradiation. The rats (except group E) were exposed to a constant dose of irradiation (x-rays) of 90 Kv and 200 mA at duration of 0.40 s twice. Blood samples were collected aseptically 48 hours after irradiation from the retro-orbital plexus of all animals into appropriately labelled plain and EDTA bottles for biochemical and haematological assays respectively. Results showed that the 500 mg/kg protected the rats from radiation as evident from the non-significant difference in the mean values of their serum enzymes level (40.40 $\pm$ 0.50 iu/L, 13.58$\pm$0.63 iu/L, 3.82$\pm$0.1 iu/L) for ALT, ALP and ACP respectively when compared with normal control values ($p>0.05$). They also showed a significant reduction in the mean serum enzyme levels (when compared with the positive control ($p<0.01$). However, results showed that the positive contol group(radiation only) had mean total white cell counts (TWCC), packed cell volume (PCV) and haemoglobin (Hb) of 14.85 ±0.18x10^9 /L, 38.13 ±0.88 % and 11.23 ± 0.59 g/dL respectively and these were significantly lower than those of the negative control (17.81±0.18 x 10^9 /L, 57.63±0.94 %, 17.80±0.39 g/dL respectively ($p<0.001$) the entire extracts treated group with the exception of the 100 mg/kg METC, did not show any significant difference in the mean values of the hematological parameters when compared to the negative control ($p<0.05$). More of the groups had significantly higher mean values for haemoglobin, Packed cell volume and total white cell count respectively when compared with the positive control ($p<0.001$, $p<0.01$). In conclusion, the result of this study indicates that Terminalia catappa has a potent radioprotective effect on albino wistar rats which is dose dependent*

I. Introduction

The development of effective radioprotectors and radiorecovery drugs is of great importance in view of their potential application during both planned radiation exposure (e.g. radiotherapy) and unplanned radiation exposure (e.g. in the nuclear industry, natural background radiation emanating from the earth or other sources) [1]. These drugs are also likely to be useful in nuclear warfare by providing protection to personnel [4]. Over the past 50 years, research in the development of radioprotectors world wide has focused on screening a plethora of chemical and biological compounds [5]. Numerous drugs of both synthetic and natural origin, eg antioxidants, cytoprotective agents, angiotensin converting enzyme (ACE) inhibitors or angiotensin II type-1(AT1) receptor antagonists (e.g Losartan, Metallo-elements, immuno modulators,and suphydryl compound) lipopolysaccharides, prostanglandins and DNA binding ligands have been tested in both invitro and invivo models, and in human clinical trials to mitigate injuries caused by ionizing radiation exposure in the sublethal to supralethal range. Combinations of agents have also been tested with little success [8]. The potential combination of differential radiomodifiers with metabolic modulator has been demonstrated in all culture and animal models [12]

Among the molecular radioprotectors, WR- 2721 [S-2-(3-annopropy 1-amino) ethyl phosphorothioc acid] also known as amifosine ethiophos (USA) or gammaphos (former USSR), is the most thoroughly investigated radioprotective drug, initially developed at the Walter Reed Army research institute USA under the Antiradiation Drug Development programme of the US Army Medical Research and Development[14].

However the radioprotective effects of phosphorothioate compounds, including amifostine, are short term, and associated with severe side effects (e.g. nausea, vomitting, diarrhoea, hypotension, hypocalcaemia, nephro and neuro toxicity) at clinically effective doses [13]. These limitations have greatly restricted their clinical use. Despite its draw backs, amifosine (ethyol) is the only radio-protector that has been approved by the Food and Drug Administration (FDA), USA Amifostine is being used clinically for ameliorating the incidence of xerostomia(mouth dryness) in patients undergoing radiotherapy for treatment of head and neck cancer [9]

Plants have been utilized since time immemorial for curing disease. Even today, nearly 70% of the world population is dependent on plants for handling their health related problem [6]

A number of Medicinal plant evaluated for their radioprotective efficiency have shown protective effects against the damaging effects of ionizing radiation [3]. Plant extracts eliciting radioprotection efficacy contain a plethora of compounds including antioxidants, Immunostimulants, cell proliferation stimulators, antiinflammatory and antimicrobial agents. Some of which may act in isolation as well as in combination with other constituents from the same plant. They may also augment the efficacy of compounds present in other plant species, to provide protection against radiation-induced damage. Most studies using natural plant products have focused on evaluation of radio-

protective efficacy of whole extracts or polyherbal formulations [2]. The facts remains that till date there is not a single radioprotective agent available which meets all the pre-requisites of an ideal radioprotector i.e. produces no cumulative or irreversible toxicity, offers effective long-term protection, posses a shelf-life 2-5 years, and can be administered easily [14]. In view of this, the search for newer, less toxic and more effective radioprotective drugs continues.

Terminalia catappa (Tropical almond) is a large, spreading tree distributed throughout the tropics. There is need to critically review the radioprotective properties of those plant products. The present research attempts to investigate the radioprotective effects of *Terminalia catappa* constituents with pharmacological activities relevant to radioprotection, radiorecovery, and treatments of radiation injuries including antioxidant, antiinflammatory, immunostimulatory, wound healing and antimicrobial effects [10]

They are also reported to contain certain photochemicals which are indications of its potential treatment of Diabetes e.g. brevifolin- carboxylic acid [15], and ellagic acid, which are a dose reductose inhibitors, Euginic acid have also show anticataract activity [15]. Terminalia is reach in tannins that are reported to be antidiabetic LD_{50} for oral route was 7500 mg/kg and for intra peritoneal administration 4500mg/kg in mice.

II. Materials and Methods

2.1 Collection of Plants Material

The *Terminalia catappa* was obtained from University of Nigeria Enugu Campus in Enugu state, Nigeria and was later identified by professor M.C. Nwosu of Botany Department of the University of Nigeria Nsukka with identification No UNH 552. The leaves were allowed to air dry under the shade and later grounded into fine powder with a mechanical blender.

2.2 Extraction of Plants Material

Eight hundred (800) g of the fine powder was weighed and macerated in 2500 ml of methanol. This was allowed to stand for 48 hours with intermittent vigorous shaking. The mixture was filtered using fine cheese cloth and then refiltered through a whatman No1 filter paper. The filterate was evaporated in an incubator at 60 ºC. The residue obtained was weighed and stored in the refrigerator. The methanolic extract had a percentage yield of 9.5 % (W/W)

2.3 Reconstitution of the Extracts

The crude methanol extract of *Terminalia catappa* (METC), 10 g was dissolved in 0.25N Na_2CO_3 and made up to 100 mls with the same solvent to give a final concentrate of 100 mg/ml.

2.4 Experimental Animals

Male albino wistar Rats weighing 140-180 g were obtained from the Animal House of the College of Medicine, University of Nigeria Enugu Campus. These rats were housed in clean cages. Under standard conditions (Temperature 27 ºC ± 3 ºC, 12 hr / 12 hr light/dark periodicity). And were fed with rat pellets (Guinea feed®and water ad libitim).

2.5 Radiation

The dose of radiation used was 90 kV and 200 mA. The duration of exposure was 0.40 sec.

2.6 Experimental Design

Rats were assign to five groups of five rats each. The test groups (B-D) received graded doses(100 mg/kg, 200 mg/kg and 500 mg/kg) of the plant extracts (*Terminalia catappa*) Once respectively. Extrtacts administration was by oral garage through oral gastric cannula. Group A & E served as the positive (Radiation only) and negative or normal control respectively. Extract administration was continued in group B-D for six (6) days. On the seventh day, the rats (except those in group E) were irradiated at the radiology unit of the National Orthopaedic Hospital, Enugu. After 48 hours, blood was collected from all the rats by retro-orbital puncture under ether anasethesia. An aliquot of blood was delivered into a tripotassium Ethylnene Diamine Tetraacetic Acid (K_3EDTA) container for haematological assays while the biochemical test was delivered into plain tubes, allowed to clot and sera separated from all the samples by centrifugation(3000 rpm for 15 min) The sera were used for biochemical studies.

3.70 Haematological Parameters

3.71 Determination of Haemoglobin Concentration using Cyanmethaemoglobin Method

3.71.1 Principle:

1 in 20 dilution of blood in Drabkins solution containing potassium cyanide and potassium ferricyanide diluents. Haemoglobin, Hi and HbCo, but not sulph Hb, are converted to HICN: The absorbance of the solution is measured in a spectrophotometer at a wave length of 540 nm.

3.71.2 Procedure

0.38 ml of Drabkin's solution was dispensed into a clean dry tube. 0.02 ml of well mixed EDTA anticoagulated venous blood was added using Hemoglobin pipette. The solution was left undisturbed for 10 mins. The colorimeter was zero with the blank and the readings were taken for the test.

3.72 Determination of Packed Cell Volume using Microhaemotocrit Method

3.72.1 Principle:

Anticoagulated blood in a glass capillary tube of specified length bore size and wall thickness is centrifuged in a microhaematocrit centrifuge at 10,000 rpm for 5 min to obtain constant packing of the red cells and readings were made with a micro haematocrit reader.

3.72.2 Procedure

Capillary tube which is 75 mm in length with an interval diameter of 1mm and wall thickness of 09.2-0.25 mm was used. Blood was allowed to enter the tube by capillary action upto two-third of the tube leaving about one-third un filled. One end was sealed with plasticine. The capillary tubes were placed in the microhematocrit centrifuge and was well balanced and centrifuged at 10,000 rpm for 5 min

3.73 Determination of Total Cell Counts (Bain *et al*)

3.73.1 Principle:

Blood was diluted in 1 in 20 dilution of Turks solution, the Glacial acetic acid lyse the cell while the Gentian violet stains the nuclei of the white cells.

3.73.2 Procedure

0.38 ml of Turks solution was dispense in to a container, 0.02 ml of well mixed EDTA anticoagulated venous blood was added. The counting chamber was assembled, sample remixed and chamber charged and filled. The chamber was left undisturbed for 20 mins. The chamber placed under the microscope using x10 and x40 objectives to focus. White cells where seen on small bright fragment stained. The white cells were counted in the inner square.

3.8 Biochemical Parameters

3.81 Alkaline Phosphatase estimation using King and King

PRINCIPLE: In an alkaline medium (PH 9.8 – 10.3) alkaline phosphatase acts on the hydrolyses disodium phenyl phosphate (sustrate to liberate phenol released is estimated by its reaction with a chromogen, 4-amino antipyrene 94AAP) in the presence of potassium ferricyanide (oxidizing agents) to produce a yellow–red colour whose absorbance is measured spectrophotometrically at 520 nm. The intensity of the colour and its absorbance is directly proportional to the activity of the ALP in the serum.

Equation of reaction:

Disodium phenyl (p) $\xrightarrow[\text{PH 10.3}]{\text{ALP}}$ Disodium phenol + Phospheric acid 1

Disodium phenol + 4AAP $\xrightarrow{(KFeCN)}$ yellow red coloured complex 2

CALCULATION: The quantity of the enzymes in the sample is calculated using the expression,

$\frac{[T-TB]}{[S-SB]} \times Concentration\ of\ standand$

T = Test

TB = Test Blank

S= Standard

SB = Standard Blank

3.8.2 Acid Phospahtase using Kinetic method

Principle:

The α-Napthol release from the substrate α - Napthylphosphate by acid phosphatase coupled with fast red TR to produce a coloured complex that absorbs light at 405 nm. The reaction can be quantitated photometrically because the coupling reaction is instantaneous.

α-Naphthylphosphate + H_2O $\longrightarrow$ α-Naphthol + Inorganic Phosphate 3

α-Naphthol + Fast Red TR $\longrightarrow$ Diazo Dye (Chromophore) 4

3.83 Aspatate and Alkaline Trasaminase using Reitman and Frankel method

Principle: Trasaminase catalyses transamination reaction in the body is given as follows.

Alanine + Oxoglutarate $\xrightarrow{\text{ALT}}$ pyruvate + glutamate 5

Aspartate + oxoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + glutamate 6

The ketoacids (pyruvate and oxaloacetate) produce are estimated by coupling them with 2,4-Dinitrophenyl hydrazine to form 2,4-Dinitrophenyl hydrazone which is already reddish brown colour is measured spectrophotometrically at 540

mm and is directly proportional to the amount of ketoacid formed which in turn depend on the activity of transaminase in the serum.

CALCULATION: The quantity of the enzymes in the sample is calculated using the expression,

$$\frac{[T-TB]}{[S-SB]} \times Concentration\ of\ standand$$

T = Test
TB = Test Blank
S= Standard
SB = Standard Blank

III. Results and Discussion

Table 1: The Effects of the Crude Methanolic Leaf Extracts of *Terminalia Catappa* on Some Biochemical Parameters of Wistar Rats Exposed to Radiation

GROUPS	ACP (IU/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
A (Positive control)	47.25 ±1.11^{e}	58.50 ±1.89^{e}	35.38 ±0.68^{e}	6.13 ±0.15^{e}
B (100 mg/kg METC+ Radiation)	43.50 ±0.65^{e,d}	53.75 ±1.25^{e}	31.03 ±0.62^{e,e}	47.0 ±.0.16^{e,f}
C (200 mg/kg METC+ Radiation)	40.75 ±0.48^{f}	53.88 ±0.97^{e}	23.15 ±0.57^{e,f}	3.78 ±0.08^{f}
D (500 mg/kg METC + Radiation)	40.40 ±0.50^{f}	51.90 ±0.71^{e,e}	13.58 ±0.63^{f}	3.82 ±0.11^{f}
E (Negative control)	38.00 ±1.47	44.0 ±1.58	14.70 ±0.58	3.68 ±0.09
F- Ratio	15.055	16.14	244.77	72.51
P- Values	P<0.001	P<0.001	P<0.001	P<0.001

Table 2: The Effects of the Crude Methanolic Leaf Extracts of *Terminalia Catappa* on Some Haematological Parameters of Wistar Rats Exposed to Radiation

GROUP:	Total White Cell	Packed cell Volume	Haemoglobin Level
A (Positive control)	14.85 ±0.18^{e}	38.13 ±0.88^{e}	11.23 ±0.59^{e}
B (100 mg/kg METC+ Radiation)	15.38 ±0.22^{e}	52.25 ±2.39^{f}	16.30 ±0.68^{f}
C (100 mg/kg METC+Radiation)	17.08 ±0.83^{e,f}	54.50 ±1.85^{f}	17.13 ±0.48^{f}
D (500 mg/kg METC+ Radiation)	17.09 ±0.10^{e,f}	51.90 ±1.71^{f}	16.30 ±0.55^{f}
E (Negative control)	17.81 ±0.18	57.63 ±0.94	17.80 ±0.39
F- Ratio	62.59	19.12	21.44
P- Values	p<0.001	p<0.001	p<0.001

KEY:

METC-Methanolic extracts of *Terminalia catappa*
Radiation dose-90V 80 MAS (constant)
a=p<0.05; b=p<0.01; c=p<0.001 with respect to normal control
d=p<0.05; e=p<0.01; f=p<0.001 with respect to positive control
Values given as mean ± standard error of mean.

Data entry and analysis were done using an SPSS-Info; means are presented as values ± standard deviation. Student's-test and Chi-Square test were used to compare the means and proportions between two groups respectively. Analysis of variance (ANOVA) and Chi-square test were utilize in comparing the means and proportions respectively between the Negative control and groups that receive different doses of extracts and likewise the Positive control and the various groups. Statistical significant was set at $P < 0.05$.

The results in table1 Showed that animals in group A (positive control) had mean serum levels of ALT,AST,ALP, and ACP of 47.25±1.11 IU/L, 58.50±1.89 IU/L, 35.38± 0.68 IU/L, 6.13±0.15 IU/L and these were significantly higher than the mean values for the negative control which were 38.00±1.47 IU/L, 44.00± 1.58 IU/L , 14.70±0.58 IU/L and 3.68±0.09 IU/L For ALT,AST,ALP, and ACP respectively (p<0.001).

Group B rats (100 mg/kg METC) also had significantly higher values for the serum enzymes when compared with the negative control (p<0.001). The 500 mg/kg dose of METC protected the rats from radiation as evident from the non significant differences in the mean values of their serum enzymes (40.40±0.50 IU/L, 13.58±0.63 IU/L 3.82±0.11 IU/L for ALT,ALP, and ACP respectively) when compared with the negative control values (p<0.01). The extracts treated rats showed a dose dependent radio-protective activity as indicated by the significant reduction in the mean serum enzyme levels of these groups when compared with the positive control. (p>0.05). For instance, the 100 mg/kg and 500 mg/kg METC treated groups had 43.50±0.65 IU/L, 31.03±0.62 IU/L, 4.70±0.16 IU/L, and 40.40±0.50 IU/L, 13.58±0.63 IU/L, 3.82±0.11 IU/L for ALT,ALP, and ACO levels respectively and these were significantly lower than mean values seen in the positive control group (p<0.001).

Table 2, Shows the effect of the crude leaf extracts of *Terminalia catappa* on some haematological parameters of rats exposed to radiation. Results showed that the positive control group (radiation only) had mean with total white cell count (TWCC), packed cell volume (PCV) and Haemoglobin (Hb) of $14.85 \pm 0.18\times10^9$/L, 38.13±0.88 % and 11.23 ±0.59 g/dL respectively and these were significantly lower than those of the negative control ($17.81\pm0.18\times10^9$/L, 57.63±0.94 %, 17.80± 0.39 g/dL respectively (p<0.001)

However, all the extracts treated groups with the exception of the 100 mg/kg. METC, did not show significant different mean values in these haematological parameters when compared with the negative control (p<0.05). Moreover, these groups had significantly higher values for Haemoglobin, packed cell volume and total white cells count respectively when compared with the positive control group (p<0.01; table 2)

IV. Conclusion and Recommendation

4.1 Conclusion

On the strength of the forgoing and the result of this research, it was observed that, the effects of the methanolic leaf extracts of Terminalia Catappa (tropical almond leaf) on radiation-exposed albino wistar rats has a radioprotective effects that prevent the rats from tissue/organ damage.

4.2 Recommendation

Further study is needed to characterize the active ingredients in the extracts of *Terminalia catappa* and to elucidate the mechanism of action. The individual fractions should be analysed for radioprotective potency in different models of radiation exposed. Observed pharmacological effects should be screened for individual fractions. Similar work should be done on the fruits and stem bark extracts of *Terminalia catappa.* Since these might provide better protection

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