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Evaluation of genotoxicity of *Anogeissus leiocarpus* roots on mouse bone marrow cells in *vivo*

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Université de Lomé

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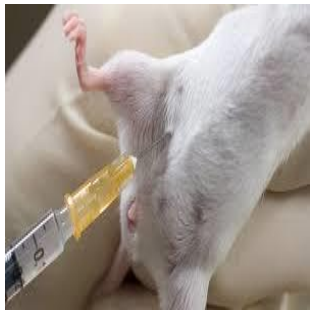
Graphical Abstract

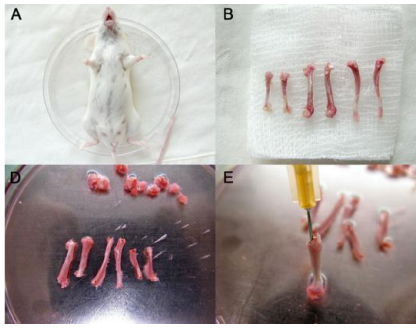
Roots of *A. leiocarpus*

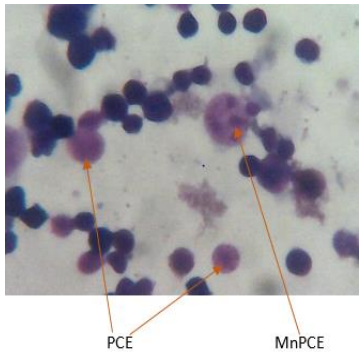
Extract



Cyclophosphamide / Nacl 0.9 %

Femur removed and bone marrow flushed for slides





Genotoxicity caused by cyclophosphamide

Extract:

- Non - genototoxic
- Prevents from genotoxicity
- Non - cytotoxic

Abstract:

Anogeissus leiocarpus (Guill and Perr) is a plant which widely spread throughout the tropical and subtropical areas. This study was designed to evaluate the potential effects of roots of *Anogeissus leiocarpus* on mice bone marrow cells *in vivo*. 250, 500, 1000 $mg.kg^{-1}$ of total hydro alcoholic extract of roots of *A. leiocarpus* were evaluated for genotoxic, antigenotoxic and cytotoxic activities by a micronucleus test. The genotoxicity was induced by the administration of cyclophosphamide IP.

At the end of the treatment, no significant difference ($p>0.05$) was found between the body weight gain of the treated groups compared to the negative controls. Evaluation of the ratio of polychromatic erythrocytes to total erythrocytes (PCEs %) in the bone marrow had shown no significant difference between the groups treated with the extract and the untreated group. In pre treated groups with the extract at the doses of 250, 500 and 1000 $mg.kg^{-1}$, there was a significant ($p<0.0001$) decrease in MnEPCs compared to the positive controls; in a dose-dependent manner.

This study has revealed that the extract of *A. leiocarpus* was not genotoxic but rather possessed a protective effect against the genotoxicity and cytotoxicity induced by cyclophosphamide.

Keywords: *Anogeissus leiocarpus* -micronucleus- genotoxicity- cytotoxicity

Introduction (1)

Genotoxicity is defined as the ability of a substance to induce damage, either direct or indirect, to genetic material resulting in breaks in DNA (clastogenic effect) or mutations in DNA (mutagenic effect).

Because of the toxic effect that chemical or natural substances can cause at the gene level, genotoxicity studies are therefore of great importance in assessing risks to human health (Quesnot, 2015; Fardel et al., 2010)

Due to the increasing use of herbal medicines in the treatment of various diseases (Folashade et al., 2012), many toxicological studies have been carried out to show the safety or harmfulness of these plants.

Introduction (2)

Anogeissus leiocarpus, a plant of the combretaceae family is widely used in traditional medicine in Togo to treat various pathologies including diabetes (Kpodar et *al*., 2015)

Previous work in our laboratory has shown the antihyperglycemic activity of the hydro alcoholic extract of the roots of the plant *in vivo* and was found to be nontoxic in rats following either a single dose or daily repeated doses for 28 days (Motto et *al*., 2020 a; Motto et *al*., 2020 b)

Still with the aim of showing the safety of this plant, the present study was undertaken to evaluate the effect of the hydro alcoholic extract of the roots of *Anogeissus leiocarpus* on erythrocytes of the bone marrow of mice.

Materials

Plant material

Roots of *Anogeissus leiocarpus* were harvested in Tsévié, Zio (TOGO) in the month of July 2018. A voucher specimen was identified and deposited in the herbarium of Laboratory of Botany and Plant Ecology under the number TOGO 15483.

Animals

ICR mice (30±5g) were housed in standard environmental conditions (temperature 24–25°C, relative humidity and a 12t/12 h light-dark cycle) and fed with standard rat diet and water *ad libitum*.

Principles of laboratory animal care as described in institutional guidelines and ethics of Laboratory of Physiology/Pharmacology of University of Lome-Togo (ref: 001/2012/ CB-FDS-UL) were followed.

Methods (1)

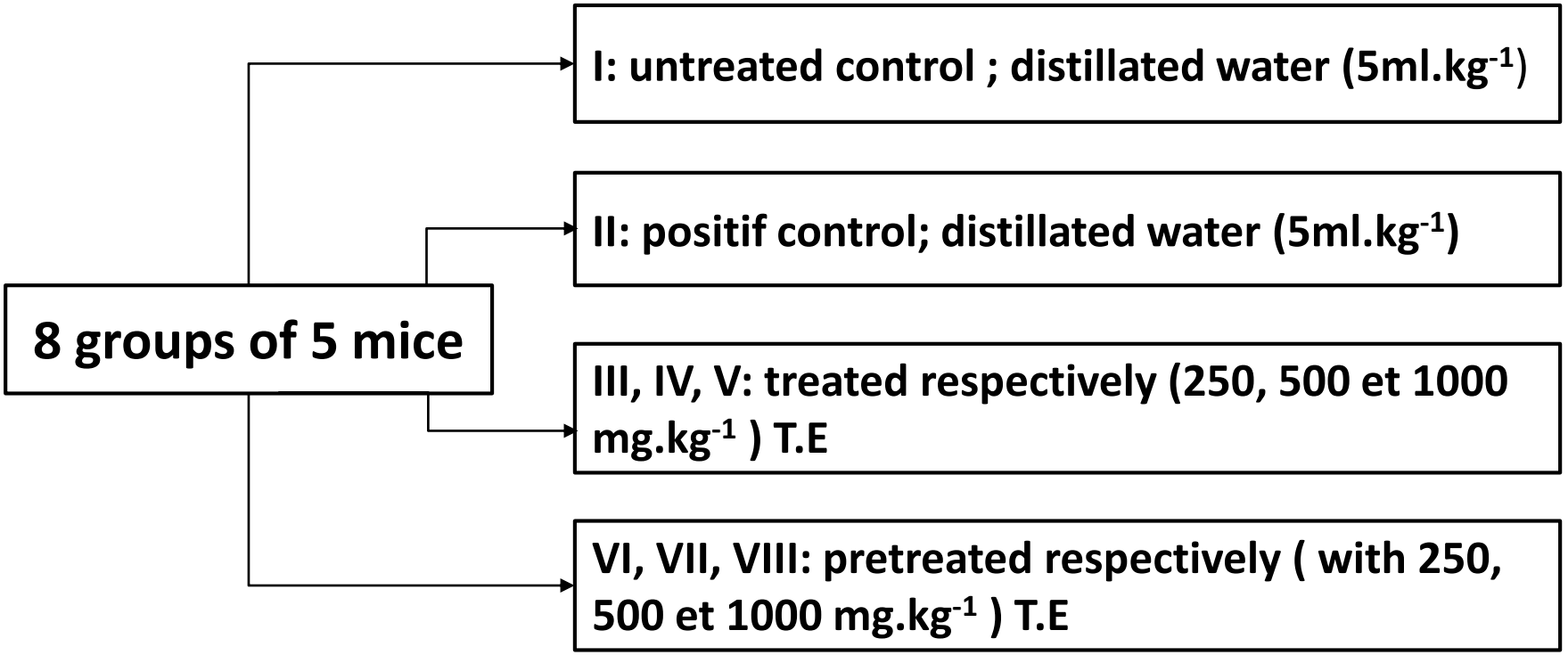
Hydro alcoholic extraction

About 400 g of roots of *Anogeissus leiocarpus* were extracted in water/ethanol (5:5) for 72 hours. The crude extract was filtered on Whatman paper and evaporated in vacuum at 45°C using a rotary evaporator (IKA® RV20 digital). The yield of the dry extract was 5.68 % and was stored at 4°C (Motto et al.,2020)

Genotoxicity by micronucleus test of roots of *Anogeissus leiocarpus* in mice bone marrow

OECD 474 test guidelines with slighty modifications (OECD, 2014)

Methods (2)



After 7 days of pre-treatment, animals of group I and groups III, IV, V received 0.9% NaCl I.P., while group II, VI, VII, VIII received a dose of 100 mg/kg of cyclophosphamide I.P. Thirty hours after injection, all animals were euthanized.

Methods (3)

Measured parameters

- ✓ Any sign of toxicity + Body weight (beginning and end)

- ✓ marrow bone was flushed for the preparation of slides (Krishna and Hayashi, 2000) and slides were observed using a binocular microscope

- Frequency of MnEPC ➔ Genotoxicity
- PCE/PCE + NCE ➔ Cytotoxicity

Micronucleus and erythrocyte counting

Micronuclei from chromosomal aberrations in polychromatic erythrocytes were recorded per 5000 polychromatic erythrocytes. % of immature erythrocytes was established per 1000 erythrocytes.

Methods (4)

Micronucleus and erythrocyte counting

% MnPCE = ((number MnPCE) / (number PCE + NCE)) x 100

% PCE = ((number of PCEs)/ (number of PCEs + number of NCE s)) x 100

MnPCE= Micronucleus in polychromatic erythrocyte
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Statistical analysis

Data were expressed as Mean ± SEM (standard error of the Mean) using the GraphPad Prism 7 software. Statistical differences between groups were determined by ANOVA followed by Tukey test and considered significant for $p < 0.05$

Results and discussion (1)

Effect of total extract on body weight of mice

During the treatment, no abnormalities were reported in behaviour in the different groups compared to controls.

No significant difference ($p>0.05$) was found between the body weight gain of the treated groups compared to the negative controls. (data not shown).

Results and discussion (2)

Effect of total extract on bone cells

An increased incidence of micronucleated polychromatic erythrocytes (MnPCE) in treated animals, is a sign of inducing of chromosomal damage (OECD, 2014)

The total extract at the doses of 250, 500 and 1000 $mg.kg^{-1}$ had no significant effect on the number of MnPCE compared to the negative control. This showed a non-genotoxic effect of the extract (Table 1)

Contrary, the administration of cyclophosphamide (positive control) induced a significant ($p<0.0001$) increase up to 69% in MnPCE compared to the negative controls (Table 1)

In fact, cyclophosphamide is an anti-cancer agent with alkylating properties used in chemotherapy that induces gene mutations, chromosomal aberrations, micronuclei and chromatid exchanges in somatic cells (Luzhna et *al*., 2013)

Results and discussion (3)

Table 1: Effect of *A. leiocarpus* on bone marrow cells

Mice	Normal		T.E 250		T.E 500		T.E 1000		Control	
	MnPCEs/ 5000 PCEs	PCEs%	MnPCEs/ 5000 PCEs	PCEs%	MnPCEs/ 5000 PCEs	PCEs%	MnPCEs/ 5000 PCEs	PCEs%	MnPCEs/ 5000 PCEs	PCEs%
1	6.55	54.57	5	41.94	5.5	41.94	4.29	43.44	15.69	39.67
2	4.47	53.63	4	38.67	4.5	42.62	7	33.64	21	40
3	7.5	40.90	5.5	44.05	5	39.39	5.71	43.44	20	45.45
4	3.33	42.30	4.85	46.43	4	42.03	4.5	47.20	15	46.66
5	4.16	41.17	4.4	45.24	6	40.32	6	37.70	21	40
M ± SEM	5.70 ± 0.78	46.52 ± 3.10	4.75 ± 0.25	43.26 ± 1.36	5 ± 0.35	41.26 ± 0.60	5.5 ± 0.5	41.08 ± 2.40	18.54 ±1.32####	42.35 ± 1.52

Normal: negative control treated with distillated water; Control: positive control given cyclophosphamide at 100 mg.kg^{-1}; T.E 250, 500 and 1000: were treated respectively with the total extract at 250, 500 and 1000 mg.kg^{-1} ; # #### *p < 0,0001* (compared to normal)

Results and discussion (4)

Effect of total extract on bone cells

Compared to positive controls, in the groups pre treated groups with the extract of *A. leiocarpus* at the doses of 250, 500 and 1000 $mg.kg^{-1}$, there was a significant decrease ($p<0.0001$) in MnEPCs of 34; 44 and 62%, respectively after the administration of cyclophosphamide

And this decrease was in dose-dependent manner (Table 2)

In pre-treated mice, the extract has shown a protective action against genotoxicity induced by cyclophosphamide in the bone marrow.

Results and discussion (5)

Table 2: Effect of *A. leiocarpus* after the administration of cyclophosphamide on bone marrow cells

Mice	Normal		Control		T.E 250 + C		T.E 500 + C		T.E 1000 + C	
	MnPCEs/5000 PCEs	PCEs%	MnPCEs/5000 PCEs	PCEs%	MnPCEs/5000 PCEs	PCEs%	MnPCEs/5000 PCEs	PCEs%	MnPCEs/5000 PCEs	PCEs%
1	6.55	54.57	15.69	39.67	12.5	44.28	11.25	41.66	8.33	40
2	4.47	53.63	21	40	12.5	45	11	40.62	6.25	43.33
3	7.5	40.90	20	45.45	10	42,5	9.33	36.36	6	37
4	3.33	42.30	15	46.66	13.33	46,66	10	42.5	7.25	41.66
5	4.16	41.17	21	40	12.3	44.2	10	45	6.95	42
M ± SEM	5.70 ± 0.78	46.52 ± 3.10	18.54 ±1.32$^{\#\#\#\#}$	42.35 ± 1.52	12.12 ± 0.56****	44.53 ± 0.67	10.31 ± 0.35****	41.23 ± 1.41	6.95 ± 0.41****	40.80 ± 1.08

Normal: negative control treated with distillated water; Control: positive control given cyclophosphamide at 100 mg.kg^{-1}; T.E 250 +C, 500+C and 1000+C: were pre- treated respectively with the total extract at 250, 500 and 1000 mg.kg^{-1} and received cyclophosphamide at 100 mg.kg^{-1} . $^{\#\#\#\#}$ *p < 0,0001* (compared to normal*);* *****p < 0,0001* (compared to controls*).*

Results and discussion (6)

Effect of total extract on bone cells

At the dose of 1000 mg.kg^{-1}, the incidence of MnEPC was 1.39. 10^{-3} compared to 2.42. 10^{-3} % at the dose of 500 mg.kg^{-1} (table 3)

General cytotoxicity can be assessed by the ratio PCE/PCE+NCE. The ratio decreased as well as the frequency of MnPCE increased (OECD, 2014)

Compared to the negative control group, there was no significant difference between the groups treated with the extract and the untreated group in the evaluation of the ratio of (PCEs %) in the bone marrow compared to the negative control group (Tables 1 and 2).

This suggested a non-cytotoxic activity of the total extract of *Anogeissus leiocarpus* on mouse bone marrow cells

Results and discussion (7)

Table 3 : Frequency of micronucleus in bone marrow cells after treatment and pre treatment with total extract of *A. leiocarpus*

mice	MnPCEs % (10^{-3})							
	Normal	Control	T.E 250	T.E 500	T.E 1000	T.E 250 + C	T.E 500 + C	T.E 1000 + C
1	1.31	3.14	1.00	1.10	0.86	2.5	2.25	1.66
2	0.89	4.2	0.80	0.90	1.40	2.5	2.2	1.25
3	1.5	4	1.10	1.00	1.14	2	1.86	1.2
4	0.66	3	0.97	0.80	0.90	2.66	2	1.45
5	0.83	4.2	0.88	1.20	1.20	2.46	2	1.39
M ± SEM	1.04±0.15	**3.70±0.26**	0.95±0.05	1.00±0.07	1.10±0.10	2.42±0.11	2.06±0.07	1.39±0.08

Normal: negative control treated with distillated water; Control: positive control given cyclophosphamide at 100 mg.kg^{-1}; T.E 250, 500 and 1000: were treated respectively with the total extract at 250, 500 and 1000 mg.kg^{-1}; T.E 250+C, 500+C and 1000+C: were pre-treated respectively with the total extract at 250, 500 and 1000 mg.kg^{-1} and received cyclophosphamide at 100 mg.kg^{-1}

Conclusions

It emerges from this study that the hydro alcoholic extract of roots of *Anogeissus leiocarpus*:

- ✓ is not genotoxic
- ✓ has the ability to prevent chromosomal damages
- ✓ is non-cytotoxic

Further studies will be carried out to evaluate the reproductive toxicity and teratogenic toxicity of the plant