Anti-inflammatory and Anti-cancer Activities of Leaf Extracts from three Datura Species found in Mali

ABSTRACT

The anti-inflammatory potential was estimated using cancer cells, by quantifying nitrites (NO) based on colorimetric method (Griess reaction) and the cell viability based on the WST-1 assay. The antiproliferative activity was assessed on cell lines (HeLa and MCF-7) using the Cell Titer Glo® assay. A phytochemical screening was conducted to identify the main groups of bioactive compounds present in these extracts. The phytochemical analysis revealed that leaf extracts from all three Datura species are sources of various secondary metabolites, including alkaloids, flavonoids and coumarins. The highest anti-inflammatory power was recorded with *D. stramonium* and *D. innoxia* extracts. with a concentration of 10 µg/mL of each extract, murine macrophages have normally grown, with NO inhibition rates of 30.05 ±3.11% and 25.70 ±2.04% for *D. innoxia* and *D. stramonium* respectively. The anticancer activity was more pronounced with *D. stramonium* extracts, which showed 13.33±3.05% of viability rate on the HeLa cell line at 50 µg/mL. In contrast, at the same concentration, the MCF-7 cell line was more sensitive to *D. innoxia* extracts, with a viability rate of 42.67±2.52%. The results of that study showed that three Datura species investigated have immunostimulant potential which could them to be useful in the treatment of breast and cervical cancers.

Keywords: Datura stramonium, Daturainnoxia, Datura ferox, Anti-inflammatory, Anti-proliferative activities.

1. INTRODUCTION

The inflammation predisposes into the cancer development and promotes all stages of tumorigenesis. Cancer cells, as well as surrounding stromal and inflammatory cells, engage in well-orchestrated reciprocal interactions to form an inflammatory tumor microenvironment [1]. This inflammatory activity is triggered by numerous pathophysiological conditions in response to microorganisms and tissue damages. In the early stages of this process, the first line of defense is provided by macrophages which, in the presence of a stimulus such as microbial lipopolysaccharide, produce several pro-inflammatory mediators, including nitric oxide (NO), cytokines and prostaglandins. Under normal conditions, the release of these molecules is vital, manifesting severely, rapidly and for a noticeably short period after injury, until neutralization of the noxious stimuli [2]. However, the abnormal production of these pro-inflammatory mediators over a long period can progress towards chronic inflammation-related diseases. The literature has reported numbers molecules, like NO, capable to attenuate the production of these pro-inflammatory mediators due to their anti-inflammatory properties [3]. The inflammation promotes cell proliferation and weakening of vascular barriers in order to facilitate immune cell migration, that process promotes cancer progression [4, 5, 6].

Cancer is a growing public health problem worldwide. According to the 2020 statistics from the data base of International Agency for Research on Cancer, there were over 19 million cases worldwide and 9.9 million deaths from cancer. The recurrence rate of cervical cancer remains high in developing countries. Moreover, this disease is the seventh most common type of cancer in the world and the fourth most common in women [7]. Breast cancer is the most frequently diagnosed cancer in women worldwide, with 11.7% out of 2.3 million new cases of cancer in 2020 [8].

That is why the research about anti-cancer drugs has become essential for cancer patients. Nowadays, the chemotherapy and radiotherapy are the conventional methods used to treat cancer. However, these methods can lead to unpleasant side effects and toxicity, as they destroy both normal and cancerous cells. Furthermore, some patients develop resistance

to therapeutic drugs during treatment, which can lead to relapse [9]. Thus, plant species have become important natural remedies in disease management due to their accessibility and very low side effects [10]. These medicinal plants are rich in secondary metabolites which are endowed with positive pharmacological responses [11, 12, 13].

The Datura genus, belonging to the Solanaceae family, has species known for their medicinal properties. It is traditionally used to treat numerous inflammatory disorders, such as wounds, ulcers, swellings, asthma and rheumatoid arthritis [14, 15]. Most of Datura's therapeutic properties could be attributed mainly to its richness in alkaloids like atropine, scopolamine, fastusine, hyoscyamine, littorine, valtropine and acetoxytropine, withonilides, and other tropanes ([16, 17, 18].

Recent works have reported the existence of three (3) species of Datura (*Datura stramonium*, *D. innoxia* and *D. ferox*) in the flora of southern Mali [15]. The same authors have mentioned the use of these species in the traditional treatment of numerous pathologies, such as wounds, inflammations and especially cancer. However, to our knowledge, no studies have been conducted in Mali to confirm these therapeutic potentialities. This is why the present work was planned to evaluate the anti-inflammatory and anti-cancer potential of leaf extracts from these three Datura species.

2. MATERIALS AND METHODS

2.1. Materials

The plant material consisted of leaves from three Datura species (*Datura stramonium* L., *Datura innoxia* Mill. and *Datura ferox* L.). Leaf samples were collected in Kolondiéba, in southern Mali. Plants were identified at the Tropical Ecology Laboratory of the University of Sciences, Techniques and Technologies of Bamako (USTTB). The leaves were carefully washed, dried at room temperature, ground to a powder and stored in a dark, dry place.
As for the biological material, it was represented by cell lines of RAW 264.7 (ATCC TIB-71) for anti-inflammatory assay and MCF-7 (ATCC HTB-22) and HeLa (ATCC CCL-2) for anticancer one. These cell lines were offered by the Integrative Biological Chemistry Platform of Strasbourg (PCBIS), France.

2.2. Methods

2.2.1. Preparation of extracts

Extracts were obtained by maceration of 50 g powder of each species in 500 mL of ethanol. The mixture was stirred for 48 h, then filtered under vacuum. The filtrates obtained were evaporated to dryness under reduced pressure using an evaporator.

2.2.2. Phytochemical screening

The research of the main chemical groups in extracts was carried out using conventional methods based on specific chemical reagents according to the protocols described by Harborne and Bruneton [19, 20]. Alkaloids were identified using Dragendorff's reagent, while tannins were characterized using ferric chloride. Acetic anhydride and concentrated sulfuric acid were used to determine triterpenes. Dilute hydrochloric alcohol, magnesium chips and isoamyl alcohol were used for flavonoids. For coumarins, we used the UV fluorescence method at 365 nm. The foam test was used to identify saponosides.

2.2.3. Assessment of anti-inflammatory activity

The anti-inflammatory activity was estimated by quantifying nitrites in RAW 264.7 cells (ATCC TIB-71).

- ***Nitric oxide (NO) production***

Macrophages were seeded in 96-well plates at a density of $2x10^5$ cells/well CORTAR 3596 and allowed to grow overnight. Then the cells were pre-treated with 20 µL of different concentrations of extracts (with DMSO + Dulbecco's Modified Eagle's Medium without serum as solvent) for 4 hours. After the pre-incubation period, cells were stimulated to generate NO by the addition of 20 µL of lipopolysaccharide (LPS) at 1 µg/mL, then incubated for 24h at 37°C under 5% CO_2. After incubation, NO production in each well was assessed by adding 100 µL of Griess reagent [21]. The absorbance was measured at 540 nm using an ELISA reader, and nitrite concentration was determined using a sodium nitrite standard curve. The IC_{50} value, i.e., the sample concentration resulting in 50% inhibition of NO production, was determined by non-linear regression analysis [22]. N-monomethyl-L-arginine (L-NMMA) was used as a positive control. The inhibition rate was calculated according to the following formula:

$$\text{Inhibition rate (\%)} = 100 \times \frac{[NO_2^-]_{LPS} - [NO_2^-]_{LPS+\text{ sample}}}{[NO_2^-]_{LPS} - [NO_2^-]\text{ untreated}}$$

- ***Cell viability: WST-1 assay***

The cell viability was assessed using the kit WST-1 OZYME 630118. WST-1. The cell proliferation reagent was added at a volume of 10 µL/well at 24th and 48th hours. Cells were incubated for 4 h in a humidified atmosphere (37ºC, 5% CO_2). Sample absorbances were measured at 450 nm with an ELISA reader [23].

2.2.3. Evaluation of antiproliferative activity

- ***Culture medium***

EMEM medium (SIGMA M5650) supplemented with 10% undecomplemented FBS (PAA A15-101), 2 mM L-glutamine 200 mM (PAA M11-004), 100 U/mL penicillin / 100 µg/mL streptomycin (PAA P11-010), 1mM sodium pyruvate (PAA S11-003) and 0.01 mg/mL Bovine insulin in 25 mM HEPES pH 7. 5 (SIGMA I6634-50MG) only for MCF-7 line.

- ***Cell viability assay***

MCF-7 and HeLa cancer cell lines were seeded in 96-well plates and treated with Datura extracts for 48 hr. Chlorpromazine (10 µg/mL) was used as a positive control. The Cell Titer-Glo assay (PROMEGA G7571) was used to assess the cell viability. It allows to quantify intracellular ATP and thus the presence of metabolically active cells. It is based on the mono-oxygenation of luciferin to oxyluciferin by luciferase in the presence of Mg^{2+} and ATP.

$$\text{Luciferin} + O_2 + \text{ATP} + Mg^{2+} \rightarrow \text{Oxyluciferin} + CO_2 + hv$$

The cell viability was assessed according to the manufacturer's instructions at two different time points, i.e., at time zero (when the extracts were added to the cells), and 48 hours after addition of the extracts. The cell number at zero point was set at 100%, and the cell number assessed after 48 hours incubation in the presence of extracts was normalized to these values.

2.3. Statistical analysis

All the experiments and reaction were conducted in triplicates. The data is represented as mean ± standard deviation (SD). The mean values with $p < 0.05$ were found to be statistically significant.

3. RESULTS

3.1. Phytochemical screening

The results of phytochemical screening of ethanolic leaf extracts from the three Datura species are summarized in Table 1. These extracts were rich in secondary metabolites such as alkaloids, tannins, flavonoids, coumarins, saponins and terpenoids.

Table 1. Phytochemical composition of extracts

Phytochemical compounds	*D. stramonium*	*D. innoxia*	*D. ferox*
Alkaloids	+	+	+
Coumarins	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+

(+) = Present

3.2. Anti-inflammatory potential

The leaf extracts from all three species (*D. stramonium*, *D. innoxia* and *D. ferox*) were shown to modulate nitrite secretion in murine macrophages (Table 2). However, we observed a more pronounced activity of *D. innoxia*, followed by *D. stramonium* and *D. ferox*. At the same concentrations, leaf extracts of *D. innoxia* followed by those of *D. stramonium* have demonstrated the best inhibition rates of nitrite secretion, with a significant rate of cell viability observed in murine macrophages. Leaf extracts (at 10 µg/mL) of both species have modulated the nitrite production without any cellular toxicity. Whereas *D.ferox* showed a cell toxicity of 23.51±1.89 with only 11.23±2.55% inhibition of nitrite levels.

IC_{50} values of NO inhibition for the various extracts are shown in Table 2. The data show that Datura extracts possess anti-inflammatory potent which varied according to species. The lowest IC_{50} (highest anti-inflammatory potent) were registered with extracts from *D. innoxia* (23.57±0.94µg/mL) and *D. stramonium* (26.84±4.09µg/mL).

Table 2. Inhibition rates of NO secretion and toxicity of extracts on murine macrophages

Concentrations (µg/mL)	*Datura stramonium*		*Datura innoxia*		*Datura ferox*	
	Inhibition of NO (%)	Toxicity of macrophages	Inhibition of NO (%)	Toxicity of macrophages	Inhibition of NO (%)	Toxicity of macrophages
10	25.70 ±2.04	0.00	30.05 ±3.11	0.00	11.23±2.55	23.51±1.89
30	51.69 ±3.71	14.01 ±4.52	59.13 ±2.33	15.25 ±5.03	24.61±3.58	56.35±7.23
50	78.07 ±2.89	52.27 ±3.34	81.86 ±6.72	59.13 ±2.33	35.12±3.11	64.59±9.08
100	89.86 ±4.53	60.33 ±2.61	92.35 ±3.01	63.59 ±4.07	66.35±7.23	76.95±2.74
IC_{50} **(µg/mL)**	26.84±4.09		23.57±0.94		72.90±5.05	

3.3. Anticancer potential

The results presented in Table 3 summarize the cytotoxic effects of extracts from the three Datura species expressed as percentage of cell viability as a function of concentration on the respective breast (MCF-7) and cervical (HeLa) cancer cell lines. These data reveals that the cytotoxic effects of extracts on these cell lines are dose-dependent (p-value<0.05). At the different concentrations used to assess cytotoxicity on MCF-7 cell lines, a slight inhibition of cell growth was observed. At a concentration of 50 µg/mL, cell viability of 84.33% and 75.33% were recorded with *D. stramonium* and *D. ferox* extracts respectively. In other side, this cancer cell line was more sensitive to *D. innoxia* extract, with a cell growth inhibition of 42.67% (Table 3). HeLa cell lines were more sensitive towards *D. stramonium* and *D. innoxia* extracts at 50 µg/mL, with cell viability rates of 13.33% and 53.33% respectively. *D. ferox* extract had virtually no effect on this cancer strain at the doses considered (Table 3).

Table 3. Cell viability rates (%) of Datura extracts on MCF-7 and HeLa cell lines

Concentrations (µg/mL)	*Datura stramonium*		*Datura innoxia*		*Datura ferox*	
	MCF-7	HeLa	MCF-7	HeLa	MCF-7	HeLa
0,5	98.33±1.54	96.33±1.15	97.67±0.58	97.67±1.53	98.67±1.53	98.33±1.15
1	95.33±1.53	93.67±1.53	95.33±1.53	95.67±1.15	96.33±2.08	96.33±1.53
5	92.67±1.15	90.33±4.0	89.33±1.53	90.67±3.21	94.67±2.52	93.67±1.53
10	90.33±2.52	84.33±4.16	86.67±2.52	86.33±2.52	78.67±1.53	90.67±3.21
50	84.33±1.53	13.33±3.05	42.67±2.52	53.33±3.21	75.33±3.05	89.33±1.53
DMSO 1%	100	-	-	-	-	-
Chlorpromazine	00.00	00.00	-	-	-	-

Microscopic analysis also showed an increase necrosis of MCF-7 cancer cells after treatment with *D. innoxia* extract. A slight suppression of this cell strain was also observed with the other two Datura species (Figure 1a-e). At 50 µg/mL, *D. stramonium* and *D. innoxia* extracts showed the best antiproliferative activity against HeLa cells after 48 h of incubation (Figure 2 a-e).

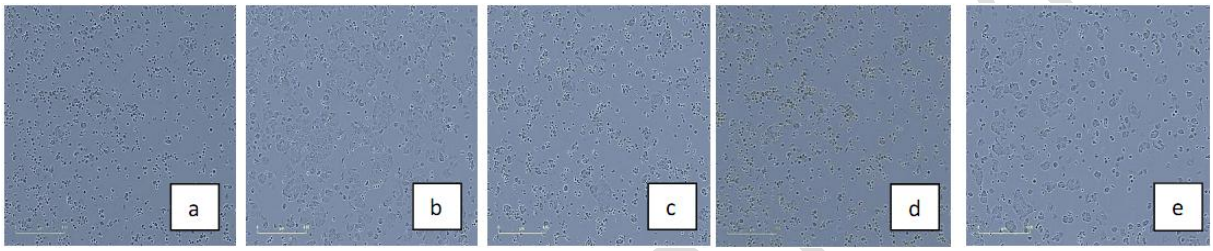

Figure 1. Photography of MCF-7 cells grown with and without Datura extracts at 50 µg/mL
(a) Effect of Chlorpromazine at 10 µg/mL, (b) Normal cell line 1% DMSO, (c) Culture in the presence of *D. stramonium* (c), *D. innoxia* (d) and *D. ferox* (e) extracts.

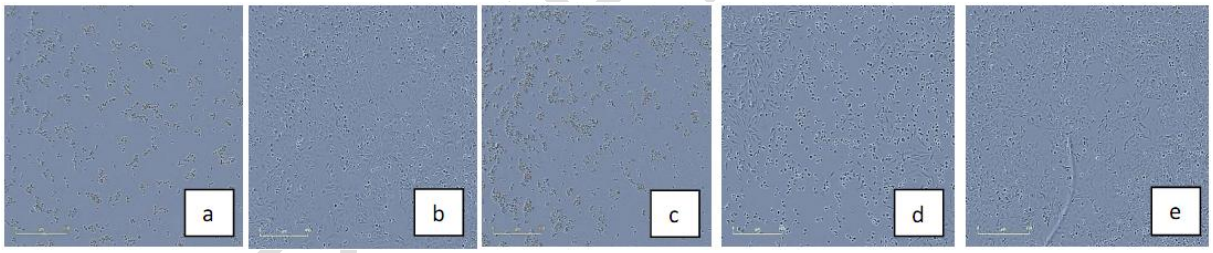

Figure 2. Photography of HeLa cells grown with and without Datura extracts at 50 µg/mL
(a) Effect of Chlorpromazine at 10 µg/mL, (b) Normal cell line 1% DMSO, (c) Culture in the presence of *D. stramonium* (c), *D. innoxia* (d) and *D. ferox* (e) extracts.

4. DISCUSSION

The aim of the present study was to evaluate the anti-inflammatory and anticancer potent of leaf extracts from three species of Datura genus, widely used in traditional Malian pharmacopoeia [15]. Ethanolic extracts were used to evaluate these biological activities of *D. stramonium*, *D. innoxia* and *D. ferox*. The phytochemical screening revealed the presence of numerous groups of secondary metabolites in the extracts of investigated species. The presence of these metabolites could explain the immunostimulant and antitumor properties of the investigated extracts. This hypothesis corroborates the findings of many studies which have shown that the anticancer potent of natural products is largely dependent on these bioactive compounds [16, 17, 24, 25].

For example, the works of Yang et al., Bing-You Yang et al. and Zhang et al. [21, 26, 27] have allowed to isolate withanolides from Datura leaf extracts and demonstrated their anti-inflammatory potential. During the inflammation, macrophage activation triggers high NO production. While this compound plays a major role as an immune regulator and neurotransmitter in various tissues [28], dysregulation of its secretion is associated with various pathologies such as inflammatory and hyper-proliferative diseases [2]. Scavenging excessive NO radicals is therefore a key therapeutic approach for fighting inflammatory disorders [29]. The present study has shown that the Datura species found in Mali possess an immunostimulant potential capable to reduce the secretion of these nitrites. This biological activity was dose

and species-dependents. Extracts from *D. innoxia* species have showed the greatest efficacy with the lowest IC_{50} of 23.57±0.94 µg/mL. The toxicity profile of *D. stramonium* and *D. innoxia* extracts showed no cytotoxic action on murine macrophages at low doses (10 µg/mL). These data are consistent with others results that have reported the ability of the Datura genus to inhibit nitrite levels produced by murine macrophages [29, 30].

Using *D. stramonium* leaf extract (at 20 µg/mL), Nasir et al. [29] have reported an inhibition rate of NO production of 27.35±0.12% and IC_{50} of 7.625±0.51 µg/mL. Also, with lipopolysaccharide-activated murine macrophage RAW 264.7 cells, Fatima et al. [31] havealso showed an inhibition rate of NO production with IC_{50} of 18.5±1.8 µg/mL with *D. innoxia* leaves. Similarly, Aboluwodi et al. [32] have demonstrated that essential oil extracted from the seeds and leaves of *D. stramonium* exhibited anti-inflammatory activity in albumin-induced rats. With 20 µg/mL of daturaolone, a substance isolated from *D. innoxia* flowers, Baig et al. [30] achieved 84±2.87 µg/mL of NO inhibition with an IC_{50} of 4.51±0.92 µg/mL.
Works had shown that Datura leaf extracts were rich in antioxidants, phenolic compounds [18] and especially alkaloids [12]. Thus, the anti-inflammatory activities recorded could be linked to this richness in bioactive compounds and antioxidants, since close links were established between the antioxidant and anti-inflammatory power of plant extracts (R^2=0.72 to 0.90) [13].

It is known that many bioactive phytochemical compounds exhibit an anticancer activity by playing a role of anti-inflammatory agents, cell cycle regulators and apoptosis regulators [33]. Indeed, preclinical and clinical studies have established that many phytomolecules are endowed with anti-inflammatory properties are promising agents for the chemoprevention of several cancers, including breast cancer [34]. Assessing the potential anti-cancer properties of plants could therefore make a significant contribution to cancer therapy studies.
Datura leaf extracts harvested in Mali thus possess molecules with anti-inflammatory properties, making them suitable candidates for further study of their antiproliferative properties. The data obtained have showed that extracts of all three species are endowed with antiproliferative activity against tested cancer cell lines (HeLa and MCF-7). However, this activity was more pronounced in *D. stramonium* and *D. innoxia*.

D. innoxia extracts were more toxic against the MCF-7 cancer strain. The same trend was observed with *D. stramonium* against HeLa. *D. ferox* has presented a moderate antiproliferative activity on both cancer cell lines tested. The cytotoxic activity of Datura on these two cancer cell strains has already been reported in the literature [33, 35, 36, 37]. Indeed, with 50 µg/mL methanoic extract of *D. stramonium* seeds, Iqbal et al. [36] have noted a toxicity rate of 72.52% against the MCF-7 cell line IC_{50} of 265.7 µg/mL and 272.5 µg/mL were observed against HeLa and MCF-7 respectively with leaf ethanolic extracts of *D. innoxia* harvested in Turkey [33]. Ethyl acetate extracts of *D. stramonium* and *D. innoxia* also showed IC_{50} of 40.2 µg/mL and 99.36 µg/mL on MCF-7 respectively [29]. Other studies have also demonstrated that ethanolic extract of *D. innoxia* induced a cell cycle arrest in G1 phase, and more specifically apoptotic cell death [35, 38]. The results of this study confirm the beneficial effects of Datura species on inflammatory and cancerous pathologies.

5. CONCLUSION

At the end of this study, Datura leaf extracts harvested in the Kolondiéba area of Mali were found to have a modulating effect on nitric oxide production by murine macrophages. An antiproliferative potential on breast and cervical cancer cells has also been demonstrated. However, these *in vitro* biological activities were more pronounced with *D. stramonium* and *D. innoxia* leaves than those from *D. ferox*. Therefore, the results of this work could justify the use of these plants in traditional Malian medicine.

ETHICAL APPROVAL

Not applicable.

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