Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(2):500-504

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Interference *in vitro* of *Aspidosperma macrocarpum* extract in the laboratory colorimetric determinations

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ABSTRACT

The application of medicinal plants as a mechanism of cure, prevention and treatment of diseases has increased significantly in recent years. Several substances can also cause adverse effects and toxicity, lead to analytical interferences in laboratory tests. This study aimed to evaluate the in vitro analytical interference caused by the application of crude ethanol extract from leaves of Aspidosperma macrocarpum in colorimetric tests. To this, were added increasing concentrations of the extract to the reaction medium on the biochemical determination. These measurements were perform with diagnostic kits and tests were conduct according to manufacturer's recommendations. According to the results, the crude ethanolic extract of leaves of Aspidosperma macrocarpum may cause analytical interference in vitro in the colorimetric determinations of glucose, cholesterol, triglycerides and urea.

Keywords: Analytical interference, Biochemistry determinations, *Aspidosperma macrocarpum*.

INTRODUCTION

A growing interest in the use of medicinal plants and their extracts have been observed in recent decades, reflecting a complement to therapy and a contribution in the primary, to reconcile care with conventional medicine. Need for such knowledge of the toxic side effects, interactions, mutagenicity, among others. The effectiveness of this type of treatment should be show by pharmacological tests and clinical trials [1]. The habit of using natural products, especially from the flora, for therapeutic purposes, came up with the human being. Traces of this use, including potentially toxic plants for healing, prevention and treatment of diseases, are find in the most remote civilizations, constituting a rich source of biologically active compounds [2]. In therapeutic practice of traditional medicine, medicinal plants are the main representatives of the medical field, having the largest number of species are use in folk medicine [3]. This fact makes them occupy important place in the therapeutic arsenal. According to the World Health Organization (WHO), about 80% of the population makes use of the popular medicines as a way to supplement private health care, moving around 22 billion dollars [4]. However, there has been a sharp increase in the indiscriminate use of natural compounds as a therapeutic alternative free of toxicity and adverse reactions, resulting in the increase in cases of poisoning by plants [5].

The urgency to gather data regarding the interference of drugs in laboratory tests at dawn on authors in the 60s intended to group the information in computerized system, forming a database available for public consultation from

internal and external sources [6]. Several laboratory tests consist of chemical determinations subject to analytical interference in varying degrees, depending on the methodology employed. Numerous substances when present in the reaction medium along with the analyte to be search, the determination can be reacted interfering [7]. Interference in the analytical methods of laboratory tests can be cause by the presence of medicinal plants and their various forms of preparations, constituting an important source of analytical errors and changes in the analysis of blood and other body fluids, compromising thus the quality of patient care [8]. This study aimed to evaluate the *in vitro* analytical interference caused by the application of crude ethanol extract from leaves of *Aspidosperma macrocarpum* in the colorimetric determinations of glucose, cholesterol, triglycerides and urea.

EXPERIMENTAL SECTION

Plant material and Extract production

A. macrocarpum was collected from Planaltina-GO, Brazil, and identify by a botanist from University of Brasilia (UnB). A voucher specimen is deposit at the UnB herbarium, with the code JEP 3767. For extraction the materials was dried and powdered in a mechanical grinder. The powder of the dried leaves (1.6 kg) was submitted to maceration with 2 L of 95% ethanol in three cycles of 72h each. The obtained ethanolic solution was concentrated under reduced pressure furnishing 436 g of crude ethanolic extract. The dry weight of the plant extracts was obtained by solvent evaporation and used to determine concentration in mg/mL.

In vitro interference

The evaluation of the in vitro assay interference was conducted by applying increasing concentrations of crude ethanolic extract of leaves of *A. macrocarpum* in the reaction medium (Table 1).

Table 1 – Analytical determination

[ ] (µg/mL)	P	0.1	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10	20	40
Standard reagent (µL)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Buffer reagent (µL)	1000	989	985	980	970	960	950	940	930	920	910	900	890	880	870
Extract of plant (µL)	-	1	5	10	20	30	40	50	60	70	80	90	100	110	120

Note: stock solution at a 100 µg/mL.

Effect of extract on the determination of glucose concentration

To test the interference to the concentration of glucose were added to the reaction medium increasing concentrations of extract (0.1; 0.5; 1; 2; 3; 4; 5; 6; 7; 8; 9; 10 ; 20: 40 µg). Glucose was enzymatically quantified by incubation for 30 min at 37 ^{o}C, using Glucox (www.doles.com.br). The standard mixture contains 3 units/ml of glucose oxidase, 0.3 unit/ml peroxidase, 0.5 mM aminoantipyrine, and 0.5 mM p-hydroxybenzoate. The product formed by the oxidation was read in a Shimadzu (Japan) U1240 spectrophotometer at 510 nm. The absorbance is directly proportional to the concentration of glucose. Three samples were analyzed for each experimental point.

Effect of extract on the determination of total cholesterol

To determine total cholesterol was used diagnostic kit Liquiform cholesterol (Labtest). This kit consists of an enzyme system for cholesterol determination by endpoint method. The mixture was stirred and place in a water bath at 37 °C for 10 minutes. The product formed was read in a Shimadzu (Japan) U1240 spectrophotometer at 500 nm. The absorbance is directly proportional to the concentration of cholesterol. Three samples were analyze for each experimental point.

Effect of extract on the determination of triglycerides

To determine triglycerides was used diagnostic kit Liquiform triglycerides (Labtest). This kit consists of an enzyme system for triglycerides determination by endpoint method. The mixture was place in a water bath at 37 °C for 10 minutes. The product formed was read in a Shimadzu (Japan) U1240 spectrophotometer at 505 nm. Three samples were analyze for each experimental point.

Effect of extract on the determination of urea

To determine urea concentration was used diagnostic kit CE Liquiform (Labtest). The mixture was stirred vigorously and place in a water bath at 37 °C for 10 minutes. The product formed was read in a Shimadzu (Japan) U1240 spectrophotometer at 600 nm. Three samples were analyze for each experimental point.

Interference of the solvent

The control to access a possible interfering effect of ethanol in the reaction was prepared. Ethanol was added to the reaction medium in equivalent amounts to be use in tests with ethanol extracts. Not the solvent interference was observe in the values of substrates for analysis.

RESULTS AND DISCUSSION

The results indicate that the use of *A. macrocarpum* sheets preparations may cause significant interference in in vitro biochemical reactions that comprise indicator systems with oxidases and peroxidases, such as the Trinder reaction method, possibly due to consumption of any component reaction. Such interference may result in falsely reduced quantification of serum components analyzed by these methods compared to the control sample. The results show a progressive reduction in the concentration of the analyte in response to increased concentrations of the extract. For all the analytes in question, the addition of *A. macrocarpum* extract in the reaction medium, appears to act by inhibiting the reaction system oxidase / peroxidase levels directly proportional to the concentration used. To evaluate the effect of the extract in the concentration of glucose concentrations were use between 0.1 and 40.0 mg / mL. The increase of the extract concentration caused a proportional decrease in the determination of glucose (Table 2). The extract appears to act by inhibiting oxidase / peroxidase reaction system, reducing the formation of antipirilquinonimine. This compound has a red color and its presence determines how much light will absorb solution in the spectrophotometer and is directly connect to the glucose content in the sample.

Table 2 – Evaluation of increasing concentrations interference of leaves crude extract of *A. macrocarpum* in the concentration of glucose.

[ ] Extract (µg/mL)	[ ] Glucose (mg/mL)
0	100.0 ± 0.00
0.1	95.05 ± 0.15*
0.5	92.05 ± 0.15**
1.0	91.90 ± 0.00**
2.0	89.35 ± 1.35**
3.0	87.55 ± 1.05***
4.0	86.95 ± 2.25***
5.0	80.80 ± 2.4***
6.0	85.45 ± 2.25***
7.0	77.80 ± 0.60***
8.0	76.45 ± 0.45***
9.0	73.00 ± 1.50***
10.0	70.30 ± 0.30***
20.0	60.15 ± 0.95***
40.0	43.40 ± 3.90***

*Statistical analysis: *p < 0.05; **p < 0.01; ***p < 0.001 compared with baseline (0 g/dL of extract). Values presented as arithmetic mean ± mean standard error, analysis of variance by One-Way ANOVA and the test Student's t followed the multiple comparison test of Newman-Kewls.*

The determination of total cholesterol also proved capable of assay interference in the presence of *A. macrocarpum* extract. Similarly, to glucose, cholesterol concentration was find to be inversely proportional to the extract concentration in the reaction medium. Concentrations were maintained between 0.1 and 40.0 µg / ml. As antipirilquinonimine formation is partially inhibit by the extract, occurs a decrease in light absorption, indicating the possibility of falsely decreased results (Table 3).

Table 3 – Evaluation of increasing concentrations interference of leaves crude extract of *A. macrocarpum* in the concentration of total cholesterol

[ ] Extract (µg/mL)	[ ] Cholesterol (mg/mL)
0	200.00 ± 0.00
0.1	154.35 ± 6.95**
0.5	157.25 ±11.85**
1.0	161.10 ± 5.40**
2.0	141.75 ± 2.05***
3.0	149.75 ± 16.75***
4.0	142.25 ± 11.35***
5.0	131.95 ± 5.65***
6.0	124.45 ± 0.25***
7.0	125.25 ± 1.55***
8.0	123.95 ± 0.25***
9.0	127.60 ± 1.30***
10.0	117.25 ± 6.45***
20.0	135.05 ± 1.05***
40.0	113.15 ± 2.35***

*Statistical analysis: *p < 0.05; **p < 0.01; ***p < 0.001 compared with baseline (0 g/dL of extract). Values presented as arithmetic mean ± mean standard error, analysis of variance by One-Way ANOVA and the test Student's t followed the multiple comparison test of Newman-Kewls.*

As in the glucose and cholesterol determinations, the methodology used to measure the triglyceride concentration is also Trinder reaction. As might be expected, similar interference was observe in this experiment. To evaluate this interference, concentrations between 0.1 and 10.0 µg / ml of extract was add to the reaction medium. In this case,

partial inhibition of peroxidases / oxidases leads to decreased formation of quinoneimine, colored compound that is directly proportional to the triglyceride concentration of the sample (Table 4).

Table 4 – Evaluation of increasing concentrations interference of leaves crude extract of *A. macrocarpum* in the concentration of triglycerides

[ ] Extract (µg/mL)	[ ] Triglycerides (mg/mL)
0	200.00 ± 0.00
0.1	200.55 ± 1.05
0.5	204.20 ± 2.10
1.0	200.20 ± 3.70
2.0	185.80 ± 3.70*
3.0	184.20 ± 3.20*
4.0	175.80 ± 4.20**
5.0	148.40 ± 5.30***
6.0	140.50 ± 1.60***
7.0	143.70 ± 7.90***
8.0	49.45 ± 5.25***
9.0	38.40 ± 2.60***
10.0	22.10 ± 2.10***

*Statistical analysis: *p < 0.05; **p < 0.01; ***p < 0.001 compared with baseline (0 g/dL of extract). Values presented as arithmetic mean ± mean standard error, analysis of variance by One-Way ANOVA and the test Student's t followed the multiple comparison test of Newman-Kewls.*

Urea was the only analyte tested that is not determined by Trinder method, however, the results show interfering effect similar to that of other experiments. The urease-Labtest method leads to formation of indophenol blue. Decreased staining (which is proportional to the urea concentration in the sample) was probably cause by the enzymatic inhibition caused by the extract (Table 5).

Table 5 – Evaluation of increasing concentrations interference of leaves crude extract of *A. macrocarpum* in the concentration of urea

[ ] Extract (µg/mL)	[ ] Urea (mg/mL)
0	70.00 ± 0.00
0.1	68.65 ± 0.05
0.5	67.90 ± 0.90
1.0	68.80 ± 0.50
2.0	69.95 ± 1.25
3.0	66.45 ± 0.45*
4.0	64.25 ± 1.25***
5.0	63.95 ± 0.15***
6.0	61.50 ± 0.20***
7.0	63.00 ± 0.30***
8.0	60.00 ± 0.10***
9.0	58.80 ± 0.00***
10.0	57.90 ± 0.90***
20.0	57.15 ± 0.45***
40.0	54.75 ± 0.55***

*Statistical analysis: *p < 0.05; **p < 0.01; ***p < 0.001 compared with baseline (0 g/dL of extract). Values presented as arithmetic mean ± mean standard error, analysis of variance by One-Way ANOVA and the test Student's t followed the multiple comparison test of Newman-Kewls.*

The occurrence of errors of analysis resulting from the influence of exogenous factors not only happens in patients with pathological state, but also in healthy individuals. These exogenous factors are usually natural herbs consumed indiscriminately. The suspension of the use of these plant preparations before the exams can prevent the emergence of such interference, depending on the time of withdrawal and dose that was being used [9]. As most laboratory assays utilize analytical methodologies based on oxide reduction reactions, the presence of antioxidant compounds in the reaction medium may lead to the consumption of components of this reaction resulting in harmful interference in determination of certain analytes. In these reactions, hydrogen peroxide and peroxidase act forming a chromogen, assigning staining solution and allowing the reading of the same spectral [7, 10, 11,]. In a survey to assess the interference activity of ascorbic acid, was demonstrated the effect of prolonged intake of vitamin C for healthy people, confirming the data in the literature showing a significant dose-dependent interference on laboratory tests. It is attribute to the powerful reducing activity ascorbic acid. It acts both reducing the hydrogen peroxide before reaction with the chromogen, or directly on the colored product, a compound generating a reduced staining or colorless, which does not absorb light in the spectrophotometer [10].

The interference in vitro by dry matter of eggplant (Solanum melongena L.) on the glucose, cholesterol, triglycerides and uric acid was verified. Therefore, this study suggested the interfering power plant extract in biochemistry. The authors attributed this effect to the presence of anthocyanins, compounds with proven antioxidant activity [12]. All these studies have in common the correlation of antioxidant compounds and their biological activities, emphasizing

the importance of these compounds in maintaining health, as well as demonstrating the effect of consumption of these products can cause.

CONCLUSION

According to the results of this study, it is suggest that the ethanol extract of *A. macrocarpum* leaves can cause analytical interference in vitro in some serum determinations. When detected any possibility of analytical interference, methodological steps should be taken to minimize their effects. It is essential that collecting information for the use of any substance from the patient so that the results obtained are reliable and assist in clinical diagnosis. For substances, that present interference confirmed by scientific experiments, such as ascorbic acid, reagents many manufacturers already provide instructional material stating that measures must be taken to ensure that these possible influences are cancel. Although species of this genus are the subject of several studies aimed at the discovery of bioactive compounds, research on *A. macrocarpum* are still incipient and little is known about its effects on the body, particularly on the potential interference that may cause biochemical tests. Moreover, the literature mainly describes the chemical species composition, with lack of description of biological effects.

Acknowledgements
This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de Alagoas (FAPEAL), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Financiadora de Estudos e Projetos (FINEP).

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