The phytochemical composition and *in vitro* antiviral activity of decoctions from galls of *Guiera senegalensis* J.F. Gmel. (*Combretaceae*) and their relative non-toxicity for chickens

C.E. LAMIEN[1]*, A. MEDA[1], E. COUACY-HYMANN[3], A.G. OUEDRAOGO[2] and O.G. NACOULMA[1]

ABSTRACT

LAMIEN, C.E., MEDA, A., COUACY-HYMANN, E., OUEDRAOGO, A.G. & NACOULMA, O.G. 2005. The phytochemical composition and *in vitro* antiviral activity of decoctions from galls of *Guiera senegalensis* J.F. Gmel. (*Combretaceae*) and their relative non-toxicity for chickens. *Onderstepoort Journal of Veterinary Research*, 72:111–118

Aqueous decoctions obtained from the galls of *Guiera senegalensis* were screened to determine their phytochemical composition and *in vitro* antiviral activity against fowlpox virus. In addition, we wanted to investigate the toxic effects, if any, of crude extracts in chickens. Steroids as well as cardiac glycosides not previously reported, an alkaloid, polyphenols and saponins were detected in the various fractions of organic solvents used for extracting the decoctions. Antiviral activity was determined by cytopathic effect inhibition assay in primary chicken embryo skin cells. The 50 % inhibitory concentration (EC_{50}) was shown to be 15.6 µg/mℓ. Toxicity for cells was established by determining the 50 % cytotoxic concentration (CCy_{50}). A value of 90 µg/mℓ and a selectivity index (CCy_{50}/EC_{50}) of 5.8 were obtained. *In vivo* studies of toxicity were performed in chickens that were dosed orally with decoctions of several concentrations for 2 weeks and then monitored for 3 months. No significant changes in several blood chemical parameters were obtained, except for a significant decline in SGOT levels in birds dosed with 100 mg/kg. These levels were nevertheless within the accepted normal range. The findings suggest that aqueous decoctions of galls from *G. senegalensis* are non-toxic for chickens when administered orally, even at a daily dose of 100 mg/kg for 14 days.

Keywords: Antiviral compounds, chickens, ethnoveterinary treatment, fowlpox virus, galls, *Guiera senegalensis*, phytochemical composition, thin-layer chromatography

INTRODUCTION

Guiera senegalensis is a popular medicinal plant for both human and veterinary use in West Africa. Its leaves have been reportedly used to treat rhinitis, bronchitis and fever, and the roots to treat diarrhoea and dysentery. The antitussive effects of the leaves have been confirmed (Bouchet, Levesque, Blond, Bodo & Pousset 1996) as has the presence of antifungal properties (Silva & Gomes 2003). Aqueous extracts from its roots and leaves have also been screened for toxicity (Pousset 1989).

The galls of *G. senegalensis* are used in Burkina Faso as an ethnoveterinary product to increase milk production in cows and to treat fowlpox infection in chickens (Nacoulma 1996). Galls in a plant are defined as a tumour-like proliferations of cells due to microbial infection or infestation with insects (Ramade 1993). In the case of *G. senegalensis*,

* Author to whom correspondence is to be directed

[1] Laboratoire de Biochimie et Chimie Appliquées, UFR/SVT, Université de Ouagadougou, Burkina Faso
E-mail: celamien@hotmail.com

[2] Laboratoire d'Enseignement et de Recherche en Santé et Biotechnologie Animales, Institut du Développement Rural, Université Polytechnique de Bobo Dioulasso, Burkina Faso

[3] LANADA/LCPA BP 206 Bingerville, Côte-d'Ivoire

Accepted for publication 4 October 2004—Editor

insect larvae and eggs from the same unidentified species were found in the galls, which we showed to be larvae of butterflies (Lamien, unpublished data 2003). Several chemical interactions are believed to occur between plant and insect, leading to synthesis in the plant of new compounds or to a change in concentration of inherent ones (Ramade 1993). Antiviral properties may be due to, amongst others, several quinic acid derivatives that have been isolated from galls (Bouchet *et al*. 1996). The antiviral activity of some of these derivatives has been reported previously (Mahmood, Moore, De Tommasi, De Simone, Colman, Hay & Pizza 1993). No data, however, are available on the effects of extracts from *G. senegalensis* galls on fowlpox virus. We report here on our findings on the general phytochemical composition of decoctions from galls obtained from this plant, their *in vitro* antiviral activity against fowlpox virus (FPV) and the *in vivo* effects of aqueous decoctions in experimental chickens. This work forms part of our ongoing research in isolating and characterising antiviral compounds against FPV.

MATERIAL AND METHODS

Plant collection and extraction

Galls from the shrub *G. senegalensis* were collected on site without damaging plants in the Ouagadougou region of Burkina Faso in 1999. A voucher specimen was deposited at the herbarium of the University of Ouagadougou (Lamien 01) and identified and verified as such. Each gall was carefully cleaned, and the enclosed insect eggs and larvae removed. The galls were then dried at room temperature and pulverised in a blender to obtain a fine powder which was suspended 1:10 (w/v) in distilled water for an hour and the suspension was then filtered through No. 1 Whatman paper. Toxicological evaluations were done using lyophilised filtrates resuspended in various volumes of distilled water.

Phytochemical screening of the aqueous decoction

Decoctions (50 mℓ) of galls from *G. senegalensis* were extracted successively with 3 x 50 mℓ petrolium ether (PE) (SDS, France), 3 x 50 mℓ dichloromethane (DCM) (SDS, France), 3 x 50 mℓ ethyl acetate (EtOAc) (SDS, France) and 3 x 50 mℓ n-butanol (BuOH) (SDS, France). The BuOH extract was air-dried while the PE, DCM and EtOAc extracts were first concentrated in a rotating evaporator (Rotavapor, Buchi, Switzerland) before being air-dried to yield various fractions. Phytochemical screening was then performed on these fractions using thin-layer chromatography (TLC) (Wagner & Bladt 1996) with Polygram SIL G/UV254 plates (Macherey-Nagel, Germany). Briefly, the presence of steroids and cardiac glycosides was screened for in the PE fraction by thin-layer chromatography (TLC) with toluene-ethyl acetate (4:1.3) as the mobile phase. The separated compounds were visualized using Liebermann-Buchard reaction (Wagner & Bladt 1996). Thin-layer chromatography was also used to screen for the presence of alkaloids in the DCM fraction. Chloroform-methanol (11.5:1) was used as the mobile phase following exposure to Dragendorff reagent (Sigma, USA) and the plates developed under the same conditions were treated with the Prochazka and Salkowski reaction (Merck 1975) to detect indole rings. The phenolic compounds were screened in the EtOAc fraction using chloroform-glacial acetic acid-methanol-water (6:3.2:1.2:0.8) as mobile phase and treated with vanillin-H_2SO_4 reagent. The saponins were separated in the BuOH fraction using ethyl acetate-methanol-water (10:1.35:1) as eluent and antimony chloride ($SbCl_3$) as spray reagent. The remaining aqueous phase was used to detect tannins and anthocyanodins as described by Ciulei (1982).

Antiviral assay

Cells and virus

Primary chicken embryo skin (CES) cell cultures were prepared from 11-day-old specific pathogen-free (SPF) embryos by an adaptation of the method of Silim, El Azhary & Roy (1981). Secondary CES cell cultures were obtained by dispersing the primary CES cells. A fowlpox virus vaccine strain was provided by the Biotechnology Division, Onderstepoort Veterinary Institute.

Cytotoxicity

The crude extract was redissolved in water to a concentration of 10 mg/mℓ and sterilized by filtration. A serial two-fold dilution was prepared in cell culture medium (DMEM/F12) containing 5 % foetal calf serum (FCS) and 1 % solution of antibiotics [100 x solution of penicillin G (10 mg/mℓ); streptomycin sulphate (10 mg/mℓ); and amphotericin (25 µg/mℓ)] (Highveld Biological Products, South Africa).

For the tetrazolium blue (MTT) assay (Mosmann 1983), confluent monolayers of primary CES cells were detached using trypsin (Highveld Biological Products, South Africa) and plated out at $2x10^4$ cells/well in 96-well, flat-bottomed plates (Cellstar,

Greiner Bio-One, Germany). After a 24-h pre-incubation period, diluted extracts were added to appropriate wells and the plates were incubated for 72 h at 37 °C in a humidified incubator (Forma Scientific, USA) with 5 % CO_2. Untreated cells were used as controls. After 72 h, the medium with extracts was removed and the wells were washed with phosphate buffered solution (PBS). Then 100 µℓ of a 0.5 mg/mℓ MTT (Sigma, USA) solution in PBS were added to each well and the plates were incubated for 3 h at 37 °C. After incubation the MTT was removed and 100 µℓ of DMSO (Associated Chemical Enterprises, South Africa) was added to each well to solubilise the MTT crystals (Rivelland, Vié, Coiffard & De Roeck-Holtzhauer 1994). The plate was shaken after 20 min and the absorbancy was read at 570 nm using a multiwell plate reader (Model EL340, BioTek Instruments, USA). The percentage toxicity was determined using the formula: (A–B)/A x 100 where A is the mean optical density of untreated wells and B is the optical density of wells treated with plant extracts.

Viral cytopathic effect inhibition

A modification of the end-point titration described by Van den Berghe, Vlietinck & Van Hoof (1986) was employed. Secondary CES cell cultures were seeded at $2x10^4$ cells/well on 96-well microtitre plates (Cellstar, Greiner Bio-One, Germany). After 24 h the growth medium was removed and replaced with a serial two-fold dilution of plant extract in growth medium (DMEM/F12, 2 % FCS, 1 % antibiotics) (Highveld Biological Products, South Africa). Then 1 000 plaque forming units (pfu) of FPV were used to infect each well. Each experiment was run in triplicate together with infected but untreated and uninfected but treated controls.

The virus-induced cytopathic effect (CPE) was recorded after 96 h using an inverted microscope (Leitz Wetzlar, Germany) (score: 0 = 0 % CPE; 1 = 0–25 % CPE; 2 = 25–50 % CPE; 3 = 50–75 % CPE; and 4 = 75–100 % CPE). The concentration reducing CPE by 50 % (EC_{50}) in comparison with the virus control was estimated from plotted data (Serkedjieva & Alan 1998). The selectivity index was calculated as follows: $SI = CCy_{50}/EC_{50}$

Animals

Chickens not immunized against FPV were obtained from the National Livestock Animal Facilities, Ouagadougou. Fertilized eggs from them were incubated. The chicks and adult birds were fed a commercial diet and given water *ad libitum*. Thirty-five chickens of both sexes weighing between 0.9 and 1.9 kg were used in the present study.

Experimental design

The birds were randomly divided into seven groups of five birds each, and each was given 2 mℓ of test reagent orally for a period of 14 days. Chickens in Group 1 (negative controls) received water in the place of an extract. Those in other groups received the following concentrations (per kg body mass) of the extracts respectively: 0.001 mg/kg (Group 2), 0.01 mg/kg (Group 3), 0.1 mg/kg (Group 4), 1 mg/kg (Group 5), 10 mg/kg (Group 6) and 100 mg/kg (Group 7).

Monitoring of chickens

The chickens were observed daily for clinical signs of weakness and mortality during the 14-day period of treatment as well as for a further 3-month period thereafter. Body mass gain was determined after 7 and 14 days after initiation of treatment and again 3 months after the last treatment.

Blood analysis

Blood samples were collected from the wing veins of each bird 1 day after the last treatment and again 3 months later. After coagulation at room temperature and refrigeration at 4 °C, blood was centrifuged at 1 000 x *g* for 10 min and serum collected.

Glucose concentration was determined immediately and the serum was then stored at –20 °C. Serum glucose, calcium, uric acid, triglycerides, total proteins, glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) were determined with a S500 spectrophotometer (Secoman, France) using commercially available test kits (BioSystems SA, Spain).

Statistical analysis

The mean and standard deviations of collected data were recorded and analysed statistically using the Sigma Stat 2.0 test (Jandel Scientific software).

One Way ANOVA analysis was done throughout the study to compare the differences in the mean values among the groups. When the One Way ANOVA analysis demonstrated significant differences among groups ($P < 0.05$), further comparisons of the means were performed using the Student-Newman-Keuls test. A P value less than or equal to 0.05 was considered significant.

RESULTS

Phytochemical composition of galls decoctions of *G. senegalensis*

The TLC analysis of the DCM fraction showed a blue-violet fluorescence after illumination at 254 nm and a dark spot after spraying with Dragendorff reagent, indicating the presence of an alkaloid (Wagner & Bladt 1996). This alkaloid also showed green and orange fluorescence after spraying with Prochazka and Salkowski reagent respectively, indicating the presence of indole rings (Merck 1975).

Thin-layer chromatological analysis of the EtOAc fraction followed by spraying with vanillin-H_2SO_4 reagent showed the presence of phenolic compounds as indicated by the presence of a pink to red colour (Merck 1975, Wagner & Bladt 1996). Thin-layer chromatological analysis of the PE fraction followed by spraying with Liebermann-Buchard reagent revealed the presence of steroidal rings (Merck 1975; Wagner & Bladt 1996). This same fraction was analysed using the Keller-Killiani test (Sofowora 1996) for detecting desoxy sugars, which showed the presence of such a sugar and suggestive of the presence of cardiac glycosides.

The TLC analyses of the BuOH fraction revealed the presence of two dark red spots after spraying with the $SbCl_3$ reagent, indicating the presence of saponins (Merck 1975). In the remaining aqueous fraction, tannins were found to be present as suggested by the positive reaction with $FeCl_3$ (Ciulei 1982). The change in the colour of the hydrolysed decoction from red to blue with the increase in the pH value was an indication for the presence of anthocyanodins (Ciulei 1982).

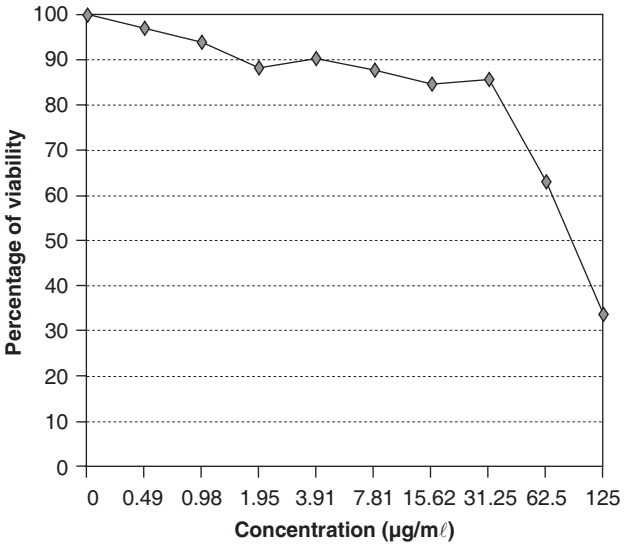

FIG. 1 Cytotoxicity in primary CES cells of an aqueous decoction from galls of *Guiera senegalensis*

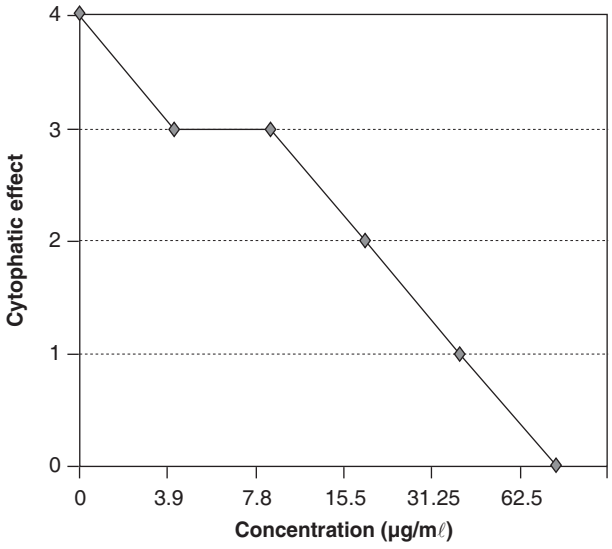

FIG. 2 Inhibition of fowlpox virus CPE by a decoction from galls of *Guiera senegalensis*

TABLE 1 Body mass of chickens taken after 7 and 14 days of continuous treatment with various concentrations of decoctions from galls of *Guiera senegalensis,* as well as after a 3-month observation period

Doses (mg/kg body mass)	Body mass gain after 1 week of treatment (kg)	Body mass gain after 2 weeks of treatment (kg)	Body mass gain 3 months after last treatment (kg)
	Mean ± SD	Mean ± SD	Mean ± SD
0	0.11 ± 0.05	0.19 ± 0.07	1.00 ± 0.56
0.001	0.13 ± 0.03	0.20 ± 0.05	1.01 ± 0.48
0.01	0.14 ± 0.07	0.19 ± 0.07	0.89 ± 0.19
0.1	0.15 ± 0.08	0.33 ± 0.25	1.09 ± 0.36
1	0.12 ± 0.04	0.18 ± 0.04	0.91 ± 0.33
10	0.14 ± 0.02	0.27 ± 0.08	0.95 ± 0.40
100	0.07 ± 0.06	0.14 ± 0.13	0.94 ± 0.27

TABLE 2 Serum chemistry profiles after 14 days of continuous treatment with increasing doses of decoctions from galls of *Guiera senegalensis*

Doses mg/kg body mass	Glucose (mmol/ℓ)	Calcium (mg/dℓ)	Uric acid (µmol/ℓ)	Triglycerides (mmol/ℓ)	SGOT (IU/ℓ)	SGPT (UI/ℓ)	Proteins (g/ℓ)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	11.47 ± 3.39	10.04 ± 0.65	208.40 ± 57.82	1.37 ± 0.59	232.00 ± 62.45	14.80 ± 12.46	47.18 ± 4.96
0.001	11.04 ± 2.99	09.68 ± 2.02	296.00 ± 70.81	1.29 ± 0.45	195.80 ± 59.33	13.80 ± 7.26	49.82 ± 4.54
0.01	11.78 ± 0.83	13.00 ± 4.90	338.00 ± 52.91	1.25 ± 0.23	216.20 ± 68.69	18.80 ± 8.26	50.90 ± 3.08
0.1	12.97 ± 2.47	12.82 ± 3.44	265.00 ± 56.93	1.38 ± 0.24	190.20 ± 65.78	13.20 ± 1.64	47.56 ± 5.06
1	13.00 ± 2.18	10.56 ± 0.74	247.60 ± 66.15	1.34 ± 0.33	209.00 ± 38.44	11.00 ± 7.31	45.22 ± 1.86
10	12.90 ± 1.11	10.36 ± 1.25	270.20 ± 15.04	1.43 ± 0.77	191.20 ± 43.60	14.50 ± 5.57	53.05 ± 6.97
100	12.47 ± 3.10	11.14 ± 2.78	392.60 ± 195.79	1.33 ± 0.51	195.20 ± 72.63	11.60 ± 4.83	46.46 ± 2.58

TABLE 3 Serum chemistry profiles after a 3-month observation period following administration of different concentrations of decoctions from galls of Guiera senegalensis

Doses mg/kg body mass	Glucose (mmol/ℓ)	Calcium (mg/dℓ)	Uric acid (µmol/ℓ)	Triglycerides (mmol/ℓ)	SGOT (IU/ℓ)	SGPT (UI/ℓ)	Proteins (g/ℓ)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	10.80 ± 1.06	14.38 ± 3.09	162.60 ± 22.50	4.98 ± 4.36	156.60 ± 15.90	13.80 ± 7.60	51.35 ± 10.20
0.001	10.40 ± 1.22	12.18 ± 2.60	222.00 ± 76.18	3.24 ± 3.15	128.00 ± 25.82	14.80 ± 2.95	51.90 ± 4.23
0.01	10.60 ± 1.19	12.84 ± 2.84	200.60 ± 54.93	3.22 ± 2.48	126.80 ± 8.23	17.60 ± 8.17	43.74 ± 9.01
0.1	09.57 ± 1.19	12.46 ± 2.47	253.80 ± 132.31	2.81 ± 2.65	123.80 ± 20.27	16.20 ± 4.66	50.32 ± 3.13
1	11.23 ± 1.54	13.68 ± 2.59	194.40 ± 59.45	3.50 ± 3.01	128.40 ± 27.65	14.60 ± 2.70	52.81 ± 7.97
10	10.87 ± 0.46	12.62 ± 3.36	238.40 ± 72.46	2.17 ± 1.96	133.00 ± 16.91	16.20 ±5.81	49.68 ± 4.50
100	12.01 ± 2.04	11.30 ± 2.63	222.20 ± 93.44	1.85 ± 1.55	113.40 ± 5.32	13.20 ± 2.49	48.88 ± 4.49

In vitro antiviral assay

Cytotoxicity

Decoctions of galls from *G. senegalensis* were tested for toxicity in CES cells. After 72 h of exposure of cells with concentrations ranging from 0.49–125 µg/mℓ, cell viability was determined using the MTT assay. Decoctions from *G. senegalensis* galls did not reduce the OD_{570} value for drug treated cells significantly at a concentration of 62.5 µg/mℓ or less, as compared with the control (Fig. 1). The CCy_{50} value for CES cells was 90 µg/mℓ.

CPE inhibition

CPE reduction assays were performed to evaluate the inhibitory effect of decoctions from galls of *G. senegalensis* on FPV. The EC_{50} value was 15.6 µg/mℓ after 4 days (Fig. 2) with a SI value of 5.8.

Effect of gall decoctions on chickens

Clinical observations

No deaths or signs of weakness were recorded in any of the control or treated groups either during the 14-day treatment period or after a 3-month observation period, even at the highest treatment dose of 100 mg/kg.

Body mass gain was recorded after 7 and 14 days of continuous treatment and after 3 months of observations for each group (Table 1). The mean values were compared between groups using the One Way ANOVA test. No statistically significant differences were found ($P > 0.05$).

Blood chemistry

Serum glucose, calcium, uric acid, triglycerides, SGOT, SGPT and protein levels were determined after 14 days and again after 3 months after the last treatment (Tables 2 and 3).

Glucose levels showed an increasing tendency for concentrations up to 0.01 mg/kg in the treatment groups in comparison with the control group. Uric acid levels were relatively higher in the treatment groups while the SGOT levels were lower in all the treated birds as compared to those in the control group. There was no important change in the levels of calcium, triglycerides, SGPT and total proteins after 14 days of treatment.

The One Way ANOVA test, which was used to statistically compare mean values, did not indicate significant differences between the groups after 14 days ($P > 0.05$). Three months after the last treatment, serum uric acid levels appeared to show an increase while triglyceride and SGOP level showed a decrease. The SGOP levels in birds treated with 100 mg/kg of galls extracts were significantly lower than the control when compared with One Way ANOVA test followed by the Student-Newman-Keul test ($P < 0.05$).

DISCUSSION

The main objectives of this work were (i) to determine the phytochemical composition of decoctions from galls of *G. senegalensis*; (ii) to evaluate their *in vitro* antiviral activity against fowlpox virus; and (iii) to determine their *in vivo* effect on chickens after oral administration. We were able to demonstrate the presence of saponins, phenolic compounds and steroids including cardiac glycosides. Although the presence of phenolic compounds in the galls (Bouchet *et al.* 1996), indole alkaloids and saponins in the leaves and the roots (Nacoulma 1996) have been described in this plant, we are unaware of any reports describing the presence of cardiac glycosides.

The antiviral evaluation gave an EC_{50} value of 15.6 µg/mℓ which is below the CCy_{50} value obtained (90 µg/mℓ). The toxic effects of aqueous decoctions are often attributed to tannins. The findings of our screening of aqueous decoctions, as well as those of others have shown that such decoctions, especially from the galls of *G. senegalensis*, are very rich in tannins. In such cases, one would expect a higher selectivity index after further purification of the aqueous decoction.

Our *in vivo* studies indicated that no undesirable clinical affects could be induced in chickens even after administration of oral dosages of up to 100 mg/kg of gall decoctions. A similar non-toxic effect was reported in mice after oral dosing with such aqueous decoctions from the leaves and the roots (Pousset 1989). The absence of any deleterious effects on body mass suggests that these aqueous decoctions do not interfere with nutrient intake or metabolism. A loss in body mass is known to often occur following intoxication (Kubena, Edrington, Harvey, Buckley, Phillips, Rottinghaus & Casper 1997).

Various blood parameters can be used as indicators of the general physiological status of birds (Rath, Balog, Huff, Huff, Kulkarni & Tierce 1999). Changes in serum chemistry also often occur fol-

lowing intoxications (Kubena *et al.* 1997) with e.g. aflatoxins or with poor nutrition (Okotie-Eboh, Kubena, Chinnah & Bailey 1997; Shapiro, Mahagna & Nir 1997; Casado, Balbontin & Ferrer 2002). Treatment of chickens with our decoctions did not induce any statistically significant differences in serum glucose, triglyceride, uric acid or protein levels between the tested groups and the control group ($P > 0.05$), suggesting that extracts from *G. senegalensis* do not cause metabolic interference in chickens. The normal levels of uric acid suggest that no deleterious effect on glomerular filtration and thus renal function occurred (Shapiro *et al*. 1997).

Although SGOT and SGPT levels did not change significantly, except at a dose of 100 mg/kg for 14 days where there was a significant decrease in SGOT levels (Tables 2 and 3) after 3 months. This was, however, not at a level indicative of liver pathology because the mean value obtained viz. 113.40 IU/ℓ, is still within the normal SGOT range of 88–208 IU/ℓ for chickens (Fontaine 1987). Increases in SGOT and SGTP levels are common findings in animals suffering from liver disorders, e.g. hepatocellular necrosis (Casado *et al*. 2002; Shanker, Pathak, Trivedi, Chansuria & Pandey 2002). A marked excess in SGOT also occurs after myocardial infarction (Shanker *et al*. 2002). This often happens following fumonisin intoxication (Kubena *et al*. 1997). According to our trials, decoctions from the galls of *G. senegalensis* do not appear to induce hepatic or cardiac lesions in chickens.

It is concluded that decoctions from galls of *G. senegalensis* contain most of the families of molecules previously described following general chemical screening of the plant (Bouchet *et al.* 1996; Nacoulma 1996) and that the gall extracts possess antiviral activity against FPV. In addition, we could not detect toxic effects even in chickens dosed with high concentrations of decoctions. These results will further facilitate investigation into the antiviral activity of these decoctions against fowlpox virus. The decoctions from galls of *G. senegalensis* are used in folk medicine to treat fowlpox virus infections**.** Our results provide a scientific basis for this ethnoveterinary use.

ACKNOWLEDGEMENTS

This work was supported by the International Atomic Energy Agency Technical cooperation project BKF/5002. The authors thank Prof. G.J. Viljoen for providing the fowlpox virus vaccine strain and his continued interest and support, as well as Dr M. Romito, Onderstepoort Veterinary Institute for critically reading and revising the manuscript. In addition, we thank Mrs J. Mans for contributing to the antiviral evaluations. We are also grateful to the head of the Laboratoire National d'Elevage and all the staff who participated in the chickens trials, as well as to Dr A. Bado for allowing us to initiate this work.

REFERENCES

BOUCHET, N., LEVESQUE, J., BLOND, A., BODO, B. & POUSSET, J.L. 1996. 1,3- di-O-galloylquinic acid from *Guiera senegalenis. Phytochemistry*, 42:189–190.

CASADO, E., BALBONTIN, J. & FERRER, M. 2002. Plasma chemistry in a booted eagle (*Hieraëtus pennatus*) during the breeding season. *Comparative Biochemistry and Physiology. Part A: Molecular & Integrated Physiology,* 131:233–241.

CIULEI, I. 1982. *Methodology for analysis of vegetable drugs*. Bucharest: Ministry of Chemical Industry.

FONTAINE, M. 1987. *Vade-mecum du vétérinaire: formulaire vétérinaire de pharmacologie, de thérapeutique et d'hygiène,* XV édition. Paris: Vigot

KUBENA, L.F., EDRINGTON, T.S., HARVEY, R.B., BUCKLEY, S.A., PHILLIPS, T.D., ROTTINGHAUS, G.E. & CASPER, H.H. 1997. Individual and combined effects of fumonisin B1 present in *Fusarium moniliforme* culture material and T-2 toxin or deoxynivalenol in broiler chicks. *Poultry Science,* 76: 1239–1247.

MAHMOOD, N., MOORE, P.S., DE TOMMASI, N., DE SIMONE, F., COLMAN, S., HAY, A.J. & PIZZA, C. 1993. Inhibition of HIV infection by caffeoylquinic acid derivatives. *Antiviral Chemistry and Chemotherapy,* 4:235–240.

MERCK, E. 1975*. Révélateurs pour la chromatographie en couches minces et sur papier*. Frankfurt: Waltherdruck.

MOSMANN, T. 1983. Rapid colorimetric assay for cellular grow and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65:55–63.

NACOULMA, O.G. 1996. Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso—Cas du plateau central. Tome 2. Ph.D. thesis, University of Ouagadougou.

OKOTIE-EBOH, G.O., KUBENA, L.F., CHINNAH, A.D. & BAILEY, C.A. 1997. Effects of beta-carotene and canthaxanthin on aflatoxicosis in broilers. *Poultry Sci*ence, 76:1337–1341.

POUSSET, J.L. 1989. *Plantes médicinales africaines: Utilisation pratiques*. Paris: Ellipses-ACCT.

RAMADE, F. 1993. *Dictionnaire encyclopédique de l'écologie et des sciences de l'environnement.* Paris: Ediscience Internationale.

RATH, N.C., BALOG, J.M., HUFF, W.E., HUFF, G.R., KULKARNI, G.B., TIERCE, J.F. 1999. Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. *Poultry Science,* 78:1232–1239.

RIVELLAND, P., VIÉ, K., COIFFARD, L. & DE ROECK-HOLTZHAUER, Y. 1994. Cytotoxicity tests of antibacterial agents on human fibroblasts cultures. *Pharmaceutica Acta Helvetiae,* 69:159–162.

SERKEDJIEVA, J. & ALAN, J. H. 1998. *In vitro* anti-influenza virus activity of a plant preparation from *Geranium sanguineum* L. *Antiviral Research,* 37:121–130.

SHANKER, K., PATHAK, N.K., TRIVEDI, V.P., CHANSURIA, J.P. & PANDEY, V.B. 2002. An evaluation of toxicity of *Taxus baccata* Linn. (Talispatra) in experimental animals. *Journal of Ethnopharmacology,* 79:69–73.

SHAPIRO, F., MAHAGNA, M. & NIR, I. 1997. Stunting syndrome in broilers: effect of glucose or maltose supplementation on digestive organs, intestinal disaccharidases, and some blood metabolites. *Poultry Science,* 76:369–380.

SILIM, A., EL AZHARY, A.S.Y. & ROY, R.S. 1981. A simple technique for preparation of chicken-embryo-skin cell cultures. *Avian Diseases,* 26:182–185.

SILVA, O. & GOMES, E.T. 2003. Guieranone A, a naphthyl butenone from the leaves of *Guiera senegalensis* with antifungal activity. *Journal of Natural Products*, 66:447–449.

SOFOWORA, A. 1996. *Plantes médicinales et médecine traditionnelle d'Afrique,* Paris: Khartala.

VAN DEN BERGHE, D.A., VLIETINCK, A.J. & VAN HOOF, L. 1986. Plant products as potential antiviral agents. *Bulletin Institut Pasteur*, 84:101–147.

WAGNER, H. & BLADT, S. 1996. *Plant drug analysis: a thin layer chromatography atlas*, 2nd ed. New York: Springer.