Int.J.Curr.Res.Aca.Rev.2020; 8(11): 47-53

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 8 Number 11 (November-2020)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2020.811.006

Invitro and *Invivo* Trypanocidal Effect of the Aqueous Extract of the Leaves of *Ochna schweinfurthiana*

S. Eteme Enama[1]*, A. N. Messi[2], R. J. Mahob[1], Augustin Siama[3], Yede[1] and A. M. Njan Nloga[4]

[1]*Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, Cameroon*
[2]*Department of Organic Chemistry, Faculty of Science, University of Yaounde I, Cameroon*
[3]*Department of Parasitology and parasitic pathology, School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon*
[4]*Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Cameroon*

**Corresponding author*

Abstract

The objective of this study was to evaluate the trypanocidal effect of the aqueous extract of *Ochna schweinfurthiana* leaves*in vitro* and *in vivo* against two trypanosome species:*Trypanosoma brucei brucei* and *Trypanosoma congolense*. The search for chemical families by screening and evaluating the trypanocidal effects of the extract *in vivo* in white mice (50, 100, 200 and 400 mg / kg) and *in vitro* (0.25;0.5;0.75 and 1 mg/kg). Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids and saponins. The doses of the extract of this plant resulted in a decreased motility of the two species of parasites *in vitro.* For the highest doses (0.5;0.75 and 1 mg/kg), the decrease is either significant ($p<0.01$) or highly significant ($p<0.001$). The activity of the extract (400 mg/kg) highly inhibited the parasite densities ($p<0.001$) compared to the negative control. Whereas the doses of 100 and 200 mg/kg did not significantly inhibit the parasite densities. The results recorded show an improvement in the hematocrit rate in the animals treated at different doses of the extract. This reflects a general decrease in anemia.

Article Info

Accepted: 08 October 2020
Available Online: 20 November 2020

Keywords

Trypanosoma brucei brucei, *Trypanosoma congolense*, *Ochna schweinfurthiana* leaves, trypanocidal effect

Introduction

African trypanosomosis is a parasitic disease caused by a blood protozoan, of the genus *Trypanosoma*. There are hundreds of species of trypanosomes in Africa and America. In Africa, this parasite causes the death of humans (OMS, 2014) and animals (FAO, 2014). According to the FAO, Africa loses more than 600 billion FCFA each year due to this parasitosis. This loss concerns cattle and buffaloes in general. The disease reduces the activity of these animals and decreases their ability to reproduce. Faced with this emergency, solutions are used to eliminate this scourge; they aim to eliminate the vector by the use of traps and screens impregnated with molecules, and also the parasite in the host by treatments with suitable molecules (diminazeneaceturate). These products are unfortunately used for several years and thus induce the resistance of trypanosomes. To improve the fight against trypanosomosis, it is therefore important to find new effective, less toxic and less expensive treatments. Under the auspices of WHO, several researchers around the

world have been moving towards the discovery of trypanocidal plant extracts (Hoet *et al.*, 2003; Bizimana *et al.*, 2006; Atawodi and Alafiatayo, 2007; Osho and Lajide, 2014).The Ochnaceae are trees, shrubs and some herbaceous plants, evergreen, subtropical to tropical. It is a family of dicotyledonous plants comprising 300 to 500 species belonging to 28 genera. The genus *Ochna* has about eighty species. *Ochna schweinfurthiana* is used in traditional medicine for treating intestinal worms, malaria, asthma, epilepsy, gastric problems, painful periods, snake bites, measles, typhoid fever, skin infections, toothache (Messi *et al.*, 2016). This herbal medicine concerns either total extracts of plants or fractionations. We proposed to assess the *in vitro* and *in vivo* trypanocidal activities of the aqueous extract of the leaves of *Ochna schweinfurthiana* in white mice.

Materials and Methods

Harvesting and conditioning of samples

The harvest of the leaves of *O.schweinfurthiana* took place in July 2014 in Mbakaou, a locality in the Adamaoua- Cameroon Region, near Mbam and Djerem National Park. *O.schweinfurthiana* has been identified at the National Herbarium of Yaounde (Cameroon) under the number N °40171HNC. The leaves were washed in tap water and dried in the shade. Then, using a mixer, the powder was obtained, sieved and stored in a glass jar.

Preparation of the aqueous extract

The leaves of *O.schweinfurthiana* were cut into small sizes with knife and air-dried on clean tarpaulins for one week at room temperature and weighed. The sufficiently dried leaves was coarsely ground in an electrical blender. The resulting powder (400 mg) was extracted 3 times with 5 L of water using the Biobase lyophilizer to yield a crude extract of 120 g. Then, 400 mg of this powder was taken and introduced into 10 ml of distilled water. A solution of 40 mg/ml concentration constituting the initial solution was administered at a volume of 10 ml/kg of body weight to the animals. This constituted a dose of 400mg/kg body weight. From this concentration, the dilutions were made with distilled water. Thus, the different doses obtained are: 200 mg/kg; 10 mg/kg; 50 mg/kg for *in vivo* tests. The same procedure were repeated to obtain 1mg/kg for the initial dose and then0.75mg/kg;0.5mg/kg;0.25mg/kg for the other doses of *in vitro* tests.

Phytochemical Screening of aqueous extract of *Ochna schweinfurthiana*

The aqueous extract of leaves of *Ochna schweinfurthiana* have been carried out in order to determine the presence of phenols, flavonoids, tannins, steroids, saponins, alkaloids and triterpenes using standard methods (Safowora, 1996).

Trypanosome

Two strains of trypanosomes were used. The first one coming from the laboratory of the university of Buea-Cameroon, *Trypanosoma bruceibrucei*. The second strain, *Trypanosoma congolense*, was collected from a cattle infested in Yoko-Cameroon. Following a blood sample for the determination of the prevalence of trypanosomoses in this locality, we were able to identify the parasite with a very high density using stained smears, and inoculated this blood in three white mice.

In vitro test

For *in vitro* studies, the stabilates were rapidly thawed in a water bath at 37°C and then cultured in 24-well plates in the minimum essential medium with Earles salts (Gibco). This medium had been previously supplemented with 2 mM L- glutamine (Gibco), 25 mM HEPES buffer (Gibco), 0.2% glucose (Sigma), 2% sodium pyruvate (Gibco) mM, penicillin to (Gibco) 150 IU/L, thymidine to 0.01 mM (Sigma), and hypoxanthine to 0.1 mM (Sigma). At the time of use, supplemented minimum essential medium was supplemented with 0.05 mM bathocuproine sulfate (Sigma), 1.5 mM L-cysteine (Sigma), 0.12 mM 2-mercaptoethanol (Sigma), and 20% (v/v) horse serum (Gibco). Before their use *in vitro*, the parasites were left in an oven at 37°C, with 5% CO_2 during an adaptation period of 14 days.

Infestation of animals

Blood collected by cardiac puncture using syringes containing EDTA in infested mice was immediately diluted with saline to serve as an inoculum. Subsequently, healthy mice were infested intraperitoneally with 0.02 ml of diluted blood containing an average of 1×10^6 trypanosomes.

In vivo test

The white mouse 22-28 g were used. They came from the University of Yaounde I. They were acclimatized for

three days before the tests in the university Buea. During this period, they received food and water *ad libitum*.

During the experiment, 72 mice were used, in which6 white mice per group. So, we had :

Negative control group consisting of infested animals receiving distilled water (DW);

Positive control group consisting of infested animals receiving Berenil (BE);

Test group receiving a dose of 50 mg/kg of extract (O - 50);

Test group receiving a dose of 100 mg/kg of extract (O - 100);

Test group receiving a dose of 200 mg/kg of extract (O - 200);

Test group receiving a dose of 400 mg/kg of extract (O-400).

In this experiment, the principles set out in the guide to the use of laboratory animals in relation to the conservation and use of these mice were respected (CCAC, 1993).

Determination of parasitaemia

Parasitaemia was determined 48 h after infestation. Therefore, we began to administer the different doses of aqueous extract (50mg/kg, 100mg/kg, 200 mg/kg, and 400mg/kg), the standard drug what Berenil (positive control) and the last group was not treated and received distilled water (negative control). The Herbert and Lumsden method allowed us to determine the level of parasites in the blood. It consisted of collecting blood from the previously sterilized tail of each mouse and observing the microscope preparation at x400 magnification (Herbert and Lumsden, 1976). Thus, we observed parasites by fields directly in the blood. Parasite rates were compared to the 14^{th} day post-treatment. The trypanocidal effect of parasitaemia doses of the extract was compared with those of the control groups (Maikai, 2011).

Hematocrit level measurements

Hematocrit is used to predict the effectiveness of the extract in preventing hemolysis resulting from the increase of parasitaemia. Heparinized tubes were used to collect the blood coming to the tail vein incision of each mouse. The hematocrit evaluation was performed at day 0, day 7 and day 14.

Evolution of body weight and temperature

The body weight and temperature readings of each mouse were measured, using a sensitive balance and a rectal thermometer, before infection and daily during treatment.

Statistical analysis

All data were analyzed by variance analysis (ANOVA) with 5% significance.

Results and Discussions

Phytochemical Screening

Screening revealed the presence in the leaves of flavonoids (++), tannins (++), steroids (+), saponins (++), alkaloids (+) and triterpenes (+).

In vitro activity of the aqueous extract of *Ochna*leaves*schweinfurthiana*

The *in vitro* activity of the aqueous extract of the leaves of *O.schweinfurthiana* against *T. bruceibrucei* and *T. congolense* shows significant variations with time and species of parasites (Figures 1 and 2). In one hour (60 min), the different doses 0.5 mg/kg, 0.75 mg/kg and 1 mg/kg of aqueous extract administered reduced the frequency of the two species with respect to the distilled water ($p < 0.05$). This was not the case for the 0.25mg/kg dose of the aqueous extract of the leaves of *Ochna schweinfurthiana* and distilled water ($p > 0.05$). The reference molecule used completely reduced the frequency of the two trypanosomal species from the 55^{th} minute. We find through these figures that no dose has totally reduced the motility of these species even if the only significant difference observed is between Berenil and the dose 0.25mg/kg at T60 ($p < 0.05$).

In vivo activity of the aqueous extract of *Ochna schweinfurthiana*

Figures 3 and 4 showed respectively *in vivo* trypanocidal activities of the different doses of the aqueous extract on *T.congolense* and *T. brucei brucei* of *Ochna schweinfurthiana*. This shows that the extract decreased

in dose dependent manner the level of *T. brucei brucei* (Figure 3) and *T. congolense* (Figure 4) compared to distilled water treated animals. The Berenil, a reference tryponocide drugs, significantly decreased the level of these parasites compared to distilled water treated mice.

Effect on hematocrit

Figure 5 shows the effects of different doses of the aqueous extract of *Ochna schweinfurthiana* on the hematocrit rate in mice infested with *T. bruceibrucei*. This shows that on day 0, there is no significant difference between the different groups of mice. On day 7 during treatment, mice receiving distilled water (ED) showed a similar hematocrit in mice receiving the dose 50, 100 and 200 mg/kg. The hematocrit of mice treated with the Berenil was different from that of mice treated at a dose 50m/kg. At the last day of treatment (day 14), the hematocrit levels of the mice receiving the Berenil were statistically different from the rate of the mice receiving distilled water, the 50mg / kg dose and the 200mg / kg dose. Between the doses 100 and 200mg/kg, 200 and 400mg/kg there was no significant difference.

Figure 6 shows the effects of different doses of the aqueous extract of *Ochna schweinfurthiana* on the hematocrit rate in mice infected with *T.congolense*. It appears that at day 0 (D0), that is to say before treatment, there is no significant difference between the different mice of the batches. On D7 during treatment, the mice receiving distilled water (ED) had a hematocrit level only similar to that of the 50mg / kg dose. The doses 100, 200 and 400mg / kg had no significant difference. The hematocrit level of Berenil was different from that of all other lots. On the last day of treatment (Day 14), the hematocrit levels of Berenil and the 400mg/kg dose were statistically similar. The same observation was made between doses of 100 and 200 mg/kg.

Fig.1 *In vitro* trypanocidal activity of the aqueous extract on *T.bruceibrucei* motility

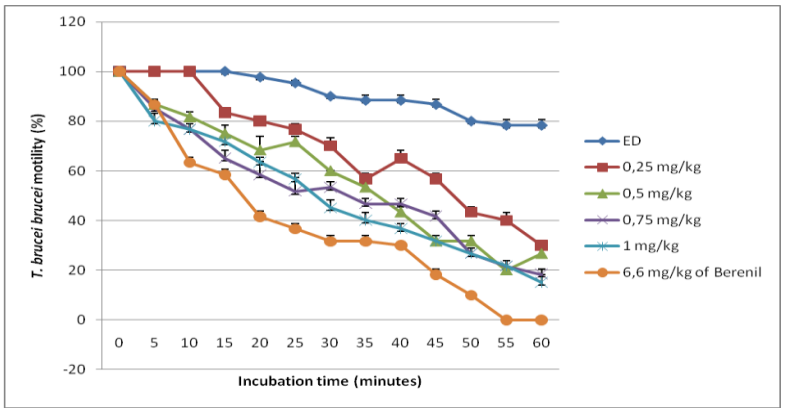


Fig.2 *In vitro* trypanocidal activity of the aqueous extract on *T.congolense* motility

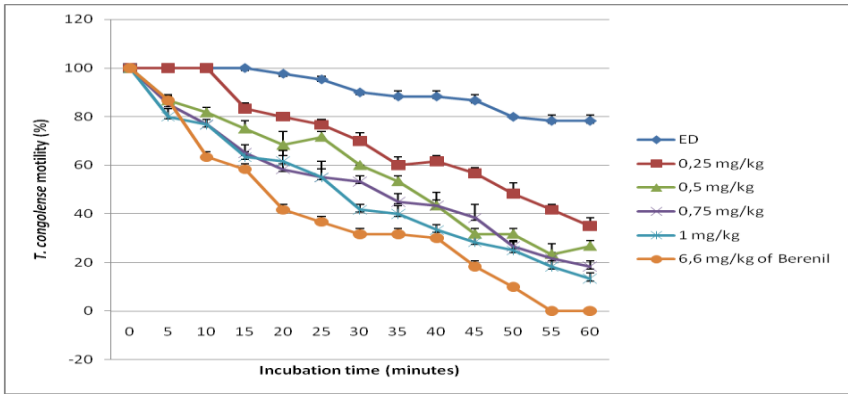


Fig.3 *In vivo* trypanocidal activity of the aqueous extract on *T.bruceibrucei* motility

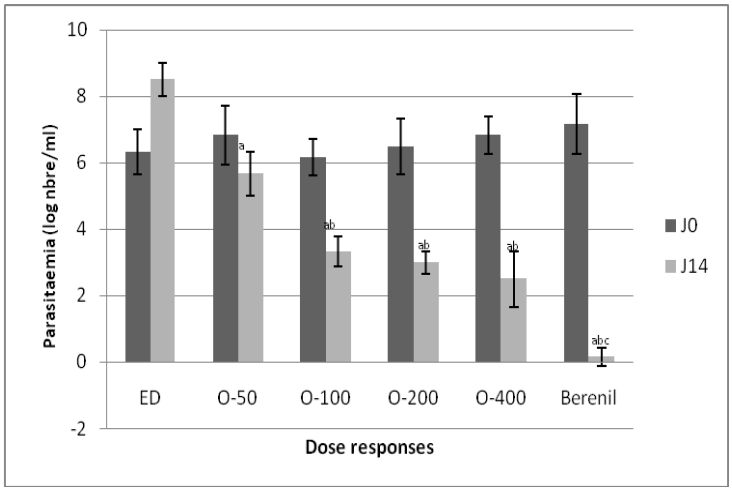


Fig.4 *In vivo* trypanocidal activity of the aqueous extract on *T.congolense* motility

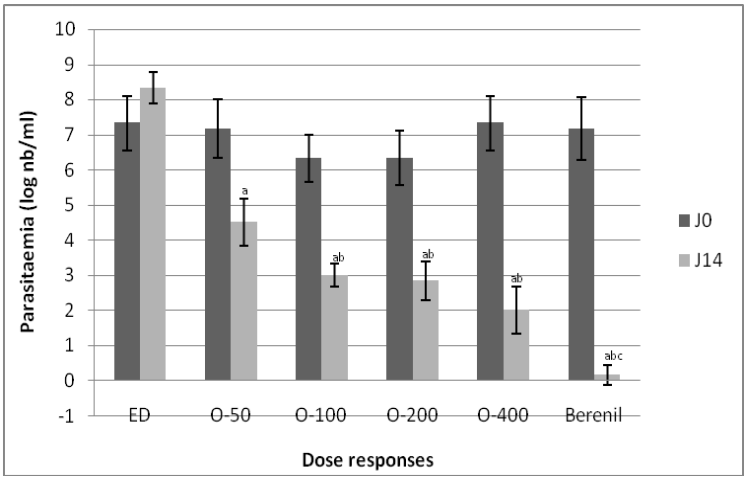


Fig.5 Effects of different doses of the aqueous extract of *Ochna schweinfurthiana* on the hematocrit rate in mice infested with *T. bruceibrucei*

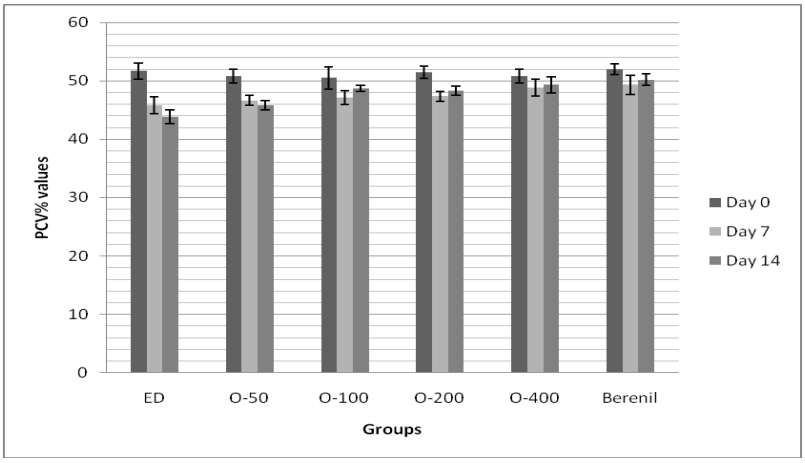
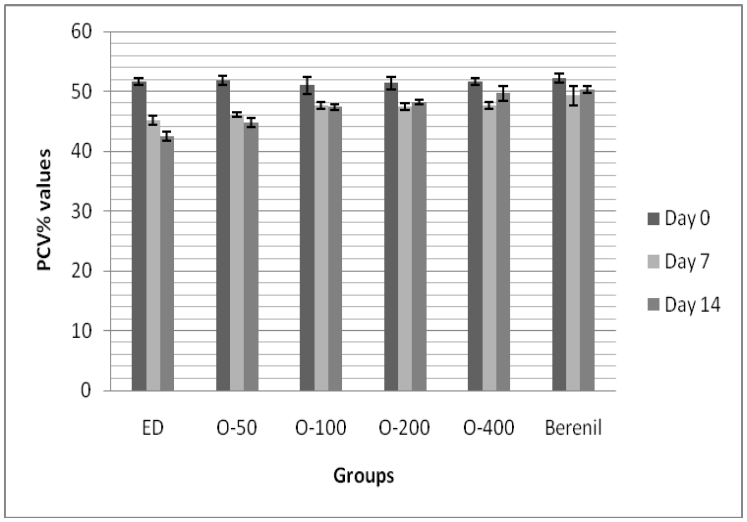


Fig.6 Effects of different doses of the aqueous extract of *Ochna schweinfurthiana* on the hematocrit rate in mice infected with *T.congolense*



Medicinal plants have always been an important source of medicines (Soh and Benoit-Vical, 2007) for both traditional medicine and for discoveries of new molecules and pharmaceutical industries (Elujoba *et al.*, 2005). The search for new trypanocidal molecules from plants involves the screening of a large number of medicinal plants from different geographical regions where local populations use them. In this study where we have preserved the formulation used by traditional therapists. The screening of the plant revealed the presence of tannins, saponins and alkaloids that may be very active compounds against parasites. Despite the difference in susceptibility of trypanosome species (Wurocheke *et al.*, 2014), the decrease in observed motility was similar in both species of trypanosomes. The *in vivo* study shows a reduction of the parasitaemia of on different doses of the aqueous extract of the *T. congolense* and *T.bruceibrucei* infested mice. Indeed, studies of this plant have shown that it had antiplasmodial and antioxidant activities (Messi *et al.*, 2016, Nyegue *et al.*, 2016).

The results obtained in this study on the aqueous extract of the leaves of the *Ochna* plant *schweinfurthiana* have shown that the different doses affect the motility of the two species of parasites and the reduction of parasitaemia. The extract also has an effect on anemia by increasing the hematocrit in mice.

Conflict of interest

No conflict of interest

References

Atawodi S.E and Alafiatayo AA, 2007. Assessment of the phytochemical and antitrypanosomal properties of some extracts of leaves, stem and root bark of *Landolphia* sp., P. Beauv. J Ethnopharmacol 114: 207-211.

Bizimana N., Tietjen U., Zessin K., Diallo D., Djibril C., Melzig MF, Clausen P., 2006. Evaluation of medicinal plants from Mali for their in vitro and in vivo trypanocidal activity. J Ethnopharmacol 103: 350-356.

Elujoba AA, Odeleye OM & Ogunyemi, 2005. Traditional medicine development for medical and dental health care delivery systems in Africa. *Afr.J. Trad.CAM*, 21: 46-61.

FAO, 2014.Food and Agriculture Organization of the United Nations, FAOSTAT, data, Live animals, consulté le 27-12-16. http://www.fao.org/faostat/en/#data/QA.

Herbert W.J. and Lumsden W.H., 1976.*Trypanosoma brucei*. A rapid matching method for estimating the host parasitemia. ExpParasitol. 40: 427-31.

Hoet S., Oppereoes F., Brun R.andQuetin-Lalercq J.,2003. Natural products active against African trypanosomes; a step towards new drugs. Nat. Prod. Rep. 21: 353-364.

Maikai V.A.,2011. Anti-trypanosomal activity of flavonoid extracted from *Ximenia americana* stem bark. IJB. 3: 115-21.

MessiAN, Ngo MbingJ., NdongoJT, NyegueMA, Tiabou ChindaA., LadohYemedaF., Michel Frédérich, PegnyembDE, 2016. Phenolic compounds from the

roots of *Ochna schweinfurthiana* and their antioxidant and antiplasmodial activities. *Phytochemistry Letters* 17; 119-125.

Nyegue MA, Ngo MbingJ., Djova1 SV, Messi AN, VoundiOluguS. Pegnyemb DE and EtoaFX, 2016. Evaluation of the antioxidant activity of the leaves, stem-bark extracts and fractions of *Ochna schweinfurthiana* F.Hoffm (Ochnaceae). Flight. 10 (17), pp. 370-378.

OMS, 2014.Trypanosomiase humaine africaine: lutte et surveillance. Rapport d'un comité d'expert de l'OMS. *Série de rapports techniques de l'OMS, 984*, 270p.

Osho IB and Lajide L., 2014. Antitrypanosomal activity of *Aristolochia ringens* against *Trypanosoma congolense* infection in mice. *Journal of Pharmacognosy andPytherapy*, Vol 6 (1), pp 1-3.

Sofowora A., 1996. Medicinal plants and traditional medicine in Africa. *J Altern Complement Med* 2: 365-372.

Soh PN and Benoit-Vical F.,2007.Are West African plants a source of future antimalarial drugs?*Journal of Ethnopharmacology*,Vol.114.2, P.130-140.

Wurochekke Abdullahi U.,Bello H.,Yusuf H.B. MahmoudandS.J., 2014. un-Nitro Trypanocidal Activity of Aqueous Stem Bark of *Haematostaphis barteri* contre Trypanosoma congolense and *Trypanosoma bruceibrucei*. *Sch.Acad.J. Pharm.*, 2014; 3 (1): 38-42.

How to cite this article:

Eteme Enama, S., A. N. Messi, R. J. Mahob' Augustin Siama, Yede and Njan Nloga, A. M. 2020. *In vitro* and *In vivo* Trypanocidal Effect of the Aqueous Extract of the Leaves of *Ochna schweinfurthiana*. *Int.J.Curr.Res.Aca.Rev.* 8(11), 47-53. doi: https://doi.org/10.20546/ijcrar.2020.811.006