Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2013, 3(6):53-59

ISSN : 2249-7412
CODEN (USA): AJPSKY

Hemolytic effect of total alkaloids from the seeds of *Peganum harmala in vitro* on erythrocytes of ruminants: Sheep, cattle and goats

Mahdeb N.[1*], Mayouf S.[1], Boukhari F.[2], Souilah S.[2] and Bouzidi A.[2]

[1]*Department of Agronomy, University of Setif, Faculty of Natural and Life Sciences, Setif, Algeria*
[2]*Department of Biochemistry, University of Setif, Faculty of Natural and Life Sciences, Setif, Algeria*

ABSTRACT

Peganum harmala has-been used in traditional medicine, but remains a poisonous plant for humans and animals. This plantis a rich source of ß-carboline alkaloids, which constitute the majority of alkaloids of Peganum harmala. The present work investigated in vitro, the hemolytic effect of alkaloids from the seeds of Peganum harmala on erythrocytes ruminant animals. The doses tested (0.22 to7.41mg/ml) showed a hemolytic effect on erythrocytes of goats, cattle and sheep. With the dose 7.41mg/ml, the erythrocytes female goats are the most sensitive with hemolysis rate 43.54% in female's species. In male animals, erythrocytes sheep were the most sensitive, with a rate of hemolysis 35.80%. Peganum harmala alkaloids are capable of disrupting the permeability of the membranes of red blood cells of ruminants.

Key words: Erythrocyte, hemolysis, alkaloids, *Peganum harmala*, Ruminant animals.

INTRODUCTION

The Zygophyllaceae plant, *Peganum harmala* is locally known as "harmel" and widely prevalent in the semi-arid climate territories in North Africa. It is also called Syrian rue, African-rue, wild rue [1; 2; 3]. Pharmacologically active principles of the plant *Peganum harmala* are β-carboline alkaloid and quinazoline. The β-carboline alkaloid is the major alkaloid of this plant which are harmaline, harmine, harmol and harmalol[4; 5; 3]. β-carboline alkaloid harmine, harmaline and tetrahydroharmine can stimulate the central nervous system by inhibiting the metabolism of amine neurotransmitters, or by direct interaction with specific receptors [6].The plantis used in traditional medicine in Central Asia, North Africa and the Middle East [7;8;9; 10], in order to treat many diseases or use for abortion[11]. The plant is also used in a hallucinogen goal and cases of *Peganum harmala* poisoning are reported[2;6]

Experiments have demonstrated the insecticidal effect[12; 13], the antibacterial effect, and the antioxidant effect of the plant *Peganum harmala*[14]. The *Peganum harmala* has applications for treating certain diseases in livestock or domestic animals[15;16;17]. *Peganum harmala* remains a toxic plant for domestic animals, especially in times of drought andin areas with semi-arid climate [2].

The objective of this work is to test in vitro the effect of total alkaloids from the seeds of *Peganum harmala* on erythrocytes of blood of goats, sheep and cattle.

MATERIALS AND METHODS

Plant material

The seeds of the plant *Peganum harmala* are purchased from an herbalist in the city market A in Azel (characterized by a semi-arid dry climate), located 50 km southwest of the town of Setif (East- Algeria). According to herbalist, seeds are harvested in this region between August and September. The seeds were stored at room in dry place. After drying, the seeds were kept in tightly-closed containers prior to use (fig. 1).

The plant has been identified on the basis of the botanical description [18; 19].

Fig. 1. *Peganum harmala* seeds

Extraction of total alkaloids

Hundred gram of Air dried powdered of seeds was defatted with petroleum ether under reflux and then the seeds were witted with150 ml of NH_4OH (25%, m m^{-1}) for 4 hours and were extracted to exhaustion with $CHCl_3$ using a soxhlet apparatus for 6 h. The organic extract (containing free alkaloids+lipophilic impurities) is then shaken three times with 150 mL aqueous sulphuric acid (2%, m m^{-1}).

The acid extracts (alkaloids salts) are treated three times with 50 ml NH_4OH (25%, m m^{-1}) to pH 10 to liberate the free alkaloids which are separated by extraction with 150 ml CH_2Cl_2 and then dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids [20]. The yield of this extract was approximately 1.2 ± 0.057 %.

Qualitative analysis of total alkaloids of seeds by TLC

Before testing the total alkaloids extracted on the blood of animals, Analytical chromatography was used to verify the presence of alkaloids at least a majority in the extract. TLC plates ready to use, silicagel 60F-Merck brand aluminum Macherey-Nagel support with dimensions 20x20cm used. The mobile phase used was methanol/ chloroform/ammonia: 80/20/1, 5 (V/V/V). The extract is dissolved in 1 ml of methanol.

After dissolution of the sample in methanol, we deposit10µl of the solution (extract) using a micropipette on the plate, previously activated in an oven at110° C for3-5min, 1 cm the lower edge of the baseline. Each deposit is dried with a hair dryer. The plate is then placed in the chamber containing the mobile phase migration. When the solvent front reaches 4cm from the top of the plate (the migration of fifteen cm takes roughly 45 minutes), the chromatograms are removed dried and pulverized with Dragendorff reagent until the appearance of colored spots, according to the slightly modified Kurt method (1971)[21].

Animal material

Samples of blood

The blood is collected from the jugular vein of ruminant animals: sheep, goats and cattle of both sexes, raised on farms in the region of Setif (North-east of Algeria), characterized by a semi-arid climate, in tubes containing of heparin as an anticoagulant. The animals are healthy and females are not gestating. All experimental procedures were conducted in accordance with guide to the care and use of experimental animals.

Treatment of erythrocytes by the total alkaloids of the *Peganum harmala* seeds

Blood was collected, centrifuged at1500 g/ minfor 5min, the supernatant was removed and the pellet was washed three times with(10 mM phosphate Na^+, pH7.4125 mM NaCl) phosphate buffer saline PBS. The last centrifugation lasts 10min [22]. The cell pellet was diluted with PBS to obtain a hematocrit of 2%.

The total alkaloids extracted from the seeds of *Peganum harmala* is dissolved in 80µl of methanol, diluted in phosphate-buffered saline to a concentration of 22.85mg/ml (stock solution). From this stock solution, a series test tube is prepared at various concentrations by dilution with phosphate buffered saline (the range of concentration extract is between 0.22and 7.41mg/ml).

The analysis performed in 96-well micro plates. In each well180µlof erythrocyte suspension prepared above are added. In 6 well was added 100µl of phosphate buffered saline (control), the other wells receive 100µl of total alkaloids extracted from the seeds of *Peganum harmala* with concentrations ranging from 0.22to7.41mg/ ml. The plate is left for 5 minutes at room temperature.

Hemolytic activity was expressed as percentage of the total hemolysis (100%) obtained by lysing erythrocytes in distilled water.

The number of hemolytic cells is determined by measuring the absorbance at 630nm using the plate reader at 96 wells (Elx800 Universal Microsoft Reader, Bio. Tek instrument, INC.) [23].

Statistical analysis

The statistical significance of the differences between means was calculated using one-way ANOVA followed by Tukey's test for multiple comparisons, * $P \leq 0.05$.

RESULTS

The evaluation of the CCM alkaloids of *Peganum harmala* seeds revealed the existence of atleast three distinct spots, which are present in large amounts (Fig. 2).

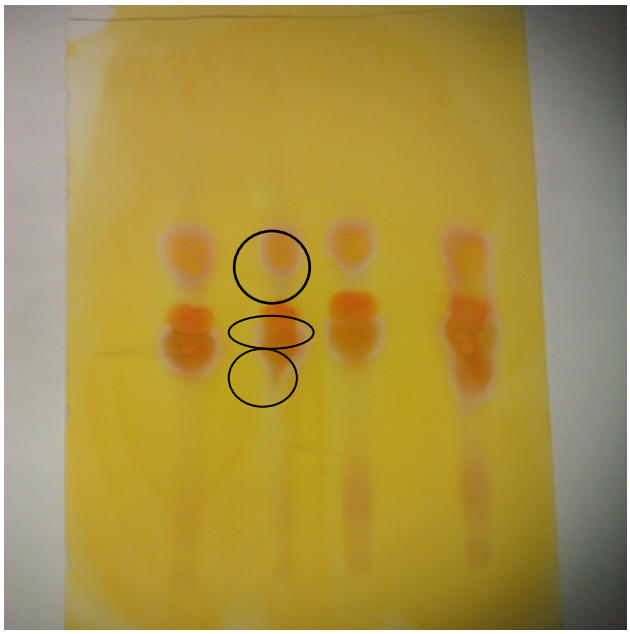

Fig. 2.Thin layer chromatography of alkaloids seeds of *Peganum harmala*

Mobile phase:methanol /chloroform/ammonia / 78.5/20/1.5 (v/ v/ v). Detection of alkaloids was performed by use of Dragendorff's reagent

Incubation of red blood cells of both sexes of ruminants(cattle, sheep and goats) in varying concentrations of total alkaloids extracted from the seeds of *Peganum harmala* plant showed hemolysis depending on the concentration of the extract. The results are shown in figures 3,4,5,6.

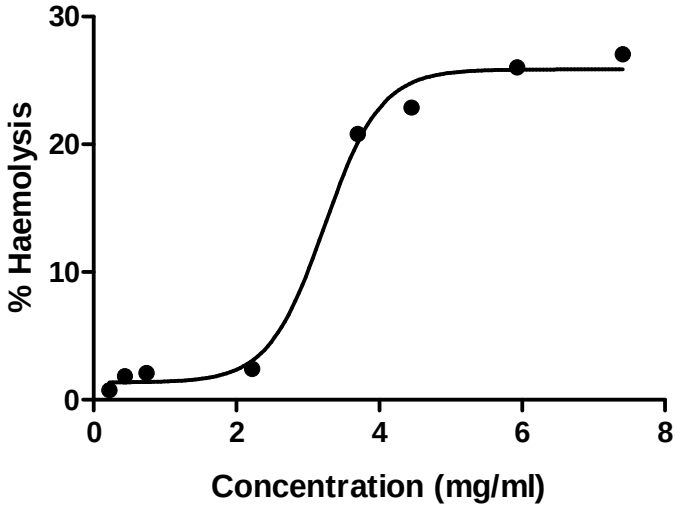

Fig. 3: Hemolytic effect of total alkaloids from the seeds of *Peganum harmala*on erythrocytes of male goats

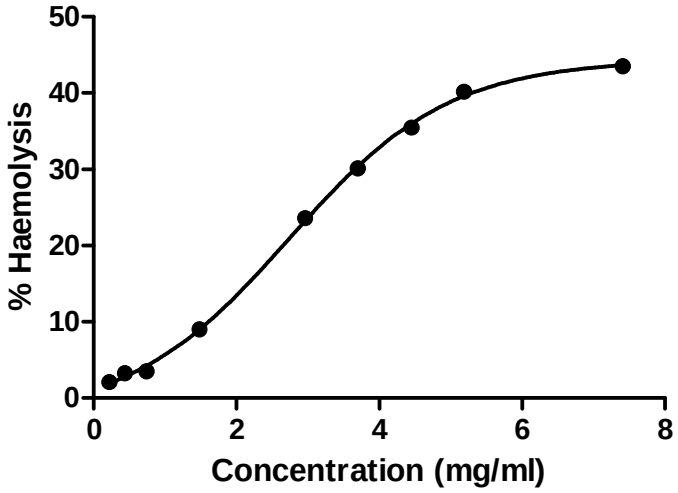

Fig. 4: Hemolytic effect of total alkaloids from the seeds of *Peganum harmala*on erythrocytes of female goats

These figures show a variation of the hemolytic effect of the extract of the total alkaloids of the plant, in the three female species. The hemolytic effect of the extract of *Peganum harmala* alkaloids is more marked on the erythrocytes of goats and it is 43.54% hemolysis, 42.53% and 11.33 % hemolysis respectively in sheep and cattle(Fig. 4,5, 6).

The effect of the extract of total alkaloids of *Peganum harmala* on erythrocytes of three male species recorded percentages 35.80%, 6.07% and 27.07% respectively for sheep, cattle and goats (Fig. 3, 5, 6).

The erythrocytes of the blood of female animals are significantly more sensitive to hemolytic effect of total alkaloids o*f Peganum harmala*(Fig. 3,4,5,6).

The red blood cells of female goats are more sensitive to the hemolytic effect of total alkaloids of *Peganum harmala* that erythrocytes are other species studied. In male animals, erythrocytes sheep are more sensitive to the hemolytic effect of the alkaloids of *Peganum harmala* (Fig. 3,4,5,6).

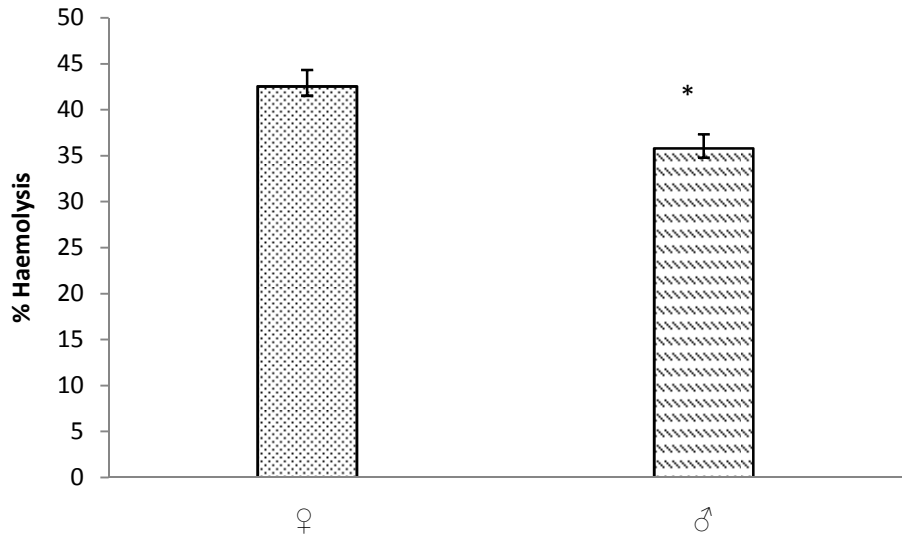

Fig. 5: Hemolytic effect of 7.41mg/ml of total alkaloids from the seeds of *Peganum harmala* on sheep erythrocytes
Values are mean ±S.D.

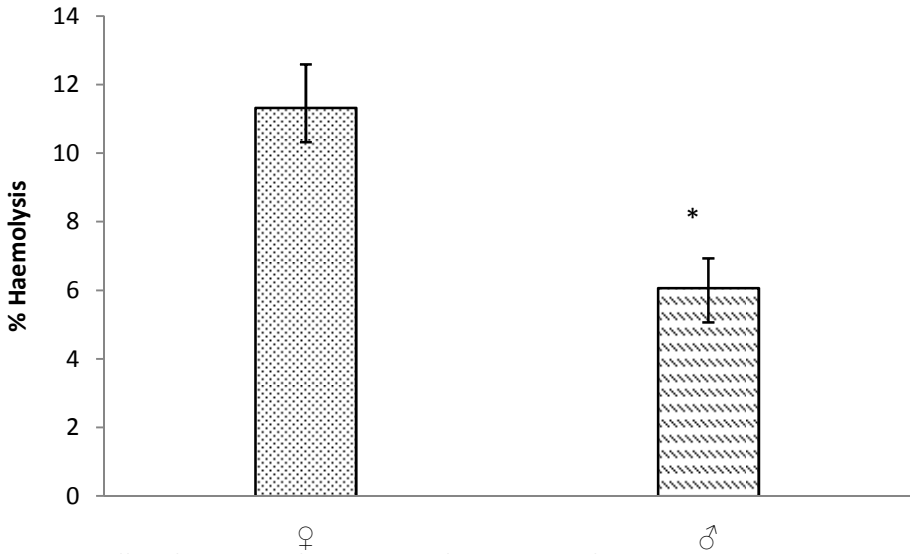

Fig. 6: Hemolytic effect of 7.41mg/ml of total alkaloids from the seeds of *Peganum harmala* on cattle erythrocytes
Values are mean ±S.D.

DISCUSSION

Peganum harmala is a plant that grows in areas of semi-arid and arid climate in North Africa. *Peganum harmala* is reported to have numerous pharmacological activities in the Middle East and North Africa. It has been used in traditional medicines [19; 11].Cases of poisoning by *Peganum harmala* were reported [11;24]. Black and Parker (1936) mentioned that *Peganum harmala* is unpalatability, but it (*Peganum harmala*) is eaten by cattle and sheep only when the animals are in a starving condition.The alkaloids are the most active principles present in the seeds of *Peganum harmala.* Extraction of total alkaloids from the seeds of *Peganum harmala* plant has achieved a high yield, but still a low yield compared to bibliographic data reported by Bukhari *et al.,(* 2008)[25] and Asgarpanah and Ramezanloo(2012). That it could be explained by the use of a different extraction technique and solvents. The evaluation of TLC of alkaloids of seeds of *Peganum harmala* revealed at least three distinct spots. In the absence of alkaloids witnesses, these spots could not be identified with precision; but it is probably the main β-carboline alkaloids plant *Peganum harmala*, which are harmaline, harmine, harmol and harmalol mentioned in the work of Idrissi Hassaniand El Hadek(1999) and Kartal *et al.*, (2003).

Plants comprise the third largest category of poisons known around the world. They form a major part of livestock feed, thus toxicosis in animals consuming these plants can be expected [26]. Toxic plants affect livestock in many ways, with or without clinical symptoms[27]. Red blood cells have a shelf life of 120 days, after which they die by aging and exhaustion of their enzyme stock. We talk about hemolysis is the premature destruction of red blood cells,

when their life is less100 days instead of 120 days, a period of normal time[28]. The relative composition of Fatty acid, phospholipids and cholesterol of red blood cells plasma membrane is inextricably connected with membrane stability and function[29]. It is generally admits that the hemolytic properties are due to their interaction with components of the erythrocyte membrane, causing a disruption of the erythrocyte permeability [30]. The hemolytic effect of total alkaloids of *Peganum harmala* on erythrocytes of the animals studied could probably explained by the interaction of alkaloids with the compounds of the membranes of erythrocytes of animals. Erythrocytes female goats are more sensitive to alkaloids of *Peganum harmala* seeds compared with the erythrocytes of other animals. The hemolytic effect of alkaloids differs from one animal species to another and from one sex to another. These variations may reflect differences in the phospholipids composition of erythrocyte plasma membrane of these species [31]. Finally, our present study is not exhaustive since these alkaloids of *Peganum harmala* may exhibit profound variations under in vivo studies because marked differences of metabolicend products of xenobiotic exist amongst organs and tissues of animals. It can be deduced from this experiment that the alkaloids from the seeds of *Peganum harmala* have a hemolytic effect on erythrocytes of ruminant animals. This is however open for further investigation.

CONCLUSION

The alkaloids of *Peganum harmala* seeds showed a hemolytic effect on erythrocytes of goats, cattle and sheep. Erythrocytes female goats are more susceptible to the hemolytic effect of *Peganum harmala* alkaloids. Studies *in vivo*, must perform to complete this work.

Acknowledgments
This work was supported by Ministry of Education and Scientific Research-Algeria (CNEPRU project N°F0122010014)

REFERENCES

[1] BlackW, Parker K.W, *New Mexico Agric. Exp. Sta. Bull.*,**1936,** 240, 1-14.
[2]Mahmoudian M, Jalilpour H, Salehian P, *Iranian J pharmacolTherap*, **2002**, 1,1-4.
[3]Monsef-Esfahani HR, Faramarzi MA, Mortezaee V, Amini M, Rouini MR, *J ApplSci*, **2008**, 8, 1761-1765.
[4] IdrissiHassani L M, EL Hadek M,*Actabotanicagallica*, **1999**, 146, 4, 353-359.
[5] Kartal M, Altun ML,Kurucu S, *JpharmaceutBiomed Anal*, **2003**, 31, 263-269.
[6] Frison G, Favretto D, Zancanaro F, Fazzin G, Ferrara SD, *Forensic Sci Inter*, **2008**,179, e37-e43.
[7] Mirdeilami S Z, Barani H, Marandarani M, HeshmatiG A, *Iran J Plant physiol*, **2011,** 2, 327-338.
[8] khan AM, Qureshi RA, Ullah F, Gilani SA, Nosheen A, Sahreen S,Laghari MK, Laghari MY, Ur-Rehman S, Hussain I, Murad W, *J Med Plants Res*, **2011**, 5, 6017-6023.
[9] Azzi R, Djaziri R, Lahfa F, Sekkal FZ, Benmehdi H, Balkacem N, *J Med Plants Res*, **2012,** 6, 2041-2050.
[10] Moshiri M, Etemad L, Javidi S, Alizadeh A, *Avicenna J Phyto med*, **2013**, 3, 288-292.
[11] Achour S, Rhalem N, Khattabi A, Lotfi H, Mokhtari A, Soulaymani A, Turcant A,SoulaymaniBenkheikh R, *Thérapie*,**2012a**, 67, 53-58.
[12] Abassi K, Mergaoui L, AtayKadiri Z, Stambouli A, Ghaout S, *ZoolBaetic.*, **2002,** 13, 203-217.
[13] Zeng Y, Zhang Y, Weng Q, HU M, Zhong G, *Molecules*, **2010**, 15, 7775-7791.
[14] Edziri H, Mastouri M, Matieu M, Zine M, Gutman L, Aouni M,*Afr J Biotech*,**2010**, 9, 8199-8205.
[15] Arshad N, Neubaeur C, Hasnain S, Hess M, *PoultSci*,**2008**, 87, 240-249.
[16] Derakhshanfar D,Mirzaei M, *J Vet Res*,**2008**, 75,67-72.
[17] Bahmani M, Eftekhari Z, *Comp Clin Pathol*, **2013,** 22, 403-407.
[18] QUÉZEL P, SANTA S, Édit. CNRS, Paris, **1962-1963**.
[19] AsgarpanahJ,Ramezanloo F,*Afr J PharmPharmaco.*,**2012,** 6, 1573-1580.
[20] BrunetonJ , Lavoisier, Paris, **1999**.
[21] Kurt R, 2sd ed. Gauthiers Villars, Paris, **1971**.
[22] Barreira CM, João, Ferreira, Isabel CFR, Oliveira MBPP, Pereira JA, *Food ChemToxicol*, **2008**,46, 2230-2235.
[23] Aurélie G, Sihem M, Farida B, Mustapha CM, Jacques B, Josiane P, *Nutrition*, **2006,**22, 758–766.
[24] Achour S, Saadi H, Turcant A, Banani A, Mokhtari A, Soulaymani A, SoulaymaniBencheikh R, *Med Sante trop*, **2012b**, 22, 84-86.
[25] Bukhari N, Choi JH, Jeon CW, Park HW, Kim WH, Khan MA, Lee SH,*ApplChem*, **2008**, 12, 101-104.
[26] Agaie BM, Salisu A, Ebbo AA, *SciResEssay*,**2007**, 2, 040-042.
[27] Torres P, Diaz GJ, Cardenas E, Lozano MC, *IJPPR.***, 2012,** 2, 14-19.
[28] Roger-Achim D, Le médecin du Quebec. **2003**, 38, 10
[29] Chikezie PC, Uwakwe AA, Monago CC,*J Cell AniBiol*, **2009**,3, 39–43.
[30] Ouedraogo Y, Nacoulma O, Guissou IP, Guedeguina F, *Pharm MédTradAfr*,**2001,** 11, 13-29.

[31] Martins LJ, de Araujo PMF, Bon C, Hyslop S, de Araujo AL, *J VenomAnimToxinsincl Trop Dis*,**2009**, 2 15, 498-508.