J. Afr. Ass. Physiol. Sci. 3 (1): 9-13, July 2015.

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

http://www.jaaps.aapsnet.org

Research Article

GROSS BEHAVIORAL EFFECTS OF ACUTE DOSES OF ARTESUNATE IN WISTAR RATS

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Keywords:
Artesunate, Central nervous effects, Gross Behavior, Modulation, Behavioral neurotransmitters, Dopaminergic system

ABSTRACT

It was the aim of this study to investigate possible modulating influences on gross behavior in doses of artesunate in wistar rats. Thirty-six healthy adult rats were placed into nine groups comprising of eight test groups and one control group. Artesunate was prepared into different doses, and two test animals were administered with one of the doses following which they were observed together for various behavioral parameters for a period of one hour. The control group received no drug treatments, but were observed for behavioral activities after they were administered with 0.4ml of normal saline. All the behavioral data were pooled and subjected to statistical analysis using the One Way Anova with Dunnet multiple comparison post adhuc test. Results were expressed as Mean ±SEM, and P values of ($p \leq 0.05$) regarded as statistically significant. Behavioral excitatory effects were observed at lower doses of artesunate in mainly grooming, rearing and feeding behaviors. Sedative effects were observed in locomotor, sniffing, climbing and scratching activities and these later effects were also seen at the lower doses of the drug. Meanwhile, sedative effects were seen in all the behavioral parameters at the higher doses. It was concluded that artesunate may have some clearly definable central nervous system properties. Specifically, it was noted that artesunate may have dual behavioral modulating properties of both excitatory and sedative effects in wistar rat. It was concluded that the mechanism of action for these alterations in gross behavior may be closely linked with the neurotransmitter systems that normally regulate behavior in the central nervous system.

.INTRODUCTION

The level of melatonin drops significantly during puberty to allow for sexual maturation (Waldhauser et al., 1988; Karasek, 2007). A high melatonin level during this stage of life has been reported to delay sexual maturation, reduce gonadal development and thus prevent reproductive capacity (Rissman, 1980, Amador et al., 1986). Short photoperiod or long photoperiod with melatonin treatment has also been reported to delay gonadal development in rodent populations (Zucker et. al. 1980, Deveson et al., 1992). The role chronic melatonin administration on reproductive function in pre-pubertal, young, middle-aged and old mice and female rats has been documented (Pierpaoli et al., 1997; López et al., 2005). Gwayi and Bernard reported a dose dependent decrease in all parameters of sperm motility with melatonin treatment in vitro on spermatozoa collected from adult Wistar rats (Gwayi and Bernard, 2002). Melatonin treatment improved epididymal sperm concentration and motility in 6-7 months old homocysteine treated Wistar rats (Sönmez et al., 2007). However, the role of chronic treatment of melatonin in male reproductive function at old age in male Sprague Dawley rats remains an unanswered. This paper presents results from a comparative study on the outcome of melatonin administration on male reproductive function during ageing in Sprague Dawley rats.

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Thirty six (36) Adult Wistar rats of comparable age, weight and size were kept in cages for about two weeks in the animal house of the department of pharmacology, University of Benin, Benin city, Nigeria, to acclimatize to the new environment before the commencement of the experiments. The animals had free access to standard feeds and water throughout the experiments. The animals were separated into nine groups by the use of body markings, with each group comprising of four rats. The control group received 0.4mls of normal saline, while the eight test groups received acute daily administration of the various doses (2, 4, 8, 16, 32, 64, 128 and 250mg/kg) of artesunate solution, respectively, for the duration of the three weeks experiments.

A standardized artesunate solution was made by dissolving known quantity in a measured quantity of normal saline, the mixture was left standing for 30minutes thereafter it was sieved to remove the debris of starch and other coatings to obtain a solution of dissolved fresh artesunate solution, and which was prepared each experimental session (Klaus *et al*.,2011).

The open field tests were conducted in standard wooden cages of 40cm x 40cm and of 20cm height of wall (Dubovicky *et al.,* 2004; Verma *et al.,* 2009). The floor of the open field apparatus was designed after the method of Krishna *et al.*(2001), and made up of four parts subdivisions of 10cm each, which allowed for clear viewing of the animals.

The doses were administered intra-peritoneally (i/p), such that two rats of same sex received the same dose and were observed together for a period of one hour after the administration. Each of the behavioral parameter was scored manually by a tally counting method. After each observation period, the open field apparatus was cleaned using 70% ethyl ethanol and allowed to dry before the next observation was conducted (Davies *et al*., 2013). The behavioral parameters scored were Climbing**,** Scratching**,** Rearing**,** Grooming**,** Stereotyped movement**,** Sniffing**,** Feeding**,** Drinking**,** Aggression and Locomotor activity.

The results of the behavioral activities were pooled and analyzed using the One Way Anova version 5.0 with Dunnet Multiple Comparison post adhoc test (Lyvia et al., 2005). The behavioral data were expressed as mean and standard error of mean while P values of $P \leq 0.05$ were considered as statistically significant.

RESULTS

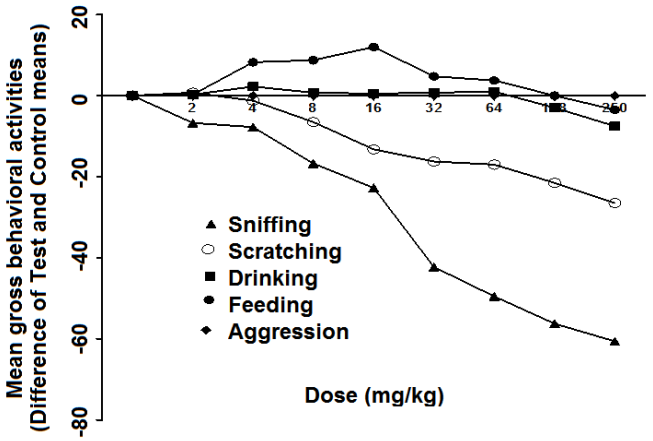

Fig.1:
Sniffing, Scratching, Drinking, Feeding and Aggression activities in open fieldTest following the administration of varying doses of artesunsate in wistar rats.

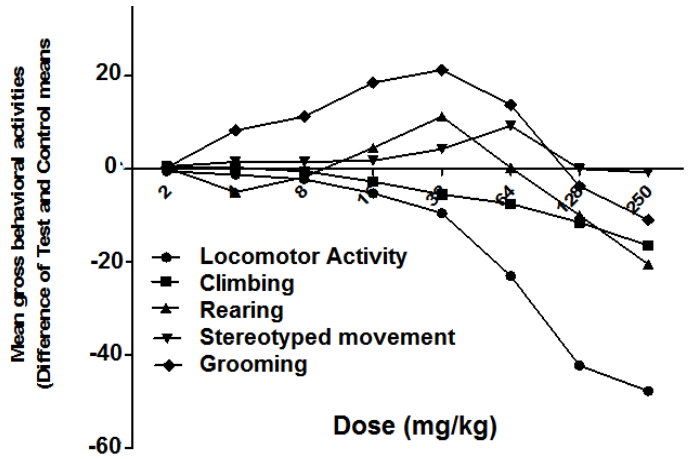

Fig. 2:
Locomotor, Climbing, Rearing, Stereotyped movement and Grooming activities in open field test after the administration of acute doses of artesunsate in wistar rats.

Table 1: Mean values of gross behavioral activities in acute administration of varying doses of artesunate in Wistar rats.

ACTIVITY / DOSE(mg/kg)	Sniffing	Scratching	Drinking	Feeding	Aggression
Control	76.7±1.93	43.8±1.18	10.8±1.12	4.8±0.85	0
2	70.0±2.20	44.5±1.28	10.5±1.32	5.0±0.91	0
4	69.0±1.41	42.5±1.47	13.0±1.68	13.0±0.41*	0
8	65.0±0.82*	37.3±1.29	11.0±2.58	13.5±1.94*	0
16	62.0±2.04*	30.5±1.28**	11.2±1.49	16.8±1.38*	0
32	34.5±1.88**	27.5±1.63***	11.5±1.19	9.5±1.19*	0
64	27.3±1.01**	26.8±1.32	11.8±1.75	8.5±1.32	0
128	20.5±1.12**	22.3±1.64***	7.8±1.25	4.8±1.11	0
250	16.1±1.92***	17.3±1.06***	3.3±0.63*	1.2±0.75	0

*Significant values (*P≤0.05, ** P≤0.01, *** P≤0.001) are Mean ±SEM compared to control (n=4)

Table 2: Mean values of some gross behavioral activities in acute doses of artesunate in Wistar rats.

ACTIVITY/ DOSE(mg/kg)	Locomotor Activity	Climbing	Rearing	Stereotype Movement	Grooming
Control	67.5±1.71	19.00±2.04	35.0±1.08	10.5±0.65	26.4±1.65
2	67.0±0.91	19.50±1.04	35.5±1.11	11.0±1.08	26.5±2.02
4	66.2±1.25	17.25±1.25	30.0±1.27	12.0±0.92	34.5±1.04*
8	65.2±1.11	19.50±1.11	33.7±1.92	11.0±0.71	37.5±2.10*
16	62.2±0.87	20.25±1.38	39.5±1.04*	16.8±1.38	44.7±1.18**
32	58.0±0.91**	13.50±1.56*	46.6±1.38**	09.5±1.19*	47.5±1.85***
64	44.5±1.56***	11.50±1.51**	35.3±1.12	08.5±1.32*	40.0±1.78***
128	25.5±1.43***	7.50±1.04***	25.0±1.08*	04.8±1.11	22.5±1.04
250	19.8±2.39***	2.50±1.04***	14.5±1.44**	07.4±0.75	15.3±1.36***

*Significant values (*P≤0.05, ** P≤0.01 and *** P≤0.001) are Mean ±SEM compared to control (n=4)

DISCUSSION

The mean values of the behavioral data in Table 1 revealed a clear decrease in sniffing and scratching activities (p ≤0.05) at lower test doses of artesunate. Decrease in locomotor and climbing activities were also seen at lower doses (Table 2). On the other hand, there was increase in feeding behavior, grooming and rearing activities (p ≤0.05) at lower doses (Tables 1&2). Aggressive behavior was not seen throughout the experiments, and there was no definite change in drinking behavior throughout the various test doses (Table 1).

In one similar study, aggressive behavior was also not observed in rats in acute doses of ascorbic acid (Ezenwanne and Anuka, 1991). The decrease in locomotor and climbing activities seen at lower doses (Table 2), were essentially at variance with the excitatory effects seen in the other behavioral parameters (feeding, grooming and rearing) at the same doses. This observation is suggestive that artesunate may have dual behavioral modulating effects in wistar rat. Nonetheless, there were sedative effects in all the behavioral parameters at higher doses of the artesunate (Tables 1&2). Sedative effects on gross behavioral activities were also reported in rats administered with

ascorbic acid (Ezenwanne and Anuka, 1991). However these observations with ascorbic acid treated rats were based on treatments at high doses of the substance. The present observation is essentially in agreement with the work of Wambebe and Sokomba (1986) in which behavioral excitatory effects were also observed in rats treated with ascorbic acid. However, it is noteworthy that these are two different compound employed in the works of the different researchers.

The open field test as was in the design of the present study, provides simultaneous measure of behavioral exploration and anxiety. It is possible to argue that artesunate possesses some clearly definable pharmaco-behavioral properties in the central nervous system that accounts for the present observed modulating influences on gross behavior in rats. From the present study, it is possible that the mode of action of artesunate seen at low doses is linked to D_1 and D_2 receptors activation through a mechanism similar to dopamine activation of D_1 and D_2 receptors as reported by komorowska and pellis (2004). For example, Amos et al. (2003) successfully demonstrated that bromocriptine is a D_2 receptor agonist that can induce locomotor activity in mice pretreated reserpine, a behavioral pattern which was attenuated by artemisinin at high doses. The result was also suggestive that artemisinin may have sedative properties at high doses, which may be mediated via postsynaptic dopamine D_2 receptor in the central nervous system.

The behavioral data in the present study show that locomotor, climbing, sniffing and scratching activities were the most profoundly depressed in the course of the administrations, while grooming and feeding were the more significantly activated (Figures 1&2). However, it should be noted that novel environments have been reported to influence the behavioral pattern of animals (Moses et al., 2010), and this has been shown to be further complicated when substances that may modify the already altered behavior are taken by the animals (Brown et al., 2007). The open field test is a widely used novel environmental apparatus in behavioral testing in wistar rats, and records of data show wider range of alterations in level of exploratory abilities and anxiety of the rat (Adjene and Ezenwanne, 2008).

CONCLUSION

It was concluded that artesunate may have some clearly definable central nervous system properties as evident from the modulating influences on gross behavioral activities observed in this study. The mechanism of action for these effects may be closely linked with the neurotransmitter systems known to normally regulate various forms of behavior.

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