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Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

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Fransiscus RG, Long JC, (1991). Variation in human nasal height and breath, Am. J. Phys. Anthropol. 85(4):419-427.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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ARTICLES

Research Articles

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Full Length Research Paper

Recovery of sertoli cells by *Allium cepa* in *Toxoplasma gondii* infected rats

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***Toxoplasma gondii* is a protozoan parasite that is globally widespread and it affects men and animals. We investigated the effect of allium cepa (onion juice) on sperm parameters, testestrone level in male rats was experimentally infected by *T. gondii.* Wistar male rats (n=40) were allocated into four groups: control group (n=10), T_1 group that received tachyzoites of *T. gondii* (n=10), T_2 group that received tachyzoites of *T. gondii* plus fresh onion juice 1cc per rat daily by gavages method (n=10), and T3 group which received fresh onion juice 1cc per rat daily by gavage method (n=10). 30 days after inducing toxoplasma, 5cc blood were collected for measuring testosterone. Testes tissues of rats in all groups were removed; then, they were prepared for sertoli cells analysis. Serum total testosterones and sertoli were significantly decreased in groups that were infected with *T. gondii*, in comparison to control and onion groups. Moreover, comparing to control group ($p<0.05$), testes weights in toxoplasma group were drastically decreased. Since, in our study, *T.gondii* had grave effect on serum total testosterones, and because of applying fresh onion juice led to removing this harmful effect, it is suggested that eating of onion is useful in infected men.**

Key words: *Allium cepa*, testes, testosterone,*Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* (Ryan and Ray, 2004). The parasite affects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family. Although, cats are often blamed for spreading toxoplasmosis, contacting with raw meat is a more significant source of human infections in many countries, and fecal contamination of hands is a greater risk factor (Torda, 2001). Up to one third of the world's human population is estimated to carry a Toxoplasma infection (Montoya and Liesenfeld, 2004). The centers for disease control and prevention noted that the overall seroprevalence in the United States, as determined with specimens collected by the National Health and Nutritional Examination Survey (NHANES) between 1999 and 2004, was found to be 10.8%, with 11% seroprevalence among women of childbearing age (15 to 44 years) (Jones et al., 2007).

Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Factors such as drug treatment, chemotherapy, toxins, infections, air pollutions, insufficient vitamins intake, and parasites like *T. gondii* tachyzoites, have harmful effects on spermatogenesis and sperm's normal production (Mosher and Pratt, 1991; Santana et al., 2010). Several studies have reported that antioxidants and vitamins A, B, C, and E can protect sperm's DNA from free radicals, and also, increase barrier stability of blood testis (Jedlinska-

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Table 1. The effect of 1cc fresh onion juice /rat on sperm parameters, testosterone, apopetosis, and testis weight of control and *T. gondii* groups.

Groups	Control	1cc fresh onion juice /rat	*T. gondii*	*T. gondii* plus,1cc fresh onion juice /rat
Testis (g)	1.39 0.55	1.38 0.54	1 0.55*	1.20 0.55
Sertoli cells apopetosis	50.11 0.11	61.22 0.33*	40.01 0.55*	44.33 4.43*
Testosterone (ng/ml)	1.22 0.11	2.46 0.11*	0.87 0.11*	1 0.11

(g=gram, ng=nanogram, ml=milliliter). Data are presented as mean ± SEM. *Significant difference at p< 0.05 level , (compared with control group).

krakowska et al., 2006). Evidence suggests that *Allium cepa* (onion juice) has antioxidative and androgenic other important molecules from oxidation and damage, improve sperm quality, and consequently, increase fertility rate in men (Yang et al., 2006). Therefore, the role of nutritional and biochemical factors in reproduction and sub-fertility treatment is very important. The present study was planned to assess the ability of *A. cepa* to promote sertoli cells parameters and testosterone concentration in *T. gondii* infected rats. The results obtained will provide further insights into appropriate treatment of infertile male patients using herbals to improve spermatogenesis.

MATERIALS AND METHODS

Preparation of onion juice

The underground yellowish-white bulbs of *A. cepa* (onion) were collected in August 2007 from Ilkhchi in the province of East Azerbaijan-Iran. Before the experiments, the skin was removed and fresh juice of onions was prepared using a Tefal fruit juice extracting machine.

Analysis of onion juice

The onion juice was tested to determine flavonoids using the Shinoda test (Yousef, 2005). Qualitative thin-layer chromatography (TLC) was employed for determining quercetin as a main flavonoid in onion. For TLC, 10 mL of fresh onion juice were dried in a vacuum and the resulting residue dissolved in 1 mL of methanol. 20 mL of methanolic solution were spotted on a silica gel plate (10 × 20 cm, silica gel 60 GF254, Merck, Darmstadt, Germany) with a solvent system of EtOAc/MeOH (80:20). Quercetin, Sigma chemical Co. (St. Louis, MO, USA), was used as a control. After developing and drying, the TLC plate was sprayed with a 2% AlCl solution in methanol. Quercetin in the onion samples appeared as a yellow spot at RF = 0.6. Separation of quercetin was performed with further purification by preparative TLC on silica gel; quantitative determination of quercetin carried out on a Model 2100 Spectrophotometer (Shimadzu, Japan) in 370 nm comparing to a pure quercetin standard curve. The amount of quercetin in fresh onion was found to be 12 mg/100 g (Khaki etal., 2009).

T. gondii infection

T. gondii strain RH was maintained by passage in mice every 2 days. Tachyzoites were collected from the peritoneal cavity of infected mice and used to inoculate rats. The rats were intraperitoneally injected with 107 tachyzoites of *T. gondii* at the Department of Veterinary Pathology, Islamic Azad University, Tabriz Branch-Iran (Berdoy et al., 2000).

effects in rats, and can promote spermatogenesis cycle (Khaki et al., 2009). Antioxidants protect DNA and

Experimental animals

Adult Wistar albino male rats (n=40) were included in the present study. The rats were 8 weeks old and weighing 250±10 g. They were obtained from animal facility of Pasture Institute of Iran. Male rats were housed in rooms with controlled temperature (25°C), constant humidity (40-70%), and 12h/12h light/ dark cycles prior to experimental protocols. All animals were treated in accordance with the Principles of Laboratory Animal Care[NIH]. All rats were fed with a standard diet and water. The daily intake of water was monitored at least one week prior to the start of treatments in order to determine the amount of water needed fire very experimental animal. Thereafter, the rats were randomly divided into control (n=10) and experimental (n=30) groups. The control group just received 4cc distilled water daily. However, the experimental infected rats (n =20) were split into two infected *T. gondii* groups; one of these groups was *T. gondii* test group (n=10) and the other was *T. gondii* group (n=10) received 1cc of fresh onion juice daily. The fourth experimental group (n=10) received 1cc of fresh onion juice daily (Khaki et al., 2009) this group was onion test group. At the end of the study, the rats were killed by carbon dioxide.

Surgical procedure

In the thirtieth day, the Pentobarbital sodium (40 mg/kg) was administered intra peritoneal for anesthesia, and the peritoneal cavity was opened through a lower transverse abdominal incision. Then, testisin control and experimental groups was immediately removed. The weights of testis in each group were registered. The animals were decapitated between 9:00 AM and 11:00 AM, and blood samples were obtained. Blood samples were centrifuged at 4°C for 10 min at 250 g, and the serum obtained was stored at −20°C until the time it was assayed.

Statistical analysis

To compare data in control and experimental groups, ANOVA test was applied. The results were expressed as mean ± S.E.M (standard error of measurements). Significant differences are written in parentheses.

RESULTS

Weight of individual male testis

The obtained results in this study are illustrated in Table 1. There was significant difference in testes weights

between *T. gondii* groups, as compared to the other groups ($p<0.05$).

Results of testosterone levels

Levels of testosterone were significantly increased in fresh onion juice group comparing to control and *T. gondii* groups ($p<0.05$). This result is higher in infected rats with *T. gondii* that received 1cc fresh onion juice, as compared to *T.gondii* group.

DISCUSSION

T. gondii infection is associated with a wide spectrum of clinical pictures in men. Onion and garlic contain a wide variety of phytochemicals and micro constituents, such as trace elements, vitamins, fructans, flavonoids and sulphur compounds, which may have a protective effect against free radicals. The present results clearly indicate that *A. cepa* (onion) has a good effect on spermatogenesis in rats. Our results showed that administration of onion juice (1 g/rat/day) for 20 consecutive days caused a marked increase in sperm number, viability, and mobility, as compared to respective controls; this agrees with our previous research (Khaki et al., 2009). These effects could be related to vitamins, vitamin C, and flavonoids of onion such as quercetin. Oxidative damage was as certained by measuring malondialdehyde levels, reactive oxygen species (ROS) generation, alterations in antioxidant defences, and the extent of protein oxidation. Quercetin, an important flavonoid, has a beneficial effect on health due to its antioxidant function. Studies on the effect of quercetin on oxidative damage in cultured chicken spermatogonial cells showed that quercetin has no deleterious effect on spermatogonial cells at doses of 1 and 10 mg/mL. Quercetin (1 mg/mL) increased the number of spermatogonial cells and decreased the mortality of Aroclor-induced oxidative damage. In this study, the effect of quercetin on serum MDA was determined, but the results indicated no obvious effect of quercetin on MDA production (Mi and Zhang, 2005; Mi et al., 2007). In the present study, *T. gondii* significantly reduced sperm amount and mobility; on the other hand, our research showed that onion fresh juice can enhance both the number of sperm and mobility in group of animals infected with *T. gondii*. These results are in agreement with other finding. They showed that Toxoplasma infection was related to infertility, so it was possibly related to the antisperm antibodies being involved in the pathogenesis of infertility (Zhou et al., 2002; Aral et al., 2011). In their study regarding to mice, Sun et al. (2008) reached to the same results. These researchers found out that acute *T. gondii* infection affects the reproductive function of male mice.

T. gondii infections have the ability to change the behavior of rats and mice, making them drawn to, rather than fearful of, the scent of cats. This effect is advantageous to the parasite, which will be able to sexually reproduce if its host is eaten by a cat (Berdoy et al., 2000). The infection is highly precise, as it does not affect a rat's other fears such as the fear of open spaces or of unfamiliar smells. Studies have also shown behavioral changes in humans, including slower reaction times and a six fold increased risk of traffic accidents among infected males (Flegr et al., 2002), as well as links to schizophrenia, such as hallucinations and reckless behavior.

Conclusion

In our study, *T. gondii* had a significant effect on sperm parameters and serum total testosterones. On the other hand, freshly prepared onion juice significantly affected the sperm number, percentage of viability, and mobility. Onion juice can both reduce and treat this malevolent effect, so it is suggested that eating of onion is useful in infected men.

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Full Length Research Paper

Effect of water-depth on the antidepressant-like actions of furosemide

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Increase in water-depth increases the sensitivity of the forced swim test (FST) and it was deemed necessary to investigate the antidepressant-like activities of furosemide, bumetanide and nifedipine vis-à-vis the currently used antidepressants, imipramine and sertraline at different water-depths. Groups of mice with six mice each were divided into two batches of 10 groups for the experiments at the different water-depths of 15 and 30 cm in labelled plastic cages for (a) two control groups for treatment with 0.25 ml of 10% Tween 80; (b) two furosemide groups for treatment with 100 mg/kg of furosemide; (c) two bumetanide groups for treatment with 75 mg/kg of bumetanide; (d) two nifedipine groups for treatment with 5 mg/kg of nifedipine; (e) two imipramine groups for treatment with 10 mg/kg of imipramine; (f) two sertraline groups for treatment with 5 mg/kg of sertraline. Injections were administered intraperitoneally (i.p). 60 min elapsed before the test of immobility was carried out at the different water depths of 15 and 30 cm. The drug combinations furosemide + nifedipine, furosemide + imipramine and furosemide + sertraline were also tested at the 15 and 30 cm water-depths and also compared to controls. The five agents and the drug combinations caused significant responses in the delay or prolongation of the period of immobility over control values and over values obtained at the 15 cm water-depth ($P < 0.05$; < 0.005). Post-hoc Student-Newman-Keuls (SNK) test showed that while imipramine produced the most significant response at the 15 cm water-depth, furosemide produced the most significant response at the 30 cm water-depth. In summary, the antidepressant-like response of furosemide is enhanced significantly by increase of water-depth above that of the other agents.

Key words: Furosemide, bumetanide, nifedipine, imipramine, sertraline, water-depth, antidepressant.

INTRODUCTION

Oxidants such as superoxide, hydroxyl radicals and lipid hydroperoxides (that is, reactive oxygen species (ROS) are now realized as signalling molecules under subtoxic conditions; stimulating signal transduction such as Ca^{2+} and protein phosphorylation (Suzuki et al., 1997) and ROS may be second messengers for apoptosis. Exercise can cause an imbalance between reactive oxygen species (ROS) and antioxidants (Belviranli and Gokbel, 2006) which is referred to as oxidative stress. Increase in water-depth increases oxidative stress which is defined as an imbalance in the pro-oxidant/anti-oxidant ratio, which favours increased pro-oxidants and results in oxidative damage (Downs et al., 2002). Also, swimming has been shown to significantly induce lipid peroxidation and decrease glutathione levels in the brain of mice (Singh et al., 2002), and natural and synthetic antioxidant treatments are shown to increase levels of superoxide dismutase (SOD) and catalase (CAT) in the forebrain and to significantly reduce the period of immobility in the

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Abbreviations: ROS, Reactive oxygen species; **SOD,** superoxide dismutase; **CAT,** catalase; **MAPK,** mitogen-activated protein kinase; **ERK,** extra-cellular signal-regulated kinase; **BDNF,** brain-derived neurotrophic factor; **SSRI,** selective serotonin reuptake inhibitors; **CCB,** calcium channel blockers; **TCA,** tricyclic antidepressants; **OSRK1,** oxidative-stress response kinase 1; **CREB, cAMP,** response element binding protein; **NKCC1,** isoform 1 of the sodium-potassium-chloride cotransporter; **KCC2,** isoform 2 of the potassium-chloride cotransporter; **FST,** forced swim test; **SEM,** standard error of mean.

forced swim test.

Abel (1993, 1994a, b) found increase in lactate, glucose, potassium, phosphorus/potassium ratio and metabolic acidosis at increased water-depths and that immobility times correlated significantly with these parameters but not with the levels of corticosterone. Acidosis enhances the formation of ROS which can also trigger mitogen-activated protein kinase (MAPK) phosphorylation, the disruption of Ca^{2+} homeostasis (Manzl et al., 2004) and inhibition of extra-cellular signal-regulated kinase (ERK) 1/2 phosphorylation, leading to necrotic cell death (Riemann et al., 2011).

Apoptosis has been proposed as a contributing cellular mechanism to the structural alteration that have been observed in stress-related mood disorders, and studies have indicated anti-apoptotic or even neurogenic effects of selective serotonin reuptake inhibitors (SSRIs) and other antidepressants through their favourable effects in inducing the growth factor, brain-derived neurotrophic factor (BDNF) and increasing B-cell lymphoma protein-2/B-cell lymphoma-associated protein X (Bcl-2/Bax) balance (Kosten et al., 2008).

Through their scavenging of ROS and protective action against oxidative stress-induced neuronal cell death (Kim et al., 2010; Garcia et al., 2009), antioxidants could also be termed anti-apoptotics. Imipramine, a tricyclic antidepressant (TCA), by increasing SOD and CAT, promote antioxidant activities in the hippocampus and prefrontal cortex (Reus et al., 2010) and prevents apoptosis (Peng et al., 2008) though Xia et al. (1999) had found that imipramine could activate apoptosis. Sertraline possesses antioxidant activity (Kumar and Kumar, 2009) but may induce apoptosis like other SSRIs (Leukovitz et al., 2005; Taler et al., 2008) at high doses. Nifedipine can induce apoptosis through interaction with an as yet uncharacterized functional site other than a calcium channel blocker (Kondo et al., 1995) but Rabkin and Kong (2000) reported that nifedipine prevented apoptosis by reducing DNA fragmentation produced by increased (Ca^{2+}).

Nifedipine has antioxidant properties (Godfraind, 2005). Bumetanide has effect against apoptosis (Marklund et al., 2001) and may possess antioxidant effects (Geng et al., 2009) by down-regulating the in-wardly directing sodium-potassium-chloride cotransporter (NKCC1) like furosemide and thereby attenuating the effects of the oxidative stress response kinase 1 (OSRK1) in activating NKCCI, an effect for which ROS may be second messenger (Pombo et al., 1997). Furosemide has a potent free radical scavenging effect *in vitro* and significant antioxidant status *in vivo* (Lahet et al., 2003; Hamelink et al., 2005).

Furosemide is a Bax blocker (Lin et al., 2005) and has overlapping role with Bcl-2 (Wang et al., 2007), attributes which enhance antiapoptotic effects. In mood disorders, there is decrease in antioxidant enzymes (Ranjekar et al., 2003) and lower superoxide dismutase, catalase, glutathione S transferase, glutathione reductase and glutathione levels than normals (Zafir et al., 2009), a situation that is reversed by antidepressants such as imipramine (TCA) and sertraline (SSRI) that have antioxidant actions. Zafir et al. (2009) postulated that the augmentation of *in vivo* antioxidant defenses could serve as a convergence point for multiple classes of antidepressants and as an important mechanism underlying the neuroprotective effect of these drugs observed clinically. Agents that also possess significant antiapoptotic actions such as furosemide may be more potent against neurodegeneration occasioned by oxidative stress.

Oriaifo and Omogbai (2010) have shown that furosemide, bumetanide and nifedipine have significant anti-depressant-like activity in the FST model of depression. The down-stream actions of antidepressants in enhancing neuroplasticity and cell resilience may be more important than their upstream effects on monoamine transporters (Krishnan and Nestler, 2010).

The aim of the study was to evaluate the effects of two different water depths on the delay or prolongation of onset of immobility induced by furosemide, bumetanide, nifedipine, imipramine and sertraline. Increasing the water-depths has been shown to increase the sensitivity of antidepressant agents (Cryan et al., 2002) probably due to the increased levels of anti-oxidants resulting from the increased production of reactive oxygen species at increased water-depths (Lesser et al., 1990; Higuchi et al., 2008).

MATERIALS AND METHODS

Groups (six mice in each group) of male albino mice (25 to 35 g) were used in this study. They were allowed to acclimatize in the animal house in labelled plastic cages for two weeks. Animals were housed at room temperature of 25 to 27°C in a 12 h light/dark cycle. They were allowed food and water *ad libitum*. All drugs were supplied by Sigma-Aldrich through Rovet Chemicals, Benin City, Nigeria. All the drugs were dissolved in 10% Tween 80 in distilled water because of furosemide's solubility. The mice were injected intraperitoneally (i.p.). The doses of drugs were chosen from previous studies (Eraly et al., 2006; Luszczki et al., 2003; Cryan et al., 2004; Kosuda et al., 1997; Hesdorffer et al., 2001; Mogilnicka et al., 1987).

Drug studies with the forced swimming test

Male albino mice, after acclimatisation and care in the departmental laboratory, were transported to the sound-proof testing area in their own labelled cages. They were allowed to adapt for one hour before the intraperitoneal injections after which there was a wait period of 60 min before the tests of immobility. A batch of mice of six in each group were forced to swim for four minutes in a vertical glass cylinder of height 27 cm, diameter 16.5 cm and containing fresh tap water to a depth of 15 cm (Abel, 1994) at 27°C. Another batch of mice also of 6 in each group were forced to swim for four minutes in another vertical glass cylinder of height 46 cm, diameter 20 cm containing fresh tap water to a depth of 30 cm at 27°C. A

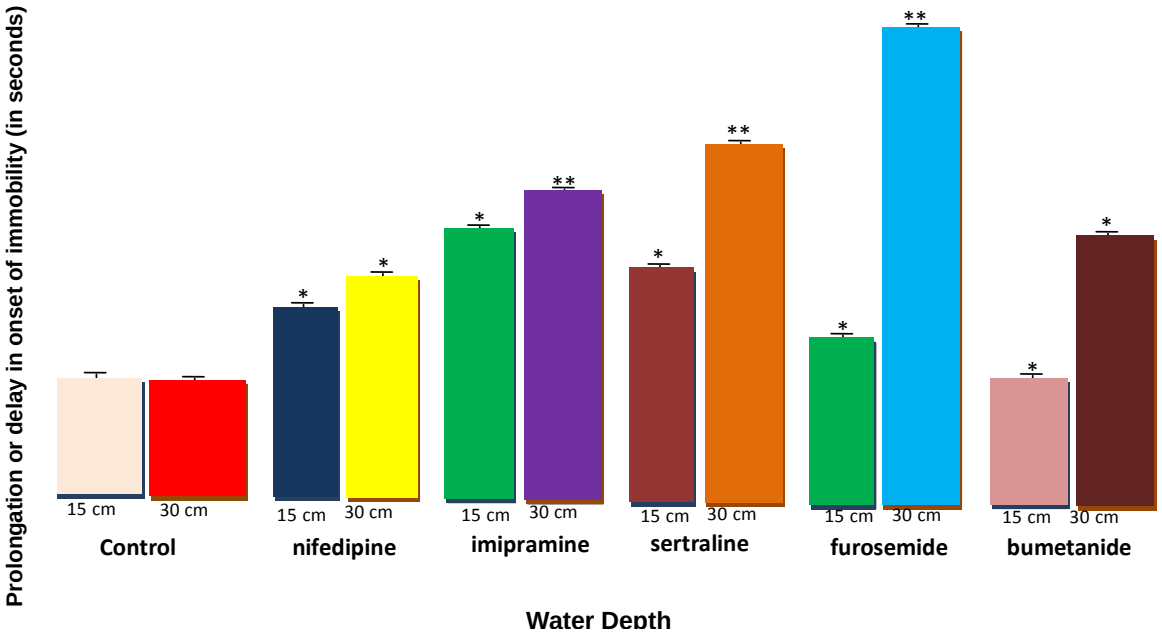

Figure 1. Effect of water-depth on the anti-depressant-like action of nifedipine, imipramine, sertraline, furosemide and bumetanide in prolongation of the onset of immobility of mice in the FST. The five agents significantly (P* < 0.05; P** < 0.005) caused prolongation of onset of immobility with furosemide producing the greatest effect at the 30 cm depth (n = 6/group).

behavioural model of immobility first postulated by Porsolt et al., (1977) and named the behavioural despair model, was used. In this model, mice are forced to swim in a restricted space from which escape is not possible. Following an initial period of vigorous activity, the mice become helpless and adopt a characteristic immobile posture with no further attempt to engage in escape-related behaviour; and this reflects a state of despair or lowered mood. The period of on-set of immobility is timed by an observer unaware of the drug given and recorded. In this experiment, same doses of drugs were given intraperitoneally (i.p) to identical groups in batches of mice as follows:

Drug administration

A - Control; 0.25 ml of Tween 80
B - Furosemide; 100 mg/kg
C - Bumetanide; 75 mg/kg
D - Nifedipine; 5 mg/kg
E - Imipramine; 10 mg/kg
F - Sertraline; 5 mg/kg
G - Furosemide + Nifedipine; (100 + 5) mg/kg
H - Furosemide + Imipramine; (100 + 10) mg/kg
I - Furosemide + Sertraline; (100 + 5) mg/kg

One hour elapsed before the test of immobility was carried out using the FST model of depression. The effects of the drug combinations furosemide + nifedipine, furosemide + imipramine and furosemide + sertraline were also studied acutely at the 15 and 30 cm water-depths.

Statistical analysis

Two-factor analysis of variance was used to determine level of significance of the treatment groups and at the different water-depths supplemented with the t-test for two groups. Result was considered significant at P < 0.05, < 0.005. The Student-Newman-Keul's (SNK) test was used as *post-hoc* test.

RESULTS

At the 15 cm water-depth (Figure 1), mean value for controls for the period of onset of immobility (Aburawi et al., 2007) was 43.75 ± 1.04 s; 70.86 ± 0.05 s for the nifedipine group; 84.43 ± 1.13 s for the imipramine group; 75.30 ± 1.20 s for the sertraline group; 63.78 ± 1.08 s for the furosemide group and 54.70 ± 4.06 s for the bumetanide group. The values for the drug agents were significantly different from control values at P < 0.05. At the 30 cm water-depth (Figure 1), mean value for controls for the period of onset of immobility was 43.75 ± 1.04 s; 74.90 ± 1.23 s for the nifedipine group; 104.78 ± 3.30 s for the imipramine group; 116.65 ± 2.00 s for the sertraline group; 176.93 ± 3.68 s for the furosemide group and 82.27 ± 0.90 s for the bumetanide group. The values at the 30 cm water-depth were significantly different from those at the 15 cm water-depth (P < 0.005).

At the 15 cm water-depth (Figure 2), mean value for controls for the period of onset of immobility was 43.75 ± 1.04 s; 79.04 ± 1.02 s for the furosemide + nifedipine group; 79.25 ± 1.19 s for the furosemide + imipramine group; 125.90 ± 1.33 s for the furosemide + sertraline group. At the 30 cm water-depth (Figure 2), mean value for controls for the period of onset of immobility was 43.75 ± 1.04 s; 150.24 ± 4.32 s for the furosemide +

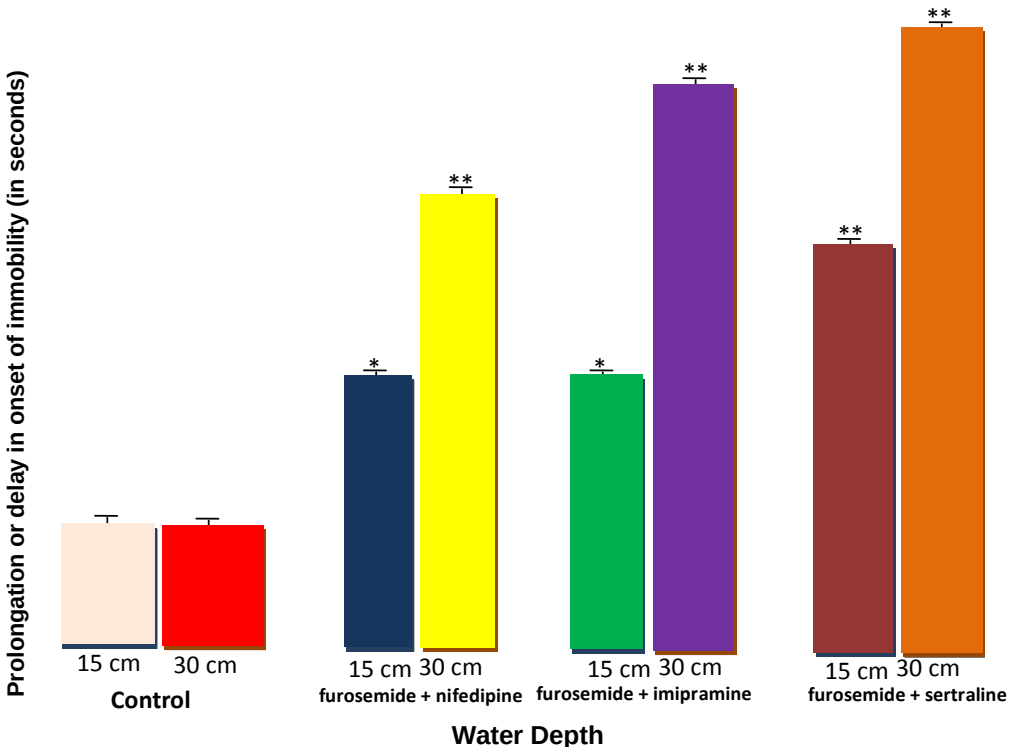

Figure 2. Effect of water-depth on the prolongation of onset of immobility in the FST by the drug combinations furosemide + nifedipine, furosemide + imipramine and furosemide + sertraline. The drug combinations significantly (P* < 0.05; **< 0.005) prolonged or delayed the onset of immobility with furosemide + sertraline combination producing the greatest effect. At the 30cm water-depth, furosemide enhanced the effect of imipramine in contrast to the antagonism at the 15cm water-depth when the combination did not achieve the value of imipramine as single drug (n = 6/group).

nifedipine group; 180.25 ± 2.69 s for the furosemide + imipramine group; 195.20 ± 4.53 s for the furosemide + sertraline group. The values at 30 cm depth were significantly different from those at the 15 cm depth (P < 0.005) and at this depth, the action of imipramine was enhanced by furosemide.

DISCUSSION

Present results show that all the five agents reduced immobility significantly at the 15 cm water-depth (P < 0.05) as we have already reported (Oriaifo and Omogbai, 2010), probably by their up-stream effects on monoamine transporters and down-stream effects on neuroplasticity and that imipramine produced the most significant response at the 15 cm water-depth; while furosemide produced the most significant response (P < 0.005) at the increased water-depth of 30 cm. Abel (1994) and Detke and Lucki (1996) had found lower immobility scores with the greater water-depth and that immersion in water impacted negatively on the physiology of rodents. Our experimental results confirm their finding that immobility in the FST is further reduced when the water-depth increased probably due to increased activity of anti-oxidants (Lesser et al., 1990).

Imipramine shows more efficacies in male mice (Kornstein et al., 2000; Raskin, 1974) and this may account for its producing the most significant response at 15 cm. There may be subtle differences in antioxidant/antiapoptotic status between imipramine and sertraline that may explain the greater potency of sertraline at the 30 cm water-depth.

The explanation for greater significant antidepressant-like action of furosemide and of the furosemide + sertraline combination above the other agents at the increased water-depth observed in the study may be that furosemide is able to correct the increase in the phosphorus/potassium ratio (Abel, 1993) due to its greater phosphaturic action more than bumetanide and the other agents studied. Also, furosemide's more potent antioxidant and antiapoptotic role may be important here. Reactive oxygen species and p38 mitogen-activated protein kinase activate Bax to induce mitochondrial cytochrome C release and apoptosis (Gomez-Lazaro et al., 2007), so the furosemide effect may be due to its greater dual function as antioxidant and Bax blocker and so reduce Bax/Bcl-2 ratio and enhance cell resilience and BDNF-ERK1/2-CREB-Bcl-2, signaling more than the other agents at the greater water-depth. The differential

pharmacodynamic and pharmacokinetic effects of the agents on the non-enzymatic oxidants such as vitamin A, E and C have not been reported, for this may partially account for the observations we are reporting. Acute administration of furosemide and imipramine gave enhanced actions at the 30 cm water-depth unlike what obtained at the 15 cm water-depth. This may be due to the damage to muscarinic acetylcholine receptors by increase in free radical generation at the 30 cm depth (Venkatesham et al., 2005) thereby and probably removing a source of antagonism between furosemide and imipramine.

Of the five agents studied in the experiment, only furosemide has a BDNF-mimetic action in down-regulating the neuron-specific cation chloride cotransporter, KCC_2 (Wardle and Poo, 2003) thereby inhibiting gamma-amino butyric acid (GABA) and this may also offer some explanations for the differences observed.

Conclusion

At a water-depth of 30 cm, furosemide displayed a more significant antidepressant-like activity in prolonging the period of onset of immobility above that of imipramine, sertraline, nifedipine and bumetanide probably due to its greater dual action as antioxidant and anti-apoptotic.

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