Pak. J. Biochem. Mol. Biol., 2012; 45(3): 117-125

Withdrawal from energy drink (Sting) precipitates behavioral toxicities in pup male rats: a comparative pilot study

*Hajra Naz, Sidra Bashir, Sadaf Naeem and Madiha Rehman**
Department of Biochemistry, University of Karachi, Karachi, Pakistan

Abstract: Previously we have demonstrated that administration of Energy Drinks (EDs) produced behavioral toxicities in male Albino Adult rats in terms of impaired memory. EDs marketed for their improved performance and feeling of alertness have gained consumer attention and popularity not only in adults but also in children. These drinks, a combination of vitamins and caffeine are not recommended for children. Since they are thought to be harmless and their safety profiles are unknown, their consumption is known to increase in children. This pilot preclinical study was designed keeping in view the current popularity of such drinks in children. ED consumption and withdrawal associated behavioral effects were monitored to evaluate its toxicity in male pup rats. 9 ml/day of sting was administered orally for 14 days with its subsequent withdrawal for 14 days; Home cage and open field activity were increased upon administration while after withdrawal home cage activity was not affected however corner sittings were significantly increased in an open field. Pups in elevated Plus maze(EPM) and light dark activity paradigms exhibited anxiolysis upon exposure while upon withdrawal only time spent in an open arm of an EPM was significantly decreased. Exposure produced no significant effect on the time to reach the escape platform in Morris Water Maze (MWM), while withdrawal decreased this time. Exposure increased the struggling time in Forced Swim Test (FST), while withdrawal decreased the struggling time. Although sting induced hyper locomotion, anxiolysis and antidepressant effects were the positive features associated with its consumption but withdrawal associated depression and anxiety may be regarded as signs of behavioral toxicity in pups. This mandates an urgent research on the safety energy drinks especially regulating pediatric energy drink consumption. This preclinical finding accompanied by the previous clinical findings help us to extrapolate the data with reference to children and a food for thought for parents to discourage their children to drink such beverages. Further studies with larger n size followed by clinical studies would help us to ascertain our notion.

Keywords: Energy Drink, pups, caffeine, Morris water maze, forced swim test, ginseng, elevated plus maze.
Received: June 2, 2012 **Accepted:** August 10, 2012
***Author for correspondence:** sidra_bch02@yahoo.com

INTRODUCTION

Energy drinks or energy drinks have exploded the market and have gained popularity in the past several years[1]. A variety of them such as Sting, Red Bull, Monster, Rock star, Full throttle, No Fear, Amp, So Be, Tab Energy are thronging the market for their performance enhancing effects and improved cognition[2-7]. Their consumption is also tremendously increasing specially in children. Documented and undocumented report conveys that 28% of 12-14 years old children regularly consume ED[8]. People are unaware about their ingredients and associated physiological effects[9]. ED may increase risk the of caffeine intake which is one of the important components of ED, hence its overdose may result toxicity in children and teenagers[10].Evidence suggest that caffeine overdose can result in seizures in humans[11]. Caffeine most commonly used as psychoactive drug legally is available over the counter to children[12,13]. Caffeine consumption should not exceed 2.5mg/kg/day in children[14,15]. Caffeine mimics epinephrine's effect increases ATP supply to muscles[16]. It also interferes with intestinal calcium absorption in early adolescence[17]. After cessation in children who habitually consume caffeine, attention decreases and reaction time increases transiently[18]. It may affect developing child's brain, reward and addiction centre[8.] Guarna, another component of these drinks, contains caffeine along with other components, thus caffeine from these sources might also contribute to over dosage.

Its additives can cause hyperactivity in children[19]. Taurine an essential amino acid, is also an ingredient of these drinks, present abundantly intracellullary[20] has a role in calcium movement and cytoprotection in cardiac and skeletal muscles [21, 22]. It exhibits antioxidant properties; however its role in CNS is not well established[23]. Preclinical studies have documented that long term taurine administration results in increased susceptibility to seizures[24]. Other additives in EDs such as Ginseng has an anxiolytic profile, produces euphoria[25] and is very effective against depression [26].it is also reported to promote learning and memory[26]. Citric acid possess antioxidant property and is effective for wound healing, by its topical application[27]. It is also an alkalinizer[28]. Niacin (Vitamin B_3) is a CNS stimulant, promotes obesity prevalence in children of US[29]. Pyridoxine (Vitamin B_6), neuroprotectant is involved in the synthesis of certain neurotransmitter[30]. Cobalamine (Vitamin B_{12}), beneficial for cognitive functions, its deficiency causes memory deficit[31]. Inositol, structural component of cell membranephospholipids[32]. It maintains cell membrane potential[33]. Azo dyes such as tartazine, allura red, sunset yellow are linked with hyperactivity in children[19]. Food preservatives as sodium benzoate and potassium sorbate used in ED are reported for causing memory impairment[34] and

genotoxic[35] respectively. Calcium disodium EDTA, a chelating agent, is used to remove heavy metals[36].

ED is consumed by 30-50% adolescents and unregulated amount of caffeine of these drugs have been reported in association with serious adverse effects [37]. Many of the components individually examined have beneficial effects. Interactions between compounds, additive and dose- dependant effects , long term consequences, and dangers of ED associated with risky behavior in children remain to be determined [8,38].Their exposure should be regarded as novel. The present study was designed to evaluate sting induced behavioral toxicity in pups. It's noteworthy to mention that previous preclinical studies with sting have demonstrated behavioral toxicities in adult animals in terms of impaired memory[39].

MATERIALS AND METHODS

Animals

Locally bred Albino Wistar male rats and male pups were purchased from Ojha campus, Dow University of Health and Sciences. Adult animals were 100-140gm and 3-4 months old, while pups were 3-4 weeks old weighing 50-75gm. The rats were housed in cages together with saw dust covered floor and in quiet room, with free access to standard diet and tap water for 3 days and were placed individually in transparent cages for one week for the purpose of acclimatization, with free access to water and rodent diet.

Energy drink (ED)

ED (sting) was purchased from local market.

Trial and error

The dose of administration of ED was decided using trial and error method. For trial we took three animals. ED in volume of 3, 6 and 12ml were administered to animal and sting induced hyperactivity was observed after 15 minutes, because its immediate effect is hyperactivity. Hyperactivity was observed at doses of 6 and 12ml, while an intermediate dose of 9ml was selected for the purpose of administration.

Experimental design

The experiment was conducted on two groups of male animals: 6 adult and 6 pups. The pup animals were orally administered with 9ml of ED with the help of syringe daily between 12:00 to 13:00 hours, while adults were administered with the same amount of water. This treatment continued for 14 days. On the 13th day following behavioral activities were monitored; home cage activity, open field activity, elevated plus maze, light/dark transition, Morris water maze and forced swim test. The animals were then divided into two groups. 3 animals from each group were decapitated and their brain was dissected out and plasma was collected for neurochemical and biochemical analysis. Samples were stored for the neurochemical analysis in the next phase of study. Only behavioral data is reported in this paper. The remaining three controls and test animals were left for withdrawal from energy drink. After withdrawal of 14 days all behavioral activities were monitored before decapitation. The experiments were carried under ethical conditions approved locally. The data of exposure was compared with adult male rats given water to drink while withdrawal data was compared with previously sting treated pups. Data was analyzed using student's t test. $P<0.05$ was regarded as significant.

Behavioral techniques

Home cage activity

A transparent square box 26×26×26 inches was used for the evaluation of locomotor activity of experimental animals. Any movement made by the animal was scored for 5 minutes.

Open field activity

This model is used for evaluation of ambulatory activity. Open field apparatus made up of perpex plastic with dimension 76×76cm and floor is divided into 25 equal squares by lines. The activity was scored by counting square crossed by the animal in 5min. Latency to move is also monitored. Corner sittings are taken as sign of lethargy.

Elevated plus maze test

The elevated plus maze, the most frequently performed test, consists of four identical arms 50×10 cm, two open arms and two closed arms with wall 40 cm high radiating from a central square. Drugs with known anxiolytic effects in human would increase the time that rodents spend in the normally aversive open arm of the maze, while anxiogenic drugs decrease this time[40].

Light dark transition test

This test is used to monitor anxiolytic effect of any drug and is conducted in two chambered compartment. The compartments are of equal size 26×26×26cm, both chambers are joined one is light and other is dark. Light chamber is illuminated with light. There is a small opening of about 12×12cm between two compartments, rats is placed in dark compartment of the light dark box, animal comes out to the light compartment to explore the environment because of anxiolytic response of drug. We monitor no. of entries and time spent in the light compartment as a sign of anxiolysis for 5 minutes[41].

Morris water maze test

In 1981, Richard G. Morris a neuroscientist developed this test for evaluation of long term spatial

memory. It is consist of a big round pool filled with water having a hidden escaped platform. The pool is 1.2-1.8m in diameter and 60cm deep. By adding milk in the water we make it opaque so that rat could not see the escape[42].

Forced swim test

This test is used to monitor depression of experimental animals. This model is consist of open glass cylindrical container of diameter 10cm, height 60cm and half filled with water. The cut off time is five minutes [43,44]. Rats are placed in this model from which they can not escape and forced to swim, after vigorously activity adopts a characteristic immobile posture which can be readily identified. This immobility is reduced by various clinically effective antidepressant drugs [45].

RESULTS

Figure 1 shows the effects of sting ED daily administration (9 ml for 14 days) on home cage activity test in pup rats. The data statistically analyzed by the t-test revealed that ED administration increased the locomotor activity (P<0.05*) as compared to water treated adults while withdrawal (figure 7) showed no significant effect as compared to the previously treated pups (P>0.05).

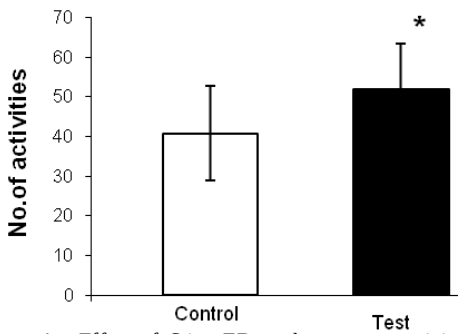

Figure 1: Effect of Sting ED on home cage activity in pups. Values are mean±SD (n=6) significant difference by t-test (*P<0.05) from respective water treated adult rats.

Figure 2(i) shows the effect of sting ED daily administration (9 ml for 14 days) on no. of square crossed in open field activity test in pup rats. The data statistically analyzed by t-test revealed that administration of ED increased the ambulatory activity but was not significant statistically (P>0.05) as compared to their water treated adults however withdrawal (figure 8) decreased it significantly (P<0.01) as compared to the previously treated pups.

Figure 2(ii) shows the effect of sting ED daily administration (9 ml for 14 days) on corner sittings in open field activity test in pup rats. The data statistically analyzed by t-test revealed that administration of ED decreased the corner sittings (P<0.01), while withdrawal (fig 8 ii) increased the corner sittings (P<0.01) as compared to previously treated pups.

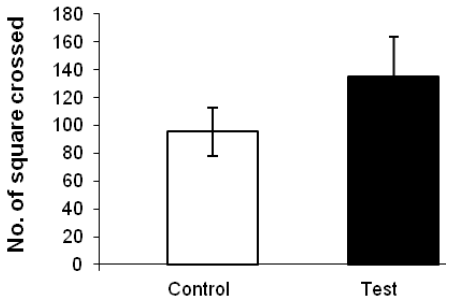

Figure 2(i): Effect of Sting ED on square crossed in open field activity in pups. Values are mean±SD (n=6). No significant difference by t-test from respective water treated adult rats.

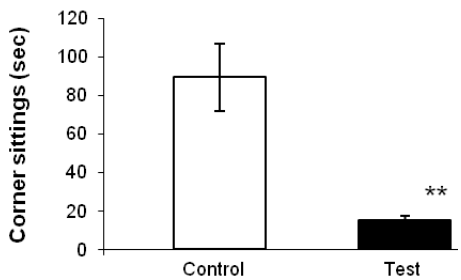

Figure 2(ii): Effect of Sting ED on corner sittings in open field activity pups. Values are mean±SD (n=6) significant difference by t-test (**P<0.01) from respective water treated adult rats.

Figure 3 (i) shows the effect of sting ED daily administration (9 ml for 14 days) on no. of entries in open arm in EPM test by pup rats. The data statistically analyzed by the t-test revealed that administration of ED significantly increased the no of entries in open arm (P<0.01) as compared to water treated controls, while withdrawal (figure 9) showed no significant effects (P>0.05) as compared to previously treated pups.

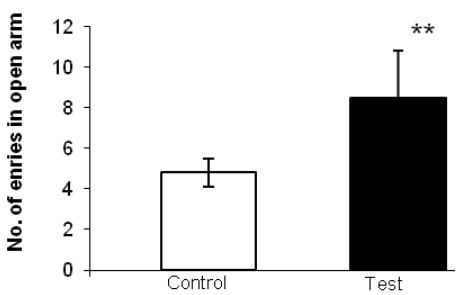

Figure 3(i): Effect of Sting ED on no. of entries in EPM in pups. Values are mean±SD (n=6) significant difference by t-test (**P<0.01) from respective water treated adult rats.

Figure 3 (ii) shows the effect of sting ED daily administration (9ml for 14 days) on time spent in EPM test by pup rats. The data statistically analyzed by t-test revealed that administration of ED significantly increased the time spent in open arm ($P<0.01$) as compared to their water treated adults while withdrawal (figure 9) decreased it ($P<0.01$) as compared to previously treated pups.

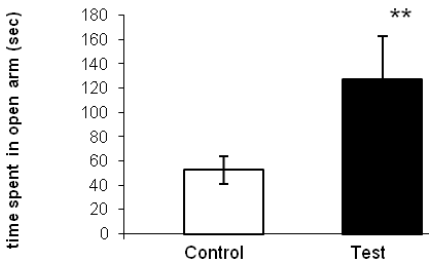

Figure 3(ii): Effect of Sting ED on time spent in EPM in pup. Values are mean±SD (n=6) significant difference by t-test (**$P<0.01$) from respective water treated adult rats.

Figure 4 (i) shows the effect of sting ED daily administration (9 ml for 14 days) on no. of entries in light compartment in light dark transition test by pup rats. The data statistically analyzed by t-test revealed that administration of ED increased the no. of entries in light compartment statistically ($P<0.05$) as compared to their water treated adults while withdrawal (figure 10) produced no significant effects ($P>0.05$) as compared to previously treated pups.

Figure 4 (ii) shows the effect of sting ED daily administration (9ml for 14 days) on time spent in light compartment in light dark transition test by pup rats. The data statistically analyzed by t-test revealed that administration of ED increased the time spent statistically ($P<0.05$) as compared to their water treated adults while withdrawal (figure 10) produced no significant effects ($P>0.05$) as compared to previously treated pups.

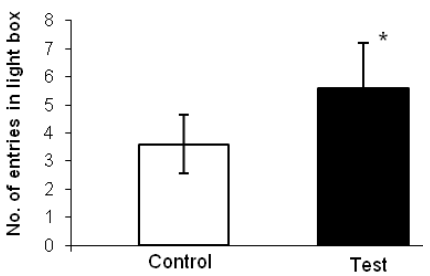

Figure 4(i): Effect of Sting ED on no. of entries in light compartment in pups. Values are mean±SD (n=6). Significant difference by t-test (*$P<0.05$) from respective water treated adult rats.

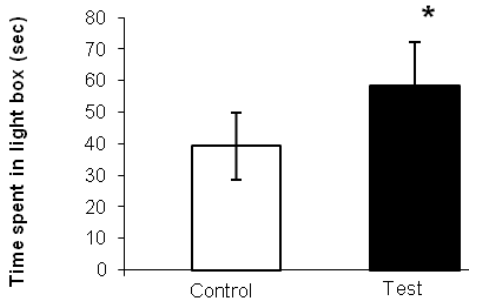

Figure 4(ii): Effect of Sting ED on time spent in light compartment in pups. Values are mean±SD (n=6). Significant difference by t-test (*$P<0.05$) from respective water treated rats.

Figure 5 shows the effect of sting ED daily administration (9ml for 14 days) on time to reach on escape platform in MWM test by pup rats. The data statistically analyzed by t-test revealed that administration of ED did not produce significant effect on time to reach the platform ($P>0.05$) as compared to their water treated adults while withdrawal (figure 11) decreased this time ($P<0.05$) as compared to previously treated pups.

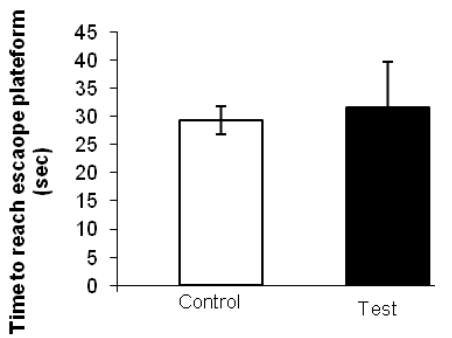

Figure 5: Effect of Sting ED on time to reach platform in MWM test in pups. Values are mean±SD (n=6). No significant difference by t-test from respective water treated adult rats.

Figure 6 shows the effect of sting ED daily administration (9ml for 14 days) on struggling time in FST by pup rats. The data statistically analyzed by t-test revealed that administration of ED increased the struggling time statistically ($P<0.01$).as compared to their water treated adults while withdrawal (figure 12) decreased this time ($P<0.05$) as compared to previously treated pups.

DISCUSSION

The objective of this pilot study was to evaluate the behavioral effects of short term exposure of sting and its withdrawal in pup rats.

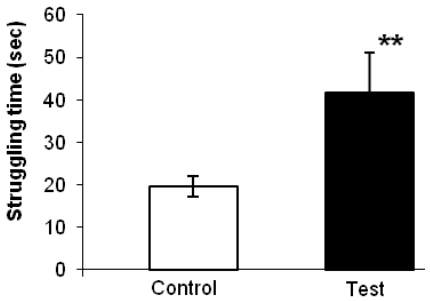

Figure 6: Effect of Sting ED on struggling time in FST in pup. Values are mean±SD (n=6). Significant difference by t-test (**P<0.01) from respective water treated adult rats.

Hyper locomotory effects of energy drink

The present findings help us to state that short term exposure produced hyperlocomotor effects in pups. These findings are also in agreement with our previous findings in adult rats[39]. Caffeine, taurine and ginseng are the important constituent of these energy drinks. Preclinical and clinical studies conducted on these individual components have shown to improve activity. Caffeine enhances Na^+/K^+ pump activity to potentiate excitation and contraction coupled with it. Caffeine acts antagonistically on adenosine receptors, thereby inhibiting the negative effects induced on neurotransmission such as arousal and pain perception.

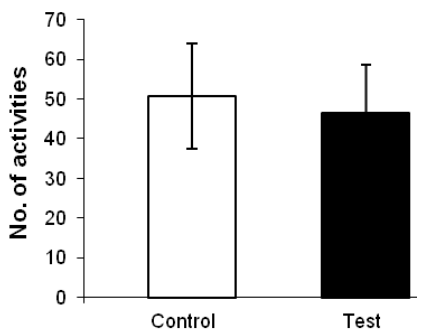

Figure 7: Effect of Sting ED withdrawal on home cage activity in pup rats. Values are mean±SD (n=3). No significant difference by t-test after withdrawal from ED from previously treated pups

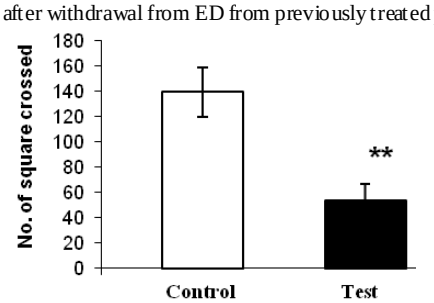

Figure 8(i): Effect of Sting ED withdrawal on no. of square crossed in open field activity by pup rats. Values are mean±SD (n=3) significant difference by t-test (**P<0.01) after withdrawal from ED in previously treated pups.

It produces hypoalgesic effects and decreases pain perception and blunted perceived exertion during exercise Moreover it increases Ca^{++} mobilization thus enhances excitation mechanism through second messenger system. It also potentially has favorable effects on negating decreased firing rates of motor units and produces forceful muscle contraction[46]. Caffeine delays depletion of muscle glycogen and enables for prolonged exercise. It mimics the effect of epinephrine, thus increases availability of ATP for muscle contraction and relaxation[16]. Caffeine activates 5'AMP-activated protein kinase (AMPK), a signalling intermediary implicated in the regulation of glucose, lipid, and energy metabolism in skeletal muscle. The two alpha isoforms of AMPK, AMPK alpha1 is predominantly activated by caffeine via an energy-independent mechanism and that activation of AMPKalpha1 increases glucose transport and (acetyl-CoA carboxylase (ACC) phosphorylation in skeletal muscle[47].

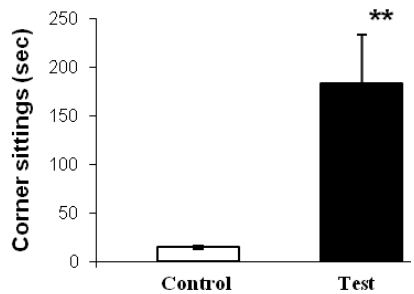

Figure 8(ii): Effect of Sting ED withdrawal on corner sitting in open field activity by pup rats. Values are mean±SD (n=3) significant difference by t-test (**P<0.01) after withdrawal from ED in previously treated pups.

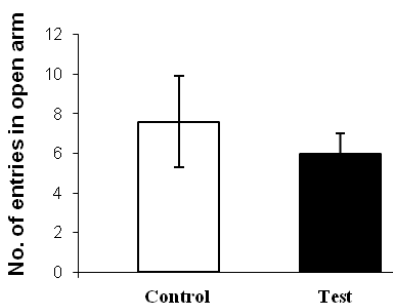

Figure 9(i): Effect of Sting ED withdrawal on no. of entries in EPM by pup rats. Values are mean±SD (n=3) no significant difference by t-test after withdrawal from ED in previously treated pup.

Taurine decreases accumulated lactate concentration thereby minimize pain and increases endurance performance during long running distance[48]. Taurine affects skeletal muscle contraction by decreasing oxidative stress via decreases the superoxide radicals' production which

causes damage in skeletal muscles after eccentric exercise[49]. Taurine supplementation for 15 days improves exhausted cycling test performance in humans because taurine promotes utilization of free fatty acid[50].It has been reported that taurine administration enhanced the activity of skeletal muscle glycolytic and oxidative enzymes (creatine kinase, lactate dehydrogenase, and phosphofructokinase), which catalyzes the required energy for muscle contraction[51].

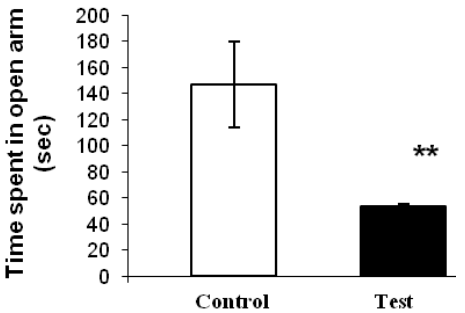

Figure 9(ii): Effect of Sting ED withdrawal on time spent in EPM by pup rats. Values are mean±SD (n=3) significant difference by t-test (**P<0.01) after withdrawal from ED in previously treated pups.

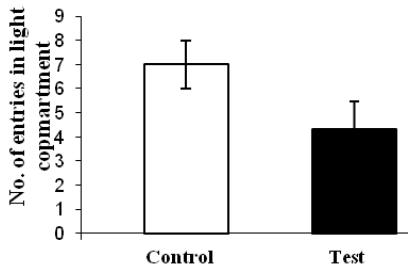

Figure 10(i): Effect of Sting ED withdrawal on no. of entries in light compartment by pup rats. Values are mean±SD (n=3). No significant difference by t-test after withdrawal from ED in previously treated pups.

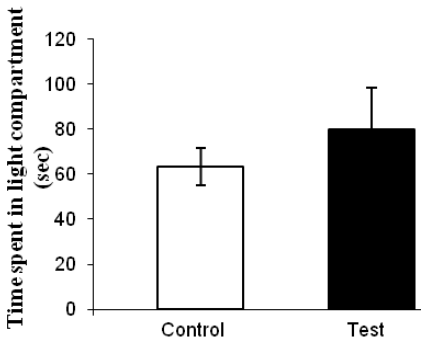

Figure 10(ii): Effect of Sting ED withdrawal on time spent in light compartment by pup rats. Values are mean±SD (n=3). No significant difference by t-test after withdrawal from ED in previously treated pups.

It has been reported that the synthesis of cyclic AMP (cAMP), a stimulator of glycolytic enzyme (phosphorylase) activity, is facilitated by the secretion of catecholamine, which occurs as a function of exercise intensity. Finally, cAMP production can be directly stimulated by taurine, through adenylyl cyclase activation[52].

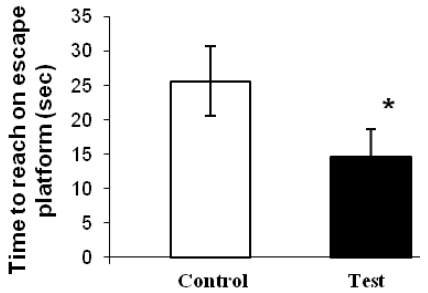

Figure 11: Effect of Sting ED withdrawal on time to reach escape in MWM test by pup rats. Values are mean±SD (n=3) significant difference by t-test (*P<0.05) after withdrawal from ED in previously treated pups.

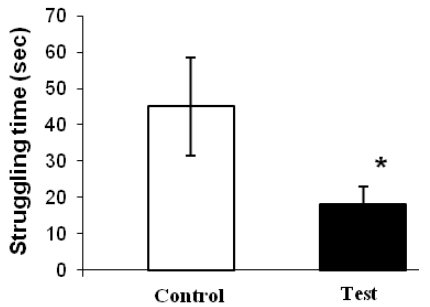

Figure 12: Effect of Sting ED withdrawal on struggling time in FST in pup rats. Values are mean±SD (n=3) significant difference by t-test (*P<0.05) after withdrawal from ED in previously treated pup.

Ginseng extracts increased performance significantly by increasing oxygen transport capacity to heart and shortening reaction time to visual stimuli[53]. Animal studies have shown that mice can swim faster and perform better in hot and cold environment and produces positive endurance after treatment with ginseng[54].Moreover highly caffeinated drinks render energy and stamina as it made up of natural ingredients[55].Caffeine, taurine and ginseng; they all potentiated the physical activity and athletic performance in present experimental animals. Many ingredients are believed to work synergistically with caffeine to boost its stimulatory power such as with taurine, caffeine enhances endurance performance[48]. Increased performance was observed during supplementation of carbohydrate, caffeine, taurine provided great strength during exercise and alternate maximal voluntary contraction (MVC) declination thus provide strength to CNS and skeletal muscles[56]

.It is possible that sting induced hyperlocomotory effects could be due to any of these mechanisms discussed above.

Anxiolytic effects of ED

It has been reported that ginseng affects the corticosterone secretion [57,] and also may inhibit synaptosomal uptake of norepinephrine, dopamine, 5-HT and GABA[58]. Ginseng produces anxiolytic effect by inactivation of GABA receptors[59,60]. Panax ginseng attenuate stress-induced brain and hypothalamic 5-HT and this mediatory action of 5-HT in anti-stress effects of panax ginseng may be modulated through prostaglandins [61].The sting induced anxiolysis observed in the present study could be due to the modulation of neurotransmitter functions or prostaglandins, which were however not monitored in this pilot study.

Effects of ED on memory

Ginseng increases learning and memory in rats in contrast to caffeine which increased physical but slow learning and mental performance[62]. Preclinical studies also demonstrate that ginseng can improve for a visual discrimination task and that the nootropic effect (memory -enhancing) may be related to changes in anxiety state[63]. Ginseng facilitates learning and memory by promoting hippocampal neuronal function of aged rats[64]. Our findings do not report any significant effects on spatial memory in pups. Its effects could have been antagonized due to the presence of sodium benzoate, a food preservative which impairs cognitive functions especially in children and produce attention deficit syndrome [34] and allura red, a food additive have long been suggested to adversely affect the learning and behavior in children[65].

Effects of ED on depression

Previous findings indicate that inositol and inositol-containing molecules, including phosphoinositides and inositol phosphates, have signaling and regulatory role in many cellular processes. This is suggested that depleting inositol may lead to perturbation of a wide range of cellular functions, may be associated with biopolar disorder[66].Ginseng also exerts antidepressant like effects in animal model of depression, this effect partly mediated through enhancing the monoamine neurotransmitter concentration in the hippocampus[67]. Ours was not although a depression model but it could be suggested that sting induced antidepressant like effects could be due to the modulation of monoamine transmitter concentration in the hippocampus.

Effects of sting withdrawal on behavioral activities

It is difficult to explain the withdrawal associated anxiogenic and depressant effects observed in pups in the present study, due to small "n" size during the withdrawal period. Some undesired responses might be elucidated due to sodium benzoate and tartazine dyes (components of ED) in children that are why it is not recommended for children. It impairs cognitive functions especially in children and produce attention deficit syndrome[48]. Sodium benzoate induced repeated episodes of acute urticaria or angio-oedema[68]. A variety of immunological responses i.e. skin disorders, depression, itching, blurred vision are associated to tartazine, an azo dye[69].Artificial food colouring and benzoate preservatives produced significant reductions in hyperactive behavior during the withdrawal phase[70].Sting induced hyperactivity Further in-depth study with a larger "n" would help us to suggest the safety of ED consumption in children, which would also assist us in devicing a mechanism relevant to its toxicity if any.

CONCLUSION

As mentioned initially, this pilot study was conducted with the aim to see the effects of sting ED in pups (that is children); although the exposure improved performance, however withdrawal exerted anxiogenic and depressant effect, so we can suggest that ED should be avoided by children. However extended preclinical and clinical studies are required to strengthen our finding. Small n size during withdrawal could be regarded as the limitation of the study.

REFERENCES

1. Canadean Wisdom Database. [Accessed August 15, 2011]. Available at: http://www.canadean.com/Products/Wisdom_Database.aspx.
2. Mucignat-Caretta C. Changes in female cognitive performance after energetic drink consumption: a preliminary study. *Prog. Neuropsychopharmacol. Biol. Psychiatry.*, 1998; 22: 1035–1042.
3. Seidl R, Peyrl A, Nicham R and Hauser E. A taurine and caffeine-containing drink stimulates cognitive performance and well-being. *Amino Acids*, 2000; 13: 635–642.
4. Alford C, Cox H and Wescott R. The effects of Red Bull Energy Drink on human performance and mood. *Amino Acids*, 2001; 21: 139–150.
5. Horne JA and Reyner LA. Beneficial effects of an "energy drink" given to sleepy drivers. *Amino Acids*, 2001; 20: 83–89.
6. Smit HJ, Grady ML, Finnegan YE, Hughes S-AC, Cotton JR and Rogers PJ. Role of familiarity on effects of caffeine- and glucose-containing soft drinks. *Physiol. Behav.*, 2006; 87: 287–297.
7. Gershon P, Shinar D and Ronen A. Evaluation of experience-based fatigue countermeasures. *Acid Anal. Prev.*, 2009; 41: 969–975.
8. Oddy WH and O'Sullivan TA. Energy drink for children and adolescent, erring on the side of caution may reduce long term health risk. *BMJ*, 2009; 339: b5268.

9. Grosz AS. The history, ingredients and effects of energy drinks. *Orv. Hetil.*, 2008; 149: 2237-2244.
10. Bigard AX. Risks of energy drink in youths. *Arch. Pediatr.*, 2010; 17: 1625-1631.
11. Cohen SM. Laurito CE and Curran MJ. Grand seizures in a postpartum patient following intravenous infusion of caffeine sodium benzoate to treat persistent headache. *J. Clin. Anaesth.*, 1992; 4: 48-51.
12. Holmgren P, Norden-Pettersson L and Ahlner J. Caffeine fatalities: four case reports. *Forensic Sci. Int.*, 2004; 139: 71-73.
13. Heatherley SV, Hancock KM and Rogers PJ. Psychostimulant and other effects of caffeine in 9- to 11-year-old children. *J. Child Psychol. Psychiatry.*, 2006; 47: 135–142.
14. Babu KM, Church RJ and Lewander W. Energy drinks: the new eye-opener for adolescents. *Clin. Pediatr. Emerg. Med.*, 2008; 9*:* 35–42.
15. Laurent D, Schneider KE, Prusaczyk WK, Franklin C, Vogel SM, Krssak M, Petersen KF, Goforth HW and Shulman GI. Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. *J. Clin. Endocriniol. Metab.*, 2000; 85: 2170-2175.
16. Heaney RP. Effects of caffeine on bone and calcium economy. *Food Chem. Toxicol*., 2002; 40: 1263-1270.
17. Temple JL. Caffeine use in children: what we know, what we have left to learn, and why we should worry. *Neurosci. Biobehav. Rev.*, 2009; 33: 793-806.
18. McCann D, barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw A, Kitchin E, Lok K , Porteous L, Prince E, Sonuga -Barke E, Warner J and Stevenson J. Food additives and hyperactive behavior in 3-year and 8/9-year-old children in the community: a randomized, double-blinded, placebo-controlled trial. *Lancet*, 2007; 370: 1560.
19. Gaull GE. Taurine inpediatric nutrition. *J. Pediatr.*, 839: 433-442.
20. Ito T, Oishi S,Takai M, Kimura Y, Uozumi Y, Fujio Y, Schaffer SW, Azuma J. Cardiac and skeletal muscle abnormality in taurine transporter-knockout mice. *J. Biomed. Sci*., 1989;17: S20.
21. Dawson R Jr, Biasetti M, Messina S and Dominy J. The cytoprotective role of taurine in exercise-induced muscle injury. *Amino Acid*, 2002; 22: 309-324.
22. Sved DW, Godsey JL, Ledyard SL, Mahoney AP, Stetson PL, Ho S, Myers NR, Renis P. Absorption, tissue distribution, metabolism and elimination of taurine given orally to rats. *Amino Acids*, 2007; 32: 459-466.
23. Eppler B, Patterson TA and Zhou W. Kianic acid (KA) induced seizures in Sprague-Dawley rats and the effect of dietary taurine supplementation or deficiency. *Amino Acids*, 1999: 16: 133-147.
24. Kennedy DO, Scholey AB. Ginseng: potential for the enhacement of cognitive performance and mood. *Pharmacol. Biochem. Behav*., 2003; 75: 687-700.
25. Kim NH, Kim KY, Kim Hm, Jeong HJ. Antidepressant-like effect of altered Korean red ginseng in mice. *J. Behav. Med.*, 2011; 37: 42-46.
26. Liu Y, Li X, Yuan HF.. Progress of research on effects of ginsenoside Rg1 in promoting capability of learning and memory. *Zhongguo Zhong Xi Yi Jie He Za Zhi*., 2006; 26): 956-960.
27. Nagoba BS, Punpale AS, Ayachit R, Gandhi RC, Wadher BJ. Citric acid treatment of postoperative wound in an operated case of synovial sarcoma of the knee. *Int.Wound J.*, 2011; 8: 425-427.
28. Zacchia M, Preisig P. Low urinary citrate: an over view. *J. Nephrol*., 2010; 16: S49-56.
29. Li D ,Sun WP, Zhou YM, Liu QG, Zhou SS, Luo N, Bian FN, Zhao ZG, Guo M..Chronic niacin overload may involve in the increased prevelance of obesity in US children. *World J. Gastroenterol*., 2010; 16: 2378-2387.
30. Lerner V, Miodownik C, Kapstson A,Cohen H, Loewenthal U, Kotler N.. Vitamin B6 as add-on treatment in chronic schizophrenic and schizoaffective patients: a double-blinded, placebo-controlled study. *J. Clin. Psychiatr*., 2002; 63: 54-58.
31. Smith AD, Refsum H. Vitamin B-12 and cognition in the elderly. 2009; 89: 707S-11S.
32. Hooper NM. Glycosyl-phosphatidylinositol anchored membrane enzyme. *Clin. Chim. Acta*., 1997; 266: 3-12.
33. Kulkulijan M, Vergara L, Stojilkovic SS. Medulation of kinetics of inositol 1,4,5-triphosphate induce calcium oscillation by calcium entry in pituitary gonadotrophs. *Biophys. J.*, 1997; 72: 698-707.
34. Boris M, Mandel FS. Foods and additives are common causes of the attention deficit hyperactive disorder in children. *J. Ann. Allergy*, 1994;72: 462-468.
35. Mamur S, Yuzbasiogle D, Unal F, Yilmaz S.. Does potassium sorbate induce genotoxic or mutagenic effects in lymphocytes? *J. Toxicol. Invitro*., 2010; 24: 790-1567.
36. Ruth DeBusk et alEthylene diamine tetracetic acid .2002;.[26.1.2012].
37. Sara M. Seifert, Judith L. Schaechter, Eugene R. Hershorin and Steven E. Lipshultz.. Health effects of Energy drinks on children, adolescent and young adults. *Pediatrics*, 2011; 127; 511.
38. Wolk BJ, Ganestsky M, Babu KM. Toxicity of energy drinks. *Curr. Opin. Pediatr.*, 2012: 24: 243-251
39. Rehman M, Bashir S, Naz H:Does short term consumtion of energy drinks and its subsequent withdrawal ptoduce behavioral toxicities?A pilot study in Adult male rats. *Pak. J. Biochem. Mol. Biol*., 2012; 45: 49-58.
40. Kliethermes CL, Finn DN and Crabbe C. Validation of a modified mirrored chamber sensitive to anxiolytics and anxiogenics in mice. *Psychopharmacology*, 2003; 169: 190–197.
41. Wallis CJ and Rezazadeh SM. GM1 ganglioside reduces ethanol intoxication and the development of ethanol dependence. *Alcohol*, 1995; 12**:** 573–580.
42. Morris RG. Spatial localization does not require the presence of local cues. *Learning and Motivation*. 1981; 12: 239-260.
43. Brocardo PS, Budni J, Kaster MP, Santos AR and Rodrigues AL. Folic acid administration produced antidepressant-like effect in mice: evidence for the involvement of the serotonergic and noradrenergic systems. *Neuropharmacology*, 2008; 54: 464-473.
44. Kaster MP, Ferreira PK, Santos AR and Rodrigues AL. Effects of potassium channel inhibitors in the forced swim test:possible involvement of l-arginine-nitric oxide-soluble guanylate cyclase pathway. *Behavior Brain Res*., 2005; 165: 204-209.
45. Porsolt RD, Anton G and Deniel M. Behavioral despair in rats: A new model sensitive to antidepressant treatments. *Eur. J. Pharmacol*., 1978; 47: 379-391.
46. Davis JK and Green JM. Caffeine and anaerobic performance: ergogenic value and mechanisms of action. *Sports Med*., 2009; 39: 813-32.
47. Imagawa TF, Hirano I, Utski K K, Horie M, Naka A, Matsumoto K and Imagawa S. Caffeine and taurine enhance endurance performance. *Int. J. Sports Med.*, 2009; 30: 485-488.
48. Silva LA, Silveira PC, Ronsani MM, Souza PS, Scheffer D, Vieira LC, Benetti M, De Souza CT and Pinho RA. Taurine supplementation decreases the oxidative stress in skeletal muscles after eccentric exercise. *Cell Biochem. Funct*., 2011; 29: 43-49.
49. Yeh TS, Chan KH, Hsu MC and Liu JF. Supplementation with soybean peptides, taurine, Pueraria isoflavone, and

ginseng saponin complex improved endurance exercise capacity in humans. *J. Med. Food*, 2011; 14: 219-225.
50. Takekura HH, Tanaka M, Watanebe T and Yoshioka MO. Effects of taurine on glycolytic and oxidattive enzyme activities of rats skeletal muscles. Sulphur -containing *Amino Acids*,1986; 125-132.
51. Raizada M and Hrishana MCR. Binding of taurine to Hartmannella culbertsoni membranes and synthesis of cyclic AMP. *Curr. Sci.*, 1973; 42: 202-204.
52. Forgo I and Schimert G. The duration of effects of standardized ginseng extract G115 in healthy competitive athletes (in German). *Notobene Mddici*., 1985; 15: 636-640.
53. Ramachandran U, Divekar HM, Grover SK and Srivastava KK. New experimental model for evaluation of adaptogenic products. *J. Ethnopharmacol*., 1990; 29: 275-281.
54. McGuinness TM and Fogger S. Update on energy drinks and youth. *J. Psychosoc. Nurs. Ment. Health Serv.*, 2011; 49: 17-19.
55. Ganjo MS, Klau JF, Lee EC, Yeargin SW, McDermott BP, Buyckx M, Maresh CM and Armstrong LE. Effects of various carbohydrate-electrolyte fluids on performance and maximal voluntary contraction. *Int. J. Sports Nutr. Exerc. Metab.*, 2010; 20: 104-114.
56. Hiai S, Yokoyama H, Oura H and Kawashima Y. Evaluation of corticosterone serration-inducing activities of ginsenoisoides and their prosapogenins and sapogenins. *Chem Pharm. Bull.*, 1983; 31: 168-174.
57. Tsang D, Yeung HW, Tso WW and Peck H**.** Ginseng saponins: Influence on neurotransmiter uptake in rat brain synaptosomes. *Planta Med*., 1985**;** 47: 221-224.
58. Jiang S, Miao B, Song X and Jiang Z. Inactivation of GABA (A) receptor reduces ginsenoside Rb3 neuroprotection in mouse hippocampal slices after oxygen-glucose deprivation. *J. Ethnopharmacol.*, 2011; 133: 914-916.
59. Kim TW, Choi HJ, Kim NJ, Kim DH. Anxiolytic-like effects of ginsenosides Rg3 and Rh2 from red ginseng in the elevated plus-maze model. *Planta Med*., 2009; 75: 836-839.
60. Bhattcharyya D, Sur TK. Effect of Panax ginseng and diazepam on brain 5-hydroxytryptamine and its modification by diclofenac in rat. *Indian J. Physiol. Pharmacol.*, 1999; 43: 505-509.
61. Saito H, Tsuchiya M, Naka S, Takagi K. Effects of panax ginseng root on conditioned avoidance response in rats. J. Pharmacol., 1977; 27: 509-516.
62. Churchill JD, Gerson JL, Hinton KA, Mifek JL, Walter MJ, Winslow CL, Deyo RA. The nootropic properties of ginseng saponin Rb1 are linked to effects on anxiety. *Inegr. Physiol. Behav. Sci*., 2002; 37: 178-187.
63. Liu M. Studies on the anti-aging and nootropic effects of ginsenoside Rg1 and its mechanisms of action. *Sheng Li Ke Xue Jin Zhan*., 1996; 27: 139-142.
64. Doguc DK, Ceyhan BM, Ozturk M and Gultekin F. Effects of maternally exposed coloring food additives on cognitive performance in rats. *Toxicol. Ind. Health*. 2012; [Epub ahead of print]
65. Deranieh RM, Greenberg ML. Cellular consequences of inositol depletion. *Biochem. Soc. Trans*., 2009; 37: 1099-1103.
66. Dang H, Chen Y, Liu X, Wang L, Jia W and Wang Y. Antidepressant effects of ginseng total saponins in the forced swimming test and chronic mild stress models of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatr.*, 2009; 33: 1417-1424.
67. Nettis E, Colanardi MC, Ferrannini A and Tursi A. Sodium benzoate-induced repeated episodes of acute urticaria/angio-oedema: randomized controlled trial. *J. Br.* 2004; 151: 898-902.
68. Rowe KS and Row KJ. Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. The J. Pediatr., 1994; 125: 691-698.
69. Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C and Stevenson J. The effects of a double blind, placebo controlled artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Arch. Dis. Child.*, 2004; 89: 506-511.