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Does short term consumption of energy drink and its subsequent withdrawal produce behavioral toxicities? A pilot study in adult male rats

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Abstract: Energy drinks such as Sting, Red bull, Full Throttle, Rockstar, Monster are thronging the local market, excessively advertised and readily incorporated into a routine, as a part of healthy life style. Some of these energy drinks have already been banned by Denmark, Germany and Australia. Since these drinks claim to boost instant energy, with prolonged physical endurance; their consumption is increasing day by day. These drinks are combination of herbs, stimulants, sugars, vitamins and other food additives and have a claim that their synergistic effects could improve energy, boost stamina and performance. Their increased consumption without awareness of the side effects might pose a threat to the well-being of a person and could precipitate adverse effects, in terms of toxicities. This concern has evoked us to focus our research based on animals, male albino wistar rats exposed to energy drink (9ml / day) for 14 days, compared to their water administered counterparts and its subsequent withdrawal (14 days). Behavioral activities were monitored on the 13th day. Home cage and open field activity were increased upon administration, while withdrawal tended to show a decline in the locomotor effects. Animals in elevated plus maze (EPM) and light/dark (anxiety measurement paradigms) exhibited anxiolysis upon administration, but not on withdrawal. Sting administered animals exhibited significant antidepressant effect in forced swim test (FST) these effects were not observed after withdrawal. Administration impaired long term memory in Morris water maze (MWM) but memory was improved upon withdrawal. These results are discussed in terms of the mechanism induced by synergistic components of Sting, associated with the behavioral toxicities observed. These results suggest that although Sting has shown very promising results in most of behavioral experimental paradigms but Sting induced impairment of memory during consumption could be related to its toxic side effects. Moreover long term studies could help us to strengthen this notion in exploring other Sting induced toxicities with larger n (number of animals). Toxicity surveillance of ED is essential because most of ingredients are under studied and not regulated. This might be a preliminary preclinical approach to establish awareness in Pakistani population with reference to energy boosting beverages.

Keywords: Energy drink, caffeine, ginseng, taurine, elevated plus maze, forced swim test, Morris water maze.
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INTRODUCTION

The history of energy drinks (ED) can be traced back to as old as 1901, where it first originated from Scotland by the name of "Iro-Bru". Then in 1992, at a hospital in UK, as an aiding supplement named as "Lucozade Energy". Gradually it became more popular around the world[1] and now also in Pakistan. They have gained popularity because media is promoting them as "Speed in a can". People prefer these drinks as they think they are harmless and are known as instant energizers. They have been incorporated as a daily requirement, to cope up with the hectic routine and to prolong efficiency in order to meet the challenging tasks. Its various constituents include herbs as ginseng, stimulants as caffeine, vitamins as B_3, B_6, B_{12}, sugars as glucoronolactone, amino acids as taurine and other food additives, such as preservative like sodium benzoate, which are known to promote beneficial effects. Many ingredients are believed to work synergistically with caffeine to potentiate working capability[2].

Caffeine is one of the major constituent of ED and legal stimulant of central nervous system (CNS). It mimics the effects of epinephrine thus increases availability of ATP for muscles contraction and relaxation[3]. It is helpful in oxidative stress[4]. It may reduce neuroinflammation directly by blocking adenosine receptors[5]. It is benzodiazepine receptor antagonist[6]. ED often contains additional amounts of caffeine through additives, including guarana, kola nut, yerba mate and cocoa[7].

Taurine, another important constituent; a sulphur containing amino acid is present abundantly intracellularly as well as in diet[8]. It has role in cytoprotection against exercise induced injury[9], its high levels produce defense against free radical mediating damage[10]. It potentiates calcium movement and cytoprotection in cardiac and skeletal muscles[11]. The role of taurine in CNS effects is unclear, as experimental animals could not demonstrate effects on brain taurine levels[12].

Ginseng is the key ingredient of ED. It is a herb which contributes in the enhancement of cognitive performance and elevates mood[13]. It possesses immunoprotectant role[14]. It is very effective against depression[15]. Panax ginseng attenuates stress induced brain and hypothalamic 5-HT levels[16]. It increases performance significantly via increasing oxygen transport towards heart[17].

Inositol another component of ED, plays a vital role in management of depression and also has role in propagation of second messenger system[18]. Citric acid, a natural preservative possess antioxidant property[19], therefore is used in ED. Citrate is potent

inhibitor of stone formation[20]. Niacin is a CNS stimulant; used for cognitive impairment therapy[21], is also a constituent of ED. It also raises high density lipoprotein[22]. Pyridoxine, Vitamin B_6, is involved in the synthesis of certain neurotransmitters[23]. It possesses antioxidant properties[24] like citric acid and prevents against neurotoxicity [25]cobalamine, vitamin B_{12} is present in ED, its deficiency cause DNA damage in children[26].

Calcium disodium EDTA, a chelating agent, and is included in composition of ED used in therapies against heavy metals. Sodium hexameta phosphate, a sequestrant and defflocculant and has antimicrobial profile[27]. Potassium sorbate, a preservative retards antimicrobial growth in food[28]. Sodium benzoate also a preservative, has bacteriostatic and sunscreen profile[29]. Other essential components include; tartazine, sunset yellow used in yellow colored azo dye and allura red, red colored azo dye.

Excessive intake of ingredients of EDs, with the changes in lifestyle and excessive marketing via media; it is envisaged that consumption of energy drinks could be increased, as they are thought to be harmless. Documented reports provide evidence that such drinks are banned in Australia, Denmark, Germany and Turkey [30], but excessively marketed locally now these days. The current preclinical study, which will be followed by investigating consumption consequences in adults, in an initiation to create awareness in general public (local population).

MATERIALS AND METHODS

Animals

Locally bred albino wistar male rats, purchased from Ojha campus (Dow University of Health Sciences). Animals weighing of 100-140gm, 3-4 months old. The rats were housed in cages together with saw dust covered floor and in quite room, with free excess 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

Energy drink (sting) was purchased from local market.

Trial and error

The administration of ED was evaluated using trial and error method. ED dose was administered in volume of 3, 6 and 12ml. Sting induced hyperactivity was monitered after 15 minutes, in an open field activity model. Hyperactivity was observed at doses of 6 and 12ml, while an intermediate dose of 9ml was selected for purpose of administration.

Experimental design

The experiment was conducted on 12 albino wistar rats of 3-4 months old, were randomly divided into two groups, six males as control and six males as test. The adult animals taken as test were orally administered with 9ml of ED with the help of syringe daily between 12:00 to 13:00 hours, while control adults were administered with the same amount of water for 14 days. On the 13th day following behavioral activities were monitored; home cage, open field activity, elevated plus maze, light/dark transition, Morris water maze and forced swim test. On the 14th day food intake and body weight were monitored. The animals were then subdivided into two groups. 3 animals from each group were decapitated and their brains were dissected out and plasma was collected. Samples were stored at -70^{o}C for the neurochemical analysis in the next phase of study. The remaining three controls and test were left for withdrawal from energy drink. After withdrawal of 14 days all behavioral activities were monitored before decapitation. The activities of previously decapitated 3 test rats were used as control and compared with withdrawal rats.

Behavioral techniques

Measurement of food intake

A weighed amount of food was placed in the hopper of the cages of the animals and food intake was calculated by subtracting the remaining food from the weighed amount after the treatment.

Measurement of body weight

Body weight was measured before and after the treatment.

Home cage activity

A transparent square box 26×26×26inches 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 apparatus was 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 and observed for 5 minutes. Latency to move was also monitored. Corner sittings were taken as sign of lethargy.

Elevated plus maze test

The elevated plus maze, currently the most frequently performing test, consisted of four identical arms 50×10cm, two open arms and two closed arms with wall 40cm high radiating from a

central square. Drugs with known anxiolytic effects in human would increase the time that rodents spend on the normally aversive open arm of the maze, while anxiogenic drugs decrease this time[31,32].

Light dark transition test

This test used to monitor anxiolytic effects of drug was conducted in a two chambered compartment. The joined chambers are of equal size 26×26×26cm. One is light and other is dark, both are of same size. There is a small opening of about 12×12cm between two compartments. Rat is placed in light compartment of the light box, animal goes to the dark box because of their nocturnal nature and comes back to explore the environment because of anxiolytic response of drug. No. of entries and time spent in the light compartment are interpreted as a signs of anxiolysis for 5 minutes[33].

Morris water maze test

In 1981, Richard G. Morris a neuroscientist developed this test for evaluation of long term spatial memory. It consists of a big round pool filled with water having a hidden escape platform. The pool is 1.2-1.8 meters in diameter and 60cm deep. By adding milk, water is made opaque so that rat could not see the escape[34]. The rat was placed facing opposite to the platform and left to explore the platform. The cut off time was recorded taken to track the platform.

Forced swim test

This test is used to monitor depression in experimental animals. This model consists of open glass cylindrical container of diameter 10cm, height 60cm and half filled with water. Rats were placed in this model, from which they cannot escape and are forced to swim, after vigorous activity they adopt a characteristic immobile posture which can be readily identified. This immobility is reduced by various clinically effective antidepressant drugs[35]. The cut off time is five minutes[36,37]

RESULTS

Effects of sting ED administration in adult male rats

Figure1 shows the effect of sting ED daily administration (9ml/day for 14 days) on food intake in adult rats. The data statistically analyzed by the t-test revealed that administration of ED decreased the food intake but not significant statistically (P>0.05). Figure 2 shows the effect of sting ED daily administration (9ml/day for 14 days) on body weight in adult rats. The data statistically analyzed by t-test revealed that administration of ED produced no significant effect (P>0.05).

Figure 3 shows the effects of sting ED daily administration (9ml/day for 14 days) on home cage activity test in adult rats. The data statistically analyzed by the t-test revealed that ED administration increased the locomotor activity but was not significant statistically (P>0.05). Figure 4(a) shows the effect of sting ED daily administration (9ml/day for 14 days) on no. of squares crossed in open field activity test in adult rats. The data statistically analyzed by t-test revealed that administration of ED increased the ambulatory activity statistically (*P<0.05). Figure 4(b) **s**hows the effect of sting ED daily administration (9ml/day for 14 days) on corner sittings in open field activity test in adult rats. The data statistically analyzed by t-test revealed that administration of ED decreased the corner sittings (**P<0.01).

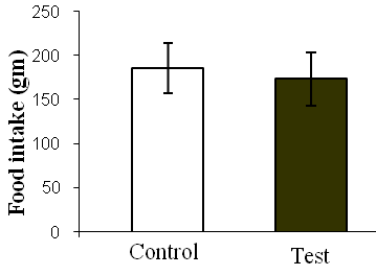

Figure 1: Effect of administration of Sting ED for 14 days in adult male rats on food intake.

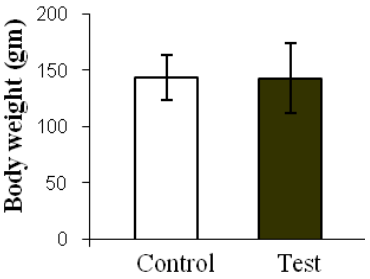

Figure 2: Effect of administration of Sting ED for 14 days in adult male rats on body weight.

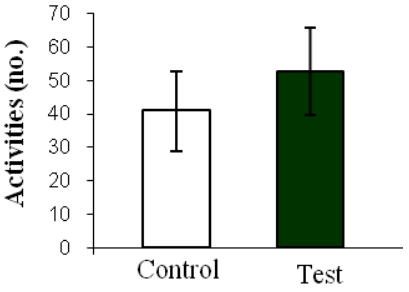

Figure 3: Effect of administration of Sting ED for 14 days in adult male rats on home cage activity.

Figure 5(a) shows the effect of sting ED daily administration (9ml/day for 14 days) on no. of entries in open arm in EPM test by adult 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). Figure 5(b) shows the effect of sting ED daily administration (9 ml/day for 14 days) on time spent in EPM test by adult rats. The data statistically analyzed by t-test revealed that administration of ED significantly increased the time spent in open arm (**P<0.01).

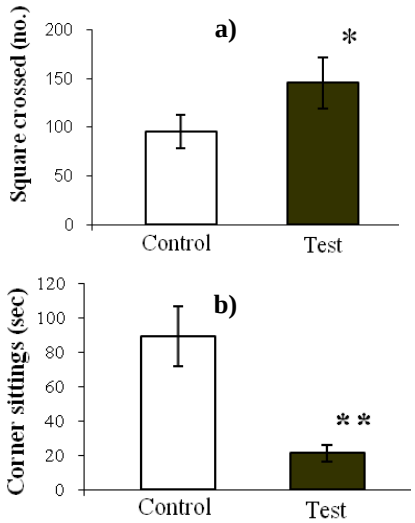

Figure 4: Effect of administration of Sting ED for 14 days in adult male rats on (a) on square crossing (b) corner sittings in open field activity. Significant difference by t-test (*P<0.05) and (**P<0.01) from 14 days administration of water treated adult male rats

Figure 6(a) shows the effect of sting ED daily administration (9ml/day for 14 days) on no. of entries in light compartment in light dark transition test by adult 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). Figure 6(b) shows the effect of sting ED daily administration (9ml/day for 14 days) on time spent in light compartment in light dark transition test by adult rats. The data statistically analyzed by t-test revealed that administration of ED increased the time spent (**P<0.01). Figure 7 shows the effect of sting ED daily administration (9ml/day for 14 days) on time to reach on escape platform in MWM test by adult rats. The data statistically analyzed by t-test revealed that administration of ED significantly increased the time to reach escape platform (**P<0.01). Figure 8 shows the effect of sting ED daily administration (9ml/day for 14 days) on struggling time in FST by adult rats. The data statistically analyzed by t-test revealed that administration of ED increased the struggling time statistically (**P<0.01).

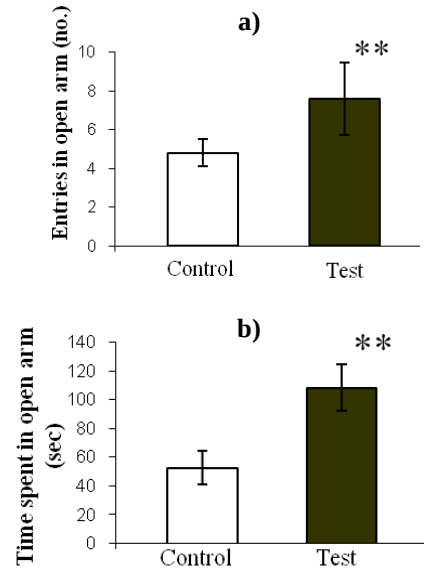

Figure 5: Effect of administration of Sting ED for 14 days in adult male rats on (a) no. of entries (b) time spent in open arm. Significant difference by t-test (**P<0.01) from 14 days administration of water treated adult male rats.

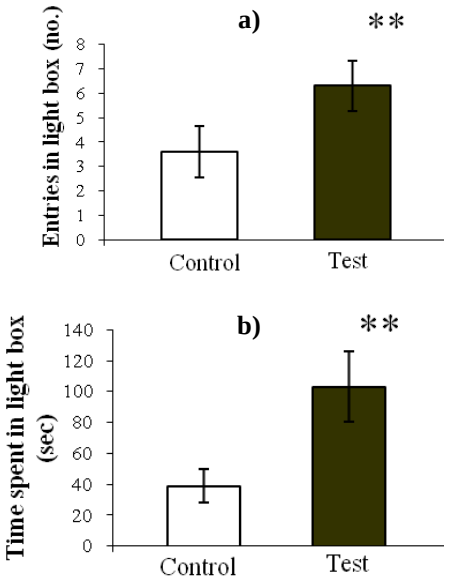

Figure 6: Effect of administration of Sting ED for 14 days in adult male rats on (a) no. of entries (b) time spent in light compartment. Significant difference by t-test (**P<0.01) from 14 days administration of water treated adult male rats.

Effects of withdrawal from sting ED in adult male rats

Figure 9 shows the effect of withdrawal of 14 days from sting ED on food intake in adult rats. The

data statistically analyzed by the t-test revealed that withdrawal from ED decreased the food intake but not significant statistically (P>0.05). Figure 10 shows the effect of withdrawal of 14 days from sting ED on body weight in adult rats. The data statistically analyzed by t-test revealed that withdrawal from ED produced no significant effect (P>0.05). Figure 11 shows the effect of withdrawal of 14 days from sting ED on home cage activity test in adult rats. The data statistically analyzed by t-test revealed that withdrawal from ED produced no significant effect (P>0.05).

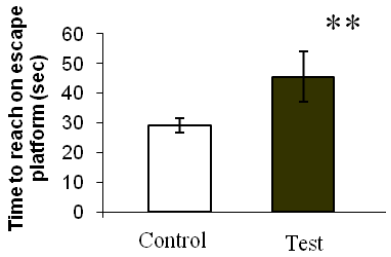

Figure 7: Effect of administration of Sting ED for 14 days in adult male rats on time to reach escape in MWM test. Significant difference by t-test (**P<0.01) from 14 days administration of water treated adult male rats.

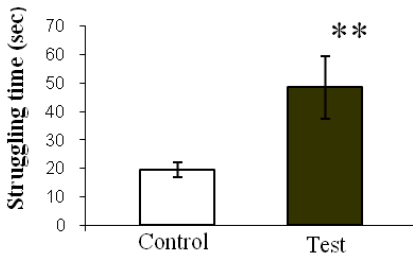

Figure 8: Effect of administration of Sting ED for 14 days in adult male rats on struggling time in FST. Significant difference by t-test (**P<0.01) from 14 days administration of water treated adult male rats.

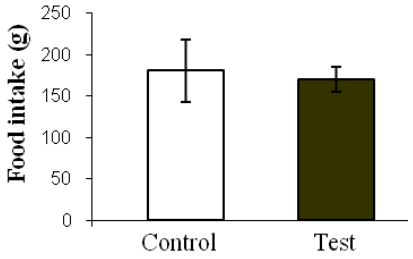

Figure 9: Effect of withdrawal of 14 days from Sting ED in adult male rats on food intake.

Figure 12(a) shows the effect of withdrawal of 14 days from sting ED on no. of squares crossed in open field activity test in adult rats. The data statistically analyzed by t-test revealed that ED withdrawal decreased the no. of square crossed but were not significant statistically (P>0.05). Figure 12(b) shows the effect of withdrawal of 14 days from sting ED on corner sittings in open field activity test in adult rats. The data statically analyzed by t-test revealed that ED withdrawal increased corner sittings statistically (**P<0.01). Figure 13(a) & (b) show the effect of withdrawal of 14 days from sting ED on no. of entries and time spent in open arm in EPM test by adult rats revealed that ED withdrawal produced no significant effect on the no. of entries and time spent in open arm (P>0.05).

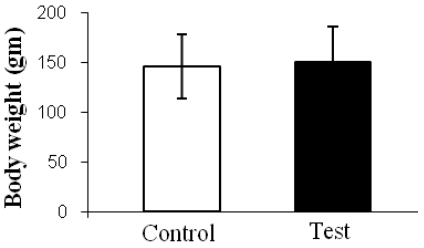

Figure 10: Effect of withdrawal of 14 days from Sting in adult male rats on body weight.

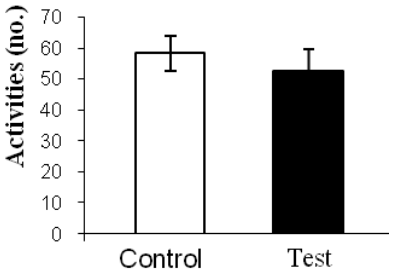

Figure 11: Effect of withdrawal of 14 days from Sting ED in adult male rats on home cage activity.

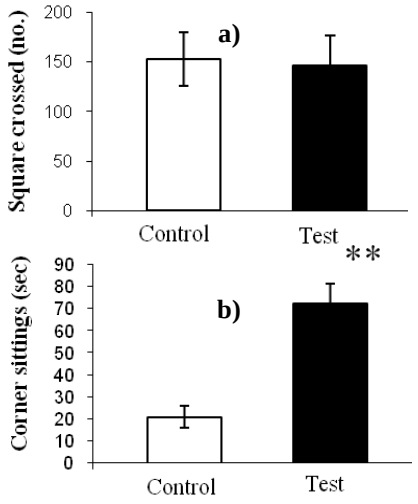

Figure 12: Effect of withdrawal of 14 days from Sting ED in adult male rats on (a) no. of square crossed (b) corner sitting in open field activity. Significant difference by t-test (**P<0.01) after respective withdrawal of 14 days in Sting ED treated adult male rats.

Figure 14 (a) and (b) show the effect of withdrawal of 14 days from sting ED on no. of entries and time spent in light compartment of light dark transition test in adult rats. The data statistically analyzed by t-test revealed that ED withdrawal produced no effect on the no. of entries and time spent in light compartment (P>0.05). Figure 15 shows the effect of withdrawal of 14 days from sting ED on time to reach on escape platform in MWM test by adult rats. The data statistically analyzed by t-test revealed that ED withdrawal decreased the time to reach on escape platform (**P<0.01). Figure 16 shows the effect of withdrawal of 14 days from sting ED on struggling time in FST by adult rats. The data statistically analyzed by t-test revealed that ED withdrawal produced no significant effect (P>0.05).

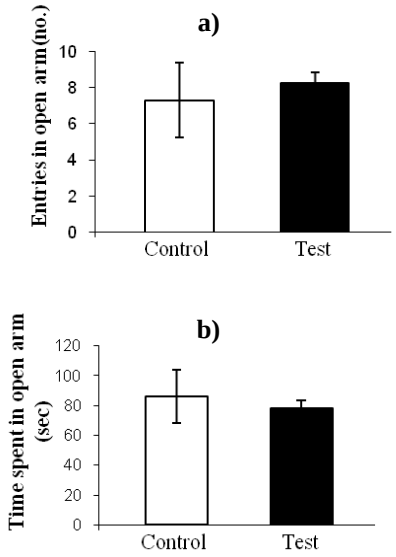

Figure 13: Effect of withdrawal of 14 days from Sting ED in adult male rats on (a) no. of entries (b) time spent in EPM.

DISCUSSION

The following study was designed to address two aspects of energy drinks consumption on behavioral toxicities: 1. Behavioral toxicities associated with consumption. 2. Behavioral toxicities precipitated after withdrawal. The preclinical findings of this multisubstance exposure have demonstrated that energy drink consumption resulted in hyperactivity, anxiolysis and antidepressant effects, which in a way meets the claims of sellers as effective energizer and stamina enhancer while Sting induced hyperactivity was not observed after withdrawal. Literature survey has also shown that these energy drinks are becoming popular day by day, despite the fact that certain countries have posed a ban on these drinks[38]. Energy drinks, a combination of caffeine, taurine, ginseng and may other food additives are taken by the adults and youth for a desire to increase energy[30]. It has been also reported that energy drinks are mixed with alcoholic drinks, while partying[39]. These beverages are marketed to improve energy, weight loss, stamina, athletic performance and concentration[40].

The popular media is heavily marketing it for beneficial effects. The data in the present study also compliments some of the beneficial effects of these energy drinks; in terms of increased activity, anxiolysis and antidepressant effects in animals as observed, in various behavioral apparatus.

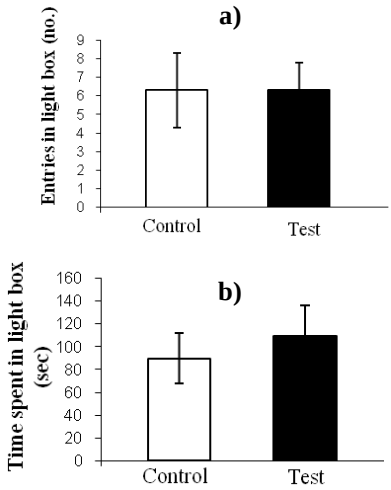

Figure 14: Effect of Sting ED withdrawal of 14 days from Sting ED in adult male rats on (a) no. of entries (b) time spent in light compartment.

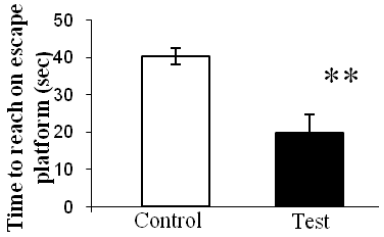

Figure 15: Effect of withdrawal of 14 days from Sting ED in adult male rats on time to reach escape in MWM. Values are means ± S.D (n=3). Significant difference by t-test (**P<0.01) after respective withdrawal of 14 days from Sting ED in adult male rats.

Caffeine is one of the main ingredients in energy drinks; many of them contain 70-80oz serving more than 3 times of the concentration in cola drinks[41]. It is possible that enhanced behavioral activity in the present study could be due to the previously described mechanism induced by

caffeine. It mimics the effect of epinephrine, thus increases availability of ATP for muscle contraction and relaxation[42]. Clinical studies have shown that caffeine consumption should not exceed 100 mg/kg/day for adolescent[43]. Since its beneficial effects are numerous and tempting there is a many fold chance of overdose or increased consumption/ excessive intake of such beverages. It has been reported that high doses of caffeine increase urine flow, sweat excretion and alter blood electrolyte levels[44]. Adverse effects due to excessive dosage can cause nervousness, irritability, anxiety, insomnia, tachycardia, palpitations, stomach upsets, vomiting, abdominal pain, rigidity, hypokalemia, altered consciousness, paralysis, hallucinations, increased intracranial pressure, cerebral edema, seizures, rhabdomyolysis, supraventricular and ventricular tachyarrhythmias[45,46].

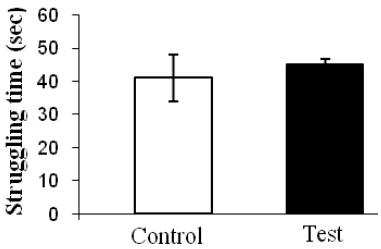

Figure 16: Effect of withdrawal of 14 days from Sting ED in adult male rats on struggling time in FST.

It has been documented that along with regulated amount of caffeine listed on the can, an additional quantity is also being pooled by guarana, which provides 40-80mg of caffeine. Manufacturers are not required to list the caffeine content from these ingredients[43]. Thus the actual caffeine dose in a single serving may exceed that of listed[47]and may cause overdose of caffeine and its associated adverse effects[41]. The enhanced activity could also due to be another component taurine, which could have increased glycolysis and oxidative enzymes in the skeletal muscles. It potentiates calcium movement and cytoprotection in cardiac and skeletal muscles [11]. Excessive consumption of taurine causes hypoglycemia, however taurine hypoglycemic consequences are less likely to precipitate because caffeine and sugar synergistically precipitate hyperglycemia[44], an increased risk to stroke[45] and diabeties[48]. Ginseng increases performance significantly via increasing oxygen transport towards heart[17]. Literature survey has shown that excessive dosage includes diarrhea, vaginal bleeding, headache, vertigo, mania, hypertension, rashes, insomnia, irritability, Steven-Johnson syndrome, agranulocytosis[46]. It is possible that an immediate effect could be due to the synergistic effects of all these ingredients that could have boosted the stimulatory power, which was translated into increased activities of the animals as observed in the present study. The same may also imply on humans but may also pose a risk to obesity[49].

Niacin, a CNS stimulant used for cognitive impairment therapy[21] and is an important constituent of these beverages. Extremely high doses of niacin can also cause niacin reversible maculopathy, a thickening of the macula and retina, which leads to blurred vision and blindness[50]. Azo dyes are reported to produce hyperactivity in children[51]. Tartazine, azo dye a component of ED, produces immunological responses such as depression skin disorder, itching[52], also can produce anaphylactic reactions[53]. Sunset yellow produces adverse biochemical and reproductive effects[54], other side effects include diarrhea, vomiting, gastric upset and migraines[55]. These azo dyes, tartazine and sunset yellow are reported as allergy producing agents[56,57], while allura red another constituent, is reported for the impairment of hepatic functions such as ureogenesis and gluconeogenesis[58]. Potassium sorbate (PS) a preservative present in ED is genotoxic to the human peripheral blood lymphocytes in vitro. PS treatment significantly increased the chromosomal abbretions and sister chromatid exchanges (SCEs) compared with vehicle control[59]. Calcium Disodium EDTA a chelating agent and is included in composition of ED. It is used in therapies against heavy metals; it may cause intestinal upsets, muscle cramps, kidney damage and blood in urine[60]. Sodium benzoate also a preservative, having bacteriostatic and sunscreen profile. It induces repeated episodes of acute urtricaria or angio-oedema[61] and impairs cognitive functions to produce attention deficit syndrome[62]. Sodium Hexameta Phosphate is a constituent of ED, reported to be mutagenic and cytotoxic to human lymphocytes in vitro[63].

It is also tempting to report the negative effects of energy drink consumption in terms of impaired memory in experimental animals. Memory is modulated by several neural systems, but predominantly by the glutamatergic and cholinergic system[64]. This impairment of memory could be due to the cholinergic system, from septum to hippocampus, modulates long-term potentiation in the hippocampus[65], implying that acetylcholine affects the glutamatergic system in the hippocampus. The cholinergic system could be the upstream effectors of memory function and cholinergic disturbance might precede glutamatergic disturbance in the onset of memory impairment.

These however were not evaluated in the present study. A further study of brain neurotransmitters which has been stored, could help us to unravel the CNS toxicity causing impaired memory function. Moreover in our studies memory reinstated in the withdrawal period. This could be due to the excretion of sodium benzoate out of the body leaving Ginseng a herb, to produce memory enhancing effects, Ginseng facilitates learning and memory by promoting hippocampal neuronal function of aged rats[66]. The in-depth mechanism by which ginseng enhances memory remains yet to be elucidated.

The findings relevant to the second part of the problem focuses on withdrawal associated studies. Although no behavioral toxicity was observed after withdrawal, from a short term exposure, this doesn't imply that energy drinks should not be avoided. Energy boosting and stamina enhancement effect subsided during withdrawal; it is possible that human utilization might increase thus leading to long term consumption. Investigating long term exposure and withdrawal toxicities with a larger sample would help in the assertion of the present findings.

CONCLUSION

The present study was a pilot study, conducted for a short period of time although it showed very promising results of short term exposure in adult rats. However memory impairment is one of the negative effects observed in the present study of ED. Moreover caffeine increases physical speed upon exposure but slow learning and mental performance[67]. It is possible that prolonged exposure to such type of drinks may lead to deleterious effects as far as the physical endurance and cognition is concerned. The withdrawal effects monitored after 14 days although showed normalization of sting induced impairment in performance and cognition, it may be possible that prolonged exposure to such drinks might produce undesirable effects associated with the use of Azo dyes used in sting which have long been suggested to adversely affect the learning and behavior in children[68]. Preclinical and clinical studies with long term exposure and withdrawal would help us to probe into the behavioral toxicities associated with consumption of such drinks.

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