Molecular and Behavioral Consequences of Concurrent Cocaine and Ethanol Use

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Abstract

Background: Concurrent cocaine and ethanol use is more common than any other drug combination, and such polydrug use often results in increased consumption of both substances. We developed an animal model of cocaine and alcohol co-abuse in which rat self-administered intravenous cocaine for 2 hours followed by oral alcohol for 6 hours. We hypothesized that subjects permitted access to both drugs would consume more ethanol and cocaine compared to groups permitted access to only one drug. We also predicted that self-administration of both drugs would reduce protein expression of mGluR5, GLT-1, and xCT and elevate levels of Homer protein expression.

Methods: Cocaine and ethanol consumption were measured over a 12-day self-administration period. Immediately following the self-administration period, subjects completed 2 weeks of extinction training, followed by reinstatement. We measured Homer, mGluR5, GLT-1, and xCT protein concentrations via microdialysis during reinstatement.

Results: No significant difference in substance consumption was found between drug-consuming groups. There was a significant interaction between cocaine and ethanol on Homer protein expression. No other significant effects were found, although mGluR5 and GLT-1 levels were decreased for all drug-consuming groups.

Conclusions: These results indicate that there are molecular and behavioral interactions between cocaine and ethanol when consumed concomitantly. The fact that cocaine and alcohol-consuming subjects chose to consume both substances indicates that this polydrug use results in a pleasurable experience, possibly reducing the negative side-effects of both substances.

Introduction

Concurrent dependence on cocaine and ethanol is an increasingly prevalent problem in the United States. High morbidity and mortality rates are associated with this growing health concern, as is the resulting high cost for healthcare. Several studies completed during the 1990s estimated that as many as 57-88% of cocaine users also suffered from comorbid alcohol dependence (5,1). When considering individuals being treated for cocaine use disorders, roughly 70-90% of those receiving inpatient treatment and 50% of those receiving outpatient treatment had also admitted to concurrent alcohol dependence (6). Grant and Harford (1990) report that in the U.S., more than 90% of individuals who admitted to using cocaine at the time of the study also reported concurrent alcohol use or abuse. Concurrent use of these substances often leads to social problems and health concerns, yet the availability of these drugs seems to be increasing, along with the amount of Americans who abuse the two drugs simultaneously. Polydrug use, in this sense, has been linked to mental health disorders, violence, criminal activity, unwanted sexual relations, and other drug use (8). Patients who suffer from addiction to both substances tend to have more psychosocial problems than patients addicted to either substance alone (1). In addition, cocaine- and alcohol-dependent individuals are much more difficult to treat than those who suffer from either cocaine-dependence or alcohol-dependence alone. With such overwhelming evidence from human trials, it is clear that simultaneous cocaine and alcohol use is a growing threat to society, and it should be treated with the utmost concern.

Despite the fact that concurrent alcohol and cocaine use is becoming more commonplace, few studies to date have analyzed the interaction between the side effects of each substance when used together. A study completed by Rubio *et al.* (2008) hypothesized that heavy drinkers who also used cocaine were more susceptible to developing alcohol dependence, which could rapidly progress to other severe alcohol use disorders. In this study, individuals in

the heavy drinker and cocaine (HD+Co) sample had significantly higher impulsivity, anxiety, and depression scores, as well as higher rates of lifetime comorbidity for psychological disorders, such as impulse control disorders and ADHD. In addition, the HD+Co patients had higher rates of alcoholism in their family histories. In the four year follow-up, ethanol consumption was twice as high as the baseline measurements in the HD+Co group, and 68% of the group met DSM-IV criteria for alcohol dependence. In particular, 100% of the subjects who used cocaine in excess of ten days per month met criteria for alcohol dependence. Almost all of the patients in the HD+Co group were using more cocaine compared to baseline levels; 30% of the group met DSM-IV criteria for cocaine use disorders (25% dependence, 5% abuse). Rubio *et al.* found that male and female cocaine users were 12 and 7 times more likely, respectively, to become alcohol-dependent than subjects who abstained from cocaine use. In fact, cocaine use significantly increased an individual's susceptibility to all substance dependent disorders. Brecht, Huang, Evans, and Hser (2008), compared the polydrug use patterns across groups of different primary drug users. Among primary cocaine users, higher levels of primary drug use were associated with higher levels of alcohol consumption. In addition, of the three primary drug groups studied (heroin, cocaine, and methamphetamine), primary cocaine users consumed more alcohol in general than members of the other two experimental groups. In fact, alcohol consumption among primary cocaine users was actually higher than cocaine usage during the majority of the ten year observation period, a trend that was not present in the other two polydrug groups. Cocaine use showed no significant changes and there was continuing stability in their alcohol use over the ten year period, although usage of other drugs declined overall. These results confirm that there is a significant correlation between cocaine use and ethanol consumption in humans.

When comparing illicit drug use patterns, alcohol dependence is more highly correlated with comorbid cocaine dependence than it is with any other drug use disorder (15). Concurrent alcohol use seems to enhance cocaine's more rewarding effects, while reducing anxiety and partially alleviating the more unpleasant effects, such as withdrawal and the "crash" associated with cocaine use (5,14). This supports the idea that simultaneous alcohol and cocaine use decreases the subjective sensation of inebriation while increasing the feeling of being "high" (16). This pattern could be explained by both the positive reinforcement theory, in which the additive effects of ethanol and cocaine produce an enhanced reward, and by the negative reinforcement theory, in which the combination of substances leads to reduction of adverse side-effects (4). Thus, concurrent heavy drinking could be a result of the stimulant and/or toxic effects of cocaine. In accordance with this idea, McCance-Katz, Kosten, and Jatlow (1998) found that, in humans, enhanced psychological effects during concomitant cocaine and alcohol abuse may encourage ingestion of larger amounts of each substance. There are also hypotheses that patients that abuse both ethanol and cocaine could be highly vulnerable to using both substances due to specific genetic alterations in brain regions associated with reinforcement and gratification, although more research in is needed in this area (17,18). Therefore, it has been shown that simultaneous alcohol and cocaine use results in a pleasurable experience that is powerful enough to drive individuals to continue to consume the two substances concomitantly.

Polydrug use can also be studied using animal models of drug seeking behavior, which can allow investigation of the underlying neural mechanics of drug addiction. For example, a study completed by Knackstedt & Ettenberg (2005) examined the unique "approach-avoidance" behavior demonstrated by rats who self-administered intravenous (IV) cocaine each day. This approach-avoidance behavior seems to be related to the varied positive and negative effects

that are associated with cocaine use and abuse in both animals and humans. The rats began the trial in a "start" box, and after a door opened, the rat would proceed into the runway, voluntarily moving towards the "goal" box at the end of the runway. Upon entering the goal box, the rat received an IV infusion of cocaine before being returned to its home cage. Following the trial, the rat was permitted to drink an alcohol solution for 30 minutes. Previous studies have associated the onset of the less pleasurable side-effects of cocaine use with the decline of drug plasma levels, which occurred about 15 minutes after the IV injection in the experimental group. In this animal model, the anxiogenic side effects induce the animal to approach the goal box but retreat back to the start box before receiving the cocaine infusion, and repeat this action many times, displaying apparent ambivalence about entering the goal box to receive cocaine. Therefore, the study drew a connection between the human tendency to supplement cocaine use with alcohol consumption and a similar tendency in animal models by allowing a group of rats to choose whether or not to consume ethanol during this phase. Over the course of the 4-week study, the rats in the cocaine and ethanol group exhibited far less "retreats" during the runway trials when compared to the experimental group that was exposed to cocaine alone. In fact, there was no significant difference between the retreat behavior of the cocaine and ethanol group and the control group which received saline in the goalbox. Thus, alcohol consumption immediately following cocaine alleviates the negative side-effects of cocaine in rodents, validating a potential motivating factor for human cocaine addicts to consume alcohol.

While the behavioral effects of concomitant alcohol and cocaine use provide valuable information as to the interactions between these substances, one cannot neglect the underlying neural networks responsible for producing such effects. The nucleus accumbens is heavily involved in several different pathways which mediate aspects of addiction in both humans and animals. The nucleus accumbens is divided into two subcompartments: the "shell" and the

"core" (9). The two main neurotransmitters associated with drug reward in the nucleus accumbens are dopamine and glutamate. The nucleus accumbens receives afferent dopaminergic input from the ventral tegmental area, as well as glutamatergic inputs from the prefrontal cortex, amygdala, hippocampus, and thalamus. Previous studies have shown that dopamine levels in the nucleus accumbens are significantly increased during intravenous cocaine self-administration, in which dopamine levels are higher in the shell than in the core. In addition, administration of dopamine antagonists increases cocaine intake. Ethanol also increases dopamine in the nucleus accumbens (14). Since both ethanol and cocaine increase dopamine levels, this could provide a possible explanation for why the two substances are often taken in conjunction.

While dopamine is integral to the addiction reward pathway, it is not necessary for cocaine relapse in the animal model of reinstatement. This model trains animals to self-administer drug in an operant chamber followed by extinction training wherein presses on the lever that previously delivered drug no longer do so. Once animals have extinguished the lever-pressing response they are presented with one of the categories of cues which induce relapse in humans (cue, drug, or stress) which can reinstate the drug-seeking response (lever-press; 23). However, an increase in the release of glutamate in the nucleus accumbens *is* associated with the relapse of cocaine-seeking behavior. In particular, AMPA and mGluR5 receptors seem to be primarily responsible for mediating reinstatement (as opposed to NMDA receptors). Overall, glutamate levels in the nucleus accumbens are significantly increased from baseline during cocaine self-administration. Both GLT-1 and xCT expression are decreased following cocaine self-administration, thereby reducing glutamate uptake. Protein expression of mGluR5 also seems to be reduced following cocaine use. The downregulation of these proteins disrupts glutamate homeostasis in the nucleus accumbens. After two weeks of extinction training following cocaine

self-administration, microdialysis data show that the glutamate levels in the core of the nucleus accumbens of rats that were trained to self-administer cocaine are lower than the glutamate levels in saline rats. In addition, glutamate levels in cocaine rats at the end of a two week extinction period are lower than previous levels during cocaine self-administration. During reinstatement, rats with a history of cocaine self-administration experience an increase in glutamate in the nucleus accumbens that is not seen in yoked cocaine and saline rats. This increase causes glutamate levels to surpass the maximum self-administration glutamate levels, although this effect falls off as the test session proceeds. Knackstedt, Melendez, and Kalivas (2009) accounted for this disruption of glutamate homeostasis and corrected the imbalance via administration of Ceftriaxone, thereby preventing relapse to cocaine-seeking, implying that glutamate homeostasis plays a vital role in reinstatement.

To our knowledge, there is only one paper to date that deals with the fluctuation of glutamate levels in the nucleus accumbens in response to ethanol abuse (13). In rats injected with ethanol, low to moderate doses of ethanol resulted in an increase in extracellular glutamate in various brain regions, whereas rats who were exposed to high doses of ethanol showed decreases in extracellular glutamate. In fact, rats with acute ethanol exposure had decreased glutamate transport, while those exposed chronically showed an increase in glutamate transport. One possible explanation for the increased levels of extracellular glutamate is via glutamate release mediated by NMDA receptors. Past research has shown that the NMDA receptor is sensitive to inhibition by ethanol, and prolonged ethanol inhibition at these sites is associated with an increase in the amount of NMDA receptor sites present in brain tissue (11). Following exposure to ethanol, there is an increase in basal glutamate levels and a decrease in the functioning of the glial transporter GLT-1, as well as a decrease in the expression of mGluR5.

The purpose of the present study was to examine the interaction between the rewarding effects of concurrent cocaine and ethanol use behaviorally, molecularly via western blots, and neurochemically via microdialysis to monitor the glutamate levels in both regions of the nucleus accumbens. We predicted that the cocaine and ethanol (CE) group would self-administer significantly higher levels of both cocaine and ethanol when compared to subjects from the other experimental groups, further validating the theory that simultaneous use of the two substances increases the pleasurable effects, while decreasing the adverse effects of both drugs. We also predicted that glutamate levels in the nucleus accumbens would be increased from baseline during cocaine self-administration, would fall below baseline during extinction, and would surpass self-administration levels during the first test session of reinstatement. We expected decreased expression of the proteins mGluR5, GLT-1, and xCT following cocaine self-administration and extinction in both the cocaine only (C) group and the CE group.

Methods

Subjects

71 male albino Sprague-Dawley rats (weighing approximately 300 g at the time of surgery) were obtained from Charles Rivers Laboratories . Subjects were individually housed in plastic holding cages with wire tops within a temperature controlled vivarium (23°C) on a 12/12 hour light/dark cycle (lights on at 0700 hours). Rats were provided ad libitum access to food and water in their home cages , with the exception of during cocaine self-administration procedures, when animas were fed 25 g food/day. Each animal was gentled via individual handling each day for approximately one week prior to surgery. The animals' care and all experimental procedures were conducted in compliance with the National Institutes of Health guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina.

Experimental Design

Four experimental groups were used: a C group (n=16) that self-administered intravenous cocaine and was not permitted to drink alcohol, a CE group (n=25) that self-administered cocaine and was permitted to drink alcohol (25% v/v) for 6 hours post-cocaine, a yoked-saline group that also drank ethanol for 6 hr (E group; n=20), and a yoked-saline group which drank water (S group; n=10). Prior to surgery, animals were assigned to one of the groups described above. Both the CE group and the E group were trained to drink ethanol using the Intermittent Drinking Paradigm (IDP), wherein animals are given unsweetened 20% ethanol in the home cage for 24 hr on alternating days (3 d/wk) without water deprivation (Simms et al., 2008). Animals were presented with 50 mL Nalgene bottles containing 25% unsweetened ethanol (v/v). Ethanol readings were taken at the end of each 24-hour period. Surgeries were performed one day after the last drinking period. Following the surgery recovery period and the pre-experimental food-training, subjects were permitted to drink ethanol for one 24 –hour session prior to the start of cocaine self-administration. Ethanol readings were taken at the end of the drinking periods. The experimental procedure began the day immediately following the last drinking period. During all ethanol pre-training sessions, animals had free access to food and water.

Surgery

Each rat was implanted with an indwelling catheter under deep anesthesia induced by an intramuscular injection of ketamine (100 mg/kg) and xylazine (2 mg/mL). Prior to surgery, the rat also received intraperitoneal Ketorolac (2 mg/mL) to help control any pain that may be caused by the surgical procedure. During surgery, one end of the catheter was inserted and secured by suture into the jugular vein. The other end was passed subdermally over the

shoulder to the animal's back, where it exited the body and was attached to a stainless steel cannula held in place by a "backpack" worn by the animal. Each animal was administered 0.3 mL Timentin (10 mg/0.1 mL) intravenously on the day of the surgery, followed by 0.1 mL Heparin to avoid blood clots in the catheter. Each animal received subsequent daily injections of 0.1 mL Timenton followed by a 0.1 Heparin injection for a period of 7 days. Rats were allowed to recover for 5 days prior to the start of experimental training.

Food-Training

After the surgery recovery period, all animals were food-trained for one 14-hour overnight period in a standard operant box chamber in order to familiarize subjects with the testing procedure and operant chamber. Each chamber contained two levers (right and left), one houselight, and an automatic pellet dispenser, located outside of the operant chamber. Food pellets (Noyes, 45 mg) were dropped into a food well located between the two levers inside the chamber. Each time the animal pressed the right liver, the animal would receive a pellet. Presses on the left lever had no consequences. After the food-training, animals were returned to their home cages, and received access to water and food.

Operant cocaine self-administration

Following the last alcohol exposure, animals were randomly assigned to one of twelve identical standard operant chambers, where that subject's testing occurred for the following 12 days of the experiment. Each operant chamber contained two levers with stimulus lights above the levers, and one houselight. Each chamber was housed in a cabinet, which would remain closed for the duration of the 2-hour self-administration period. Animals placed in the chamber were tethered, and drug delivery was accomplished via PE tubing that was attached to the animal's

cannula and ran up through a flow-through swivel apparatus above the animal to a fluid-filled syringe (containing either cocaine or saline solution) set into a syringe pump. Animals were tested daily (at roughly the same time each day), where they were able to self-administer drug via right lever presses; left lever presses were used as a control. Each infusion contained either 0.25 mg/kg cocaine or 0.9% physiological saline; infusions were administered via intravenous catheter. When the animal would press the right lever, the experimental light would illuminate briefly and a tone would sound. All experimental contingencies were programmed using Med Associates software (St. Albans, VT). After the operant period, each rat received 0.1 mL Heparin to maintain the catheter efficiency.

Immediately following the self-administration period, the rats were returned to their home cages to begin the 6-hour drinking period. During this drinking period, each animal was given access to two identical bottles, one containing a 25% ethanol solution and one containing plain tap water, for a 6-hour drinking period. The bottles were placed next to one another, offering the subject a free choice. The orientation of the bottles (left side or right side) was alternated daily to control for inherent or learned side/location preferences. Ethanol and water readings were taken at the 2-hour point and at the end of the 6-hour period, as more frequent readings may have disturbed the subjects. Rats who did not receive ethanol only received one bottle of tap water during the 6-hour period, and readings were taken at the same intervals. Following this session, the rats' regular water bottles were returned, and they were given food. This schedule was in effect for 12 days.

Data Analysis

The self-administration data were analyzed using 2-way RM ANOVAs. The between-group variable was exposure to cocaine and/or ethanol, and the repeated measure was time. The western blotting data was analyzed with2-way ANOVAS. Significance was set at $p < 0.05$.

Results

In CE rats, ethanol consumption did not affect cocaine consumption, and cocaine consumption did not affect ethanol consumption. Mean amount (g/kg) of alcohol consumed in the first 2 hours did not differ between CE and E groups. A 2-way RM ANOVA revealed no significant effect of group ($F(1,15) = 0.001$, n.s.) or day ($F(1,15) = 2.295$), and no significant interaction ($F(10,150) = 0.584$, n.s.). When considering the mean number of cocaine infusions during the self-administration period using a 2-way ANOVA, a significant effect of day was found ($F(11,108) = 7.250$, $p = 0.001$), but there was no significant effect of group ($F(1,28) = 0.463$) and no significant interaction ($F(1,28) = 1.299$). A t-test also revealed no difference in total cocaine (mg) consumed between CE and C groups during the entire 12 days of self-administration ($t(1,28) = 7.88$). However, there was a significant effect of day for both the 2 hour ($F(11,363) = 3.514$, $p < 0.001$) and the 6 hour ($F(11,363) = 6.094$, $p < 0.001$) time points when considering the percentage ethanol of total fluid consumed. For the 2 hour time point, there was no significant effect of group ($F(1,33) = 0.008$, n.s.) and no day x group interaction ($F(1,33) = 0.600$, n.s.). For the 6 hour time point, there was no significant effect of group ($F(1,33) = 0.566$, n.s.) and no day x group interaction ($F(1,33) = 1.322$, n.s.).

Some fluctuation in protein levels in the nucleus accumbens was observed following self-administration. A 2-way ANOVA of Homer levels revealed no significant effect of cocaine ($F(1,3) = 0.043$, n.s.), no significant effect of ethanol ($F(1,3) = 0.338$), but a significant cocaine x ethanol interaction was observed ($F(3,29) = 5.166$, $p < 0.05$). Homer levels were elevated in all experimental groups, relative to saline controls. When considering mGluR5, there was no significant effect of cocaine ($F(1,3) = 0.033$, n.s.) or of ethanol ($F(1,3) = 0.263$, n.s.), and no significant cocaine x ethanol interaction ($F(3,29) = 0.366$, n.s.), although mGluR5 concentrations

did appear to decrease in all experimental groups slightly. GLT-1 levels were also unaffected. While no significant effect of cocaine ($F(1,3) = 0.251$, n.s.) or of ethanol ($F(1,3) = 0.069$, n.s.) was found, and there was no significant cocaine x ethanol interaction ($F(3,30) = 1.77$, n.s.), there was a slight decrease in GLT-1 concentrations in all experimental groups. In xCT, there was no significant effect of cocaine ($F(1,3) = 0.004$, n.s.) or of ethanol ($F(1,3) = 0.819$), nor any significant cocaine x ethanol interaction ($F(3,26) = 2.499$). Due to low sample size (n=5), the present study had low power. However, xCT concentrations did appear to be slightly decreased in CE and C groups, and slightly elevated in the E group.

Since the trends observed validate prior findings and fail to reject our hypothesis, the lack of significant results is most likely due to small sample size. Thus, it would be beneficial to repeat the experiment with more subjects in order to increase the sample. In doing so, it is possible that the results would be more statistical significant.

Discussion

This study aimed to observe and analyze the behavioral and molecular interactions consequences of cocaine and ethanol co-administration. We predicted that the CE group would self-administer significantly higher levels of both ethanol and cocaine compared to the other experimental groups. Since glutamate levels in the nucleus accumbens typically drop below baseline during extinction, yet spike to levels higher than those seen during self-administration during the first reinstatement session, we predicted a reduced expression of the proteins mGluR5, GLT-1, and xCT in all drug-consuming groups. We predicted an increase in Homer protein expression in all drug-consuming groups.

As seen in Figure 1, there was a significant effect of day on the mean number of cocaine infusions. Cocaine consumption appeared to be the highest on Day 1, after which it dropped drastically before it began to slowly increase until the end of the 12 day self-administration

period. This could be due to the novel side-effects following the first session of cocaine self-administration. Figure 1 shows that while it appears that the CE group consumed slightly more cocaine, there was no statistically significant difference between the CE group and the C group. As observed in Figure 3 and Figure 6, there was a significant effect of day on the percent ethanol of total fluid consumed for both the 2 hour and 6 hour time points, although there was no difference observed between the CE and E groups. Interestingly, both groups seemed to consume more ethanol on the second day of self-administration and less on day 10, compared to other days. Prior studies with animal models have shown that concurrent ethanol and cocaine use typically results in increased cocaine consumption; concurrent ethanol and cocaine abuse in humans has been linked to increased consumption of both substances (2,3,19). While these results may not have been statistically significant, it is important to note that the CE subjects still chose to use both substances, rather than favoring one or the other. This behavior mimics what is observed in humans, and it could validate the idea that ethanol consumption following cocaine use helps negate the more unpleasant side-effects of cocaine use.

Figure 2 and Figure 5 illustrate the mean amount of ethanol consumed (g/kg) over the 12 day self-administration period. There is no significant difference between the CE group and the E group when considering ethanol consumption in the 2 hour period following cocaine self-administration. According to Figure 4 and Figure 7, it seems that the S (control) group drank more water than the other experimental groups at both the 2 hour period and the 6 hour period following cocaine self-administration. This observed trend is in line with earlier evidence by Obara et al. (2009), who found that rats that consumed ethanol drank significantly less water.

Homer proteins are part of a post-synaptic scaffolding complex that regulates and maintains basal glutamate release within the nucleus accumbens, as well as the activity of the metabotropic glutamate receptor (mGluR) family and *N*-methyl-D-aspartate (NMDA) receptors

(13). Homer gene products control behavioral and biochemical sensitivity to cocaine, and they have a similar role in ethanol consumption and abuse. However, while the increases in Homer levels can be observed in the nucleus accumbens, the forebrain, and the temporal lobe following cocaine use, the protein concentration increase due to ethanol exposure has only been observed in the nucleus accumbens thus far (13). Since Homer is known to play an active role in reinforcing ethanol reward, maintaining homeostasis of this protein concentration seems to be an integral part of understanding and controlling addiction and relapse behavior. As seen in Figure 8 (Homer), protein concentrations were elevated in all drug-consuming groups relative to yoked-saline controls. However, it appears that the effect of these substances on Homer concentrations is not additive, as the protein concentration in the nucleus accumbens of the CE group is not as highly elevated as the concentrations in either the C group or the E group.

The mGluR5 receptor is a signaling partner for Homer proteins. Ethanol has been shown to inhibit the activity of mGluR5, and mGluR5 concentrations are decreased after cocaine administration. In addition, mGluR5 antagonists have been shown to prevent cocaine reinstatement. Figure 9 shows that there is an observed slight decrease in the mGluR5 concentrations in the nucleus accumbens of all experimental groups during reinstatement, although it is not statistically significant.

The glial glutamate transporter GLT-1 aids in the regulation of extracellular glutamate levels in the nucleus accumbens. It aids in glutamate reuptake and recycling following an action potential. In fact, GLT-1 is responsible for 90% of glutamate reuptake in the brain (20). GLT-1 expression is generally reduced following cocaine self-administration, as can be observed in Figure 10. This decrease in GLT-1 expression results in reduced glutamate uptake, which causes an increase in extracellular glutamate in the nucleus accumbens. At the present time, the role of GLT-1 in the regulation of basal glutamate levels is not well known, but it has been observed

that basal glutamate levels are decreased following chronic cocaine consumption (9). The functioning of the GLT-1 transporter is also downregulated following ethanol exposure, but in this case, basal glutamate levels increase.

The protein xCT is the catalytic subunit of the cystine-glutamate exchanger, which accounts for most of the non-synaptic extracellular glutamate in the nucleus accumbens (21). It functions by exchanging one intracellular glutamate with one extracellular cystine molecule (22). xCT expression in the nucleus accumbens is reduced as a result of chronic cocaine use. It has been postulated that xCT function and glutamate uptake via the GLT-1 transporter are co-regulated (9). According to Figure 11, the xCT concentration was decreased in the C group and the CE group, but it was elevated in the E group (although these results are not statistically significant). However, Figure 10 shows that GLT-1 expression is reduced in all experimental groups, hinting that the above mentioned postulate may only apply to glutamate regulation following certain drugs of abuse such as cocaine and nicotine.

In summary, the data trends seem to support our hypothesis concerning protein expression in the nucleus accumbens, although not all of the results were statistically significant. There was a significant interaction between cocaine and ethanol that affected Homer protein expression, affirming that concurrent use of these two substances results in different neurochemistry compared to that resulting from use of either substance alone. While there was no significant difference in cocaine or ethanol self-administration levels between drug-consuming groups, there was a significant effect of day on both cocaine infusions and ethanol consumption. Although the CE group failed to consume higher levels of either drug, it is important that CE subjects still chose to consume both substances, behaviorally asserting that there is an interaction between cocaine and ethanol. This agrees with prior findings that suggest

that ethanol enhances the pleasurable effects of cocaine use, while lessening or negating the negative side-effects (4,5,14,16).

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Figure Captions

Figure 1. There is no significant difference between the mean number of cocaine infusions per day during self-administration for the Cocaine + H2O (C) group and for the Cocaine + EtOH (CE) group. The effect of day on average number of infusions is statistically significant ($p = 0.001$), but no significant interaction was found. Cocaine consumption seems to be the highest on the first day of the self-administration period, after which it drops suddenly, then begins to rise slowly until the end of the 12-day experiment.

Figure 2. The average amount of alcohol consumed 2 hours following the cocaine self-administration period is not significantly different between the Saline + EtOH (E) group and the Cocaine + EtOH (CE) group.

Figure 3. The percent ethanol of total fluid consumed 2 hours after cocaine self-administration is not significantly different between the Cocaine + EtOH (CE) group and the Saline + EtOH (E) group. However, there is a significant effect of day ($p < 0.001$) on the percentage ethanol consumed. There is no significant interaction between day and group.

Figure 4. There is no statistically significant difference in the amount of water (mL) consumed on average by all groups 2 hours after cocaine self-administration. However, it appears that the Saline + H2O (S) control group consumed slightly more water than the drug-consuming groups.

Figure 5. The average amount of alcohol consumed 6 hours following the cocaine self-administration period is not significantly different between the Saline + EtOH (E) group and the Cocaine + EtOH (CE) group.

Figure 6. The percent ethanol of total fluid consumed 6 hours after cocaine self-administration is not significantly different between the Cocaine + EtOH (CE) group and the Saline + EtOH (E) group. However, there is a significant effect of day ($p < 0.001$) on the percentage ethanol consumed. There is no significant interaction between day and group.

Figure 7. There is no significant difference in the amount of water (mL) consumed on average by all groups 6 hours following cocaine self-administration. It seems that the Saline + H2O (S) control group drank slightly more water than the other 3 experimental groups.

Figure 8. Concentrations of Homer protein complex during reinstatement seem to increase in all drug-consuming groups, but the effects on the Saline + EtOH (E) group and on the Cocaine + H2O (C) group are insignificant. The Saline + H2O (s) group served as a control. Only the cocaine x ethanol interaction observed in the Cocaine + EtOH (CE) group is statistically significant ($p < 0.05$). However, this effect is not additive.

Figure 9. mGluR5 concentrations seem to drop slightly during reinstatement in the Cocaine + EtOH (CE) group and the Saline + EtOH (E) group, and more noticeably decreased in the Cocaine + H2O (C) group. However, the effect of cocaine and the effect of alcohol are both insignificant, as is the interaction between the two substances.

Figure 10. GLT-1 protein expression in the nucleus accumbens during reinstatement seems to decrease slightly for all drug-consuming groups. The Saline + H2O (S) group served as a control. However, there are no significant results.

Figure 11. xCT concentrations in the nucleus accumbens during reinstatement appear to drop in both the Cocaine + H2O (C) group and the Cocaine + EtOH (CE) group, but increase slightly in the Saline + EtOH (E) group. However, no effects or interactions are significant.

Figures

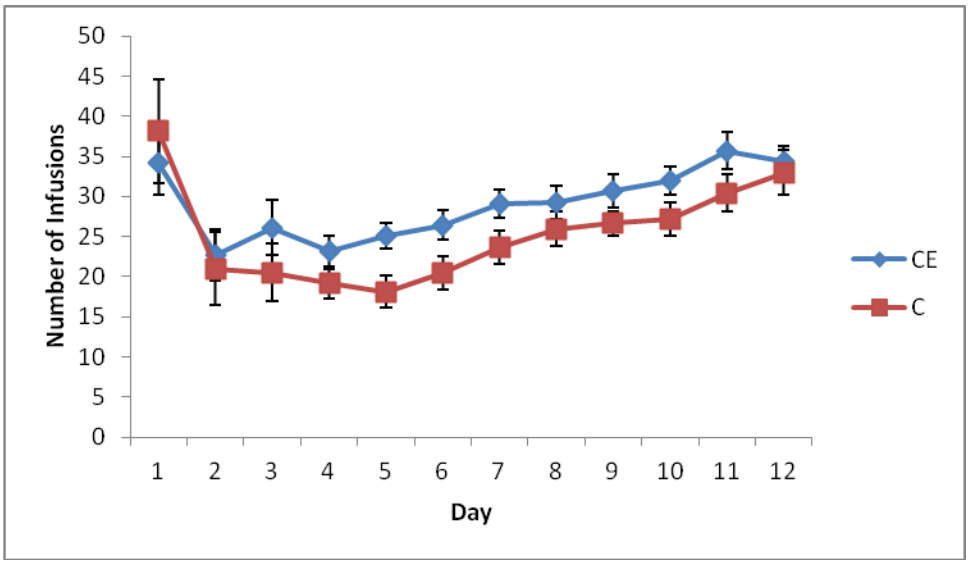
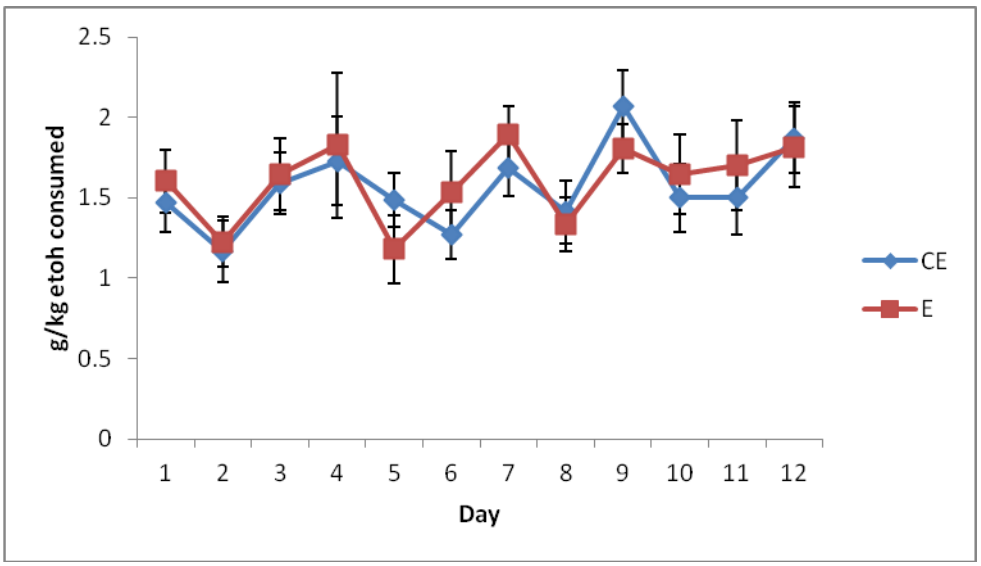


Figure 1.



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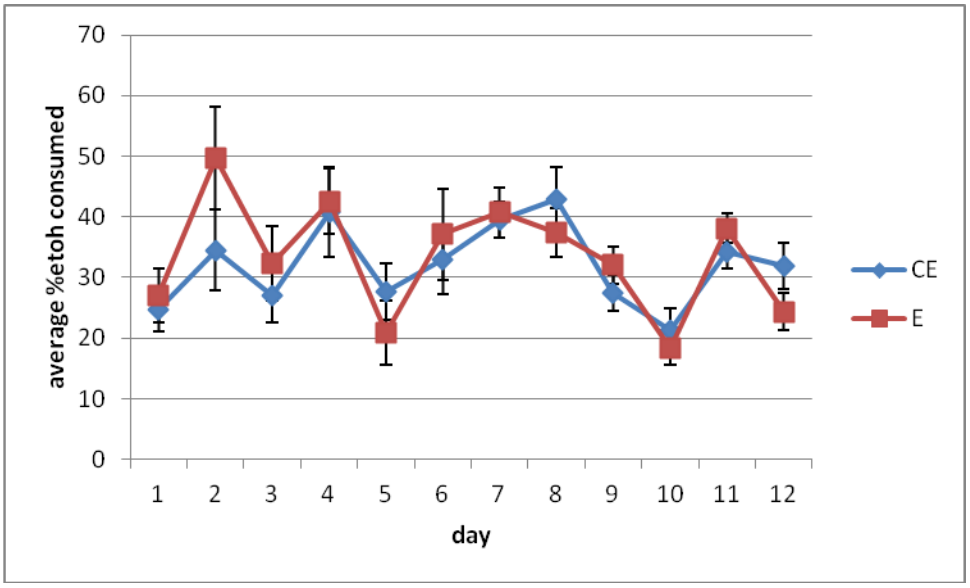

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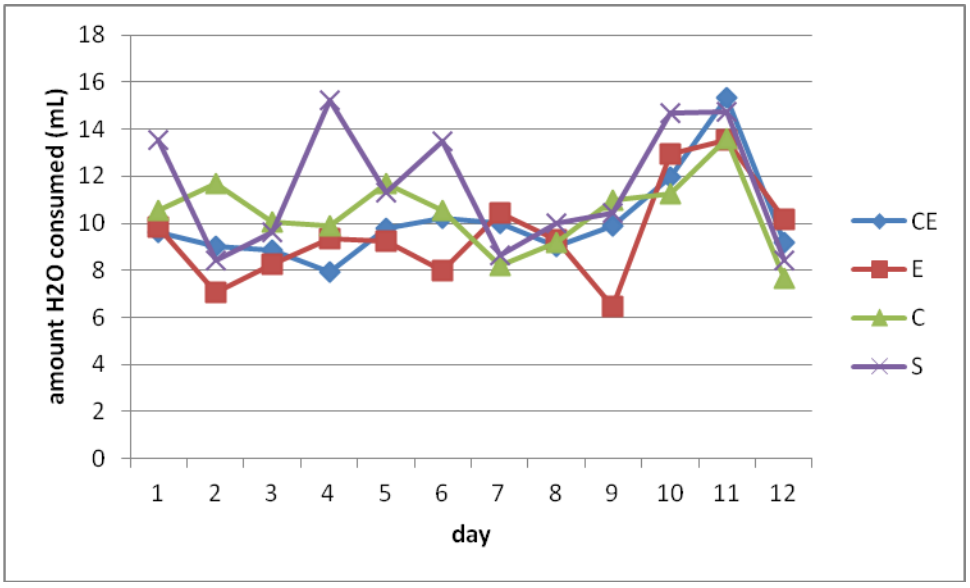

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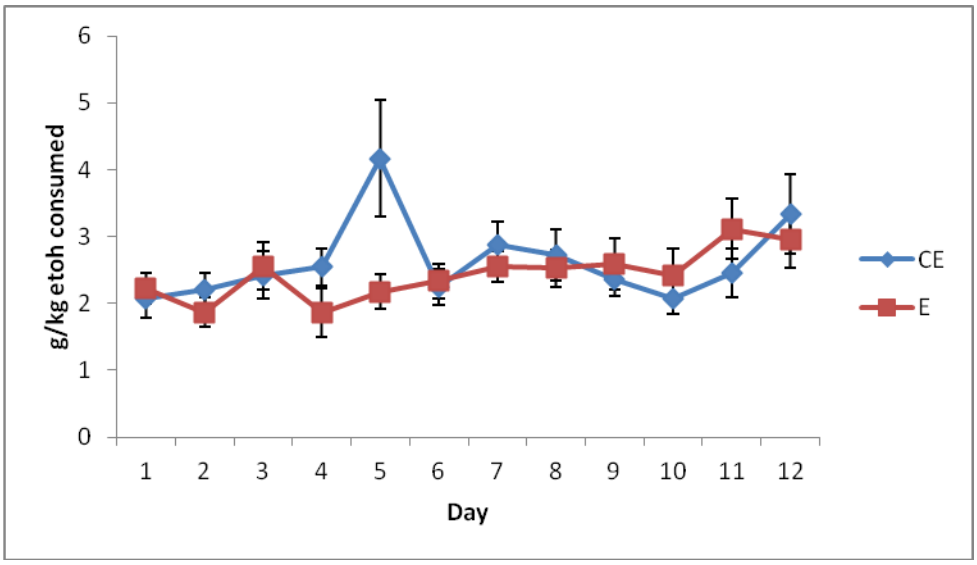

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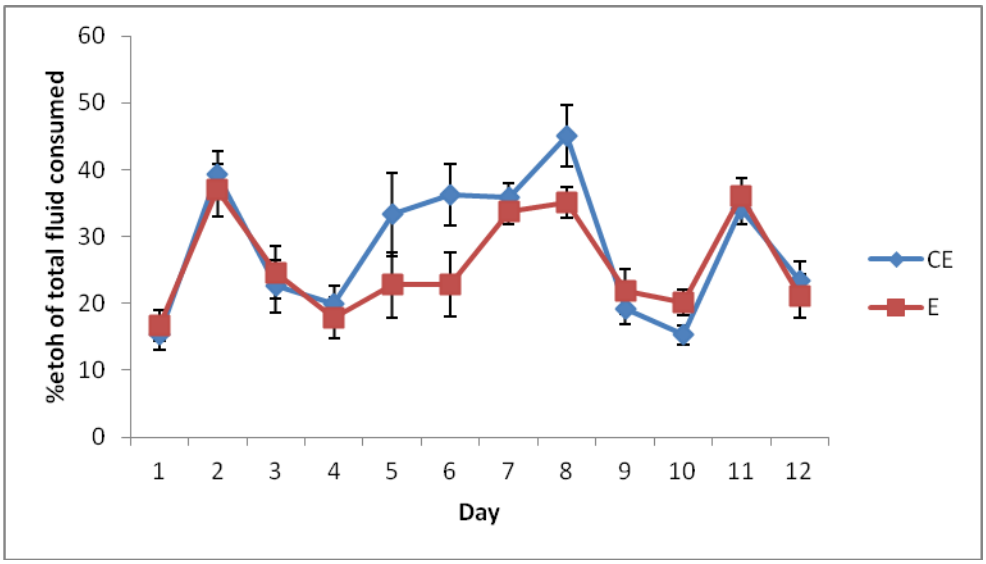

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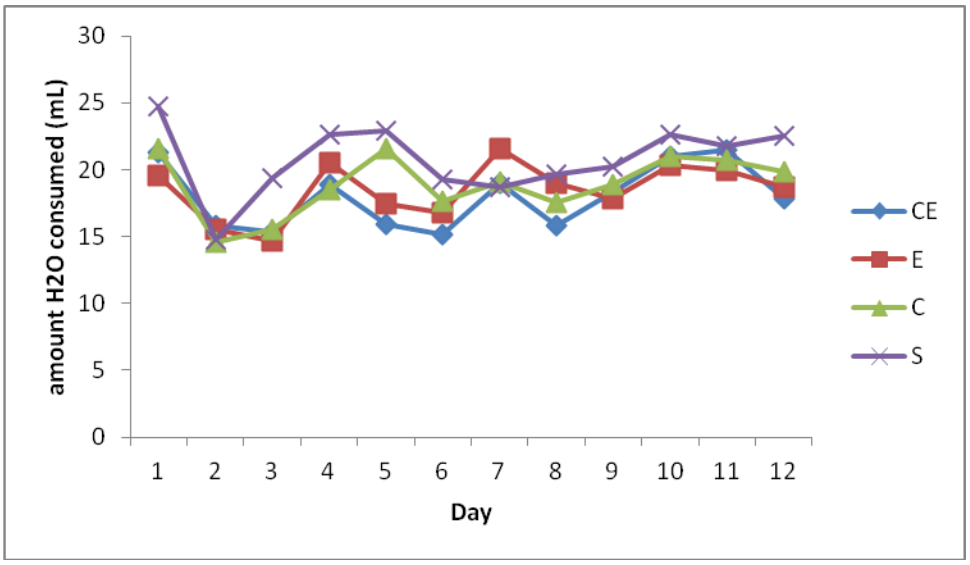

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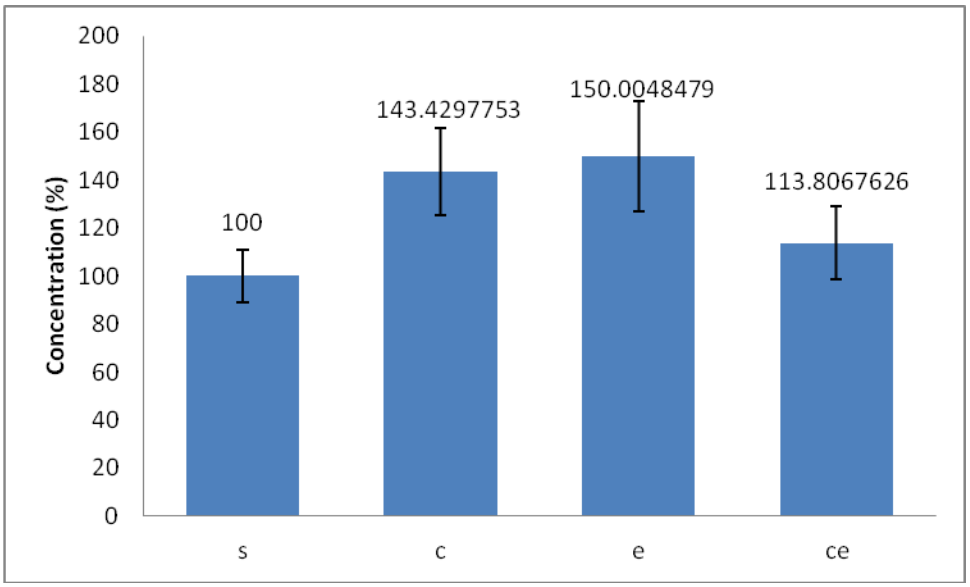

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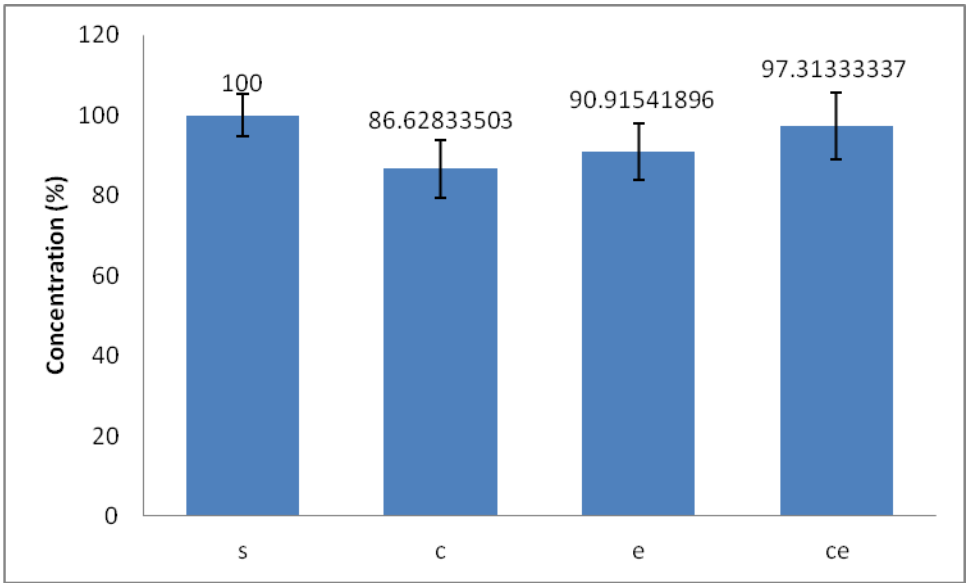

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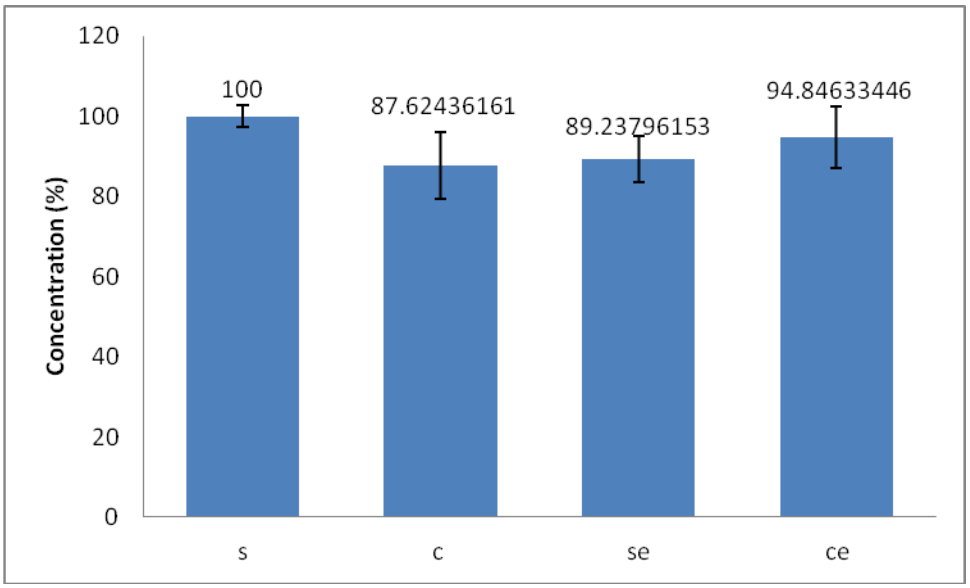

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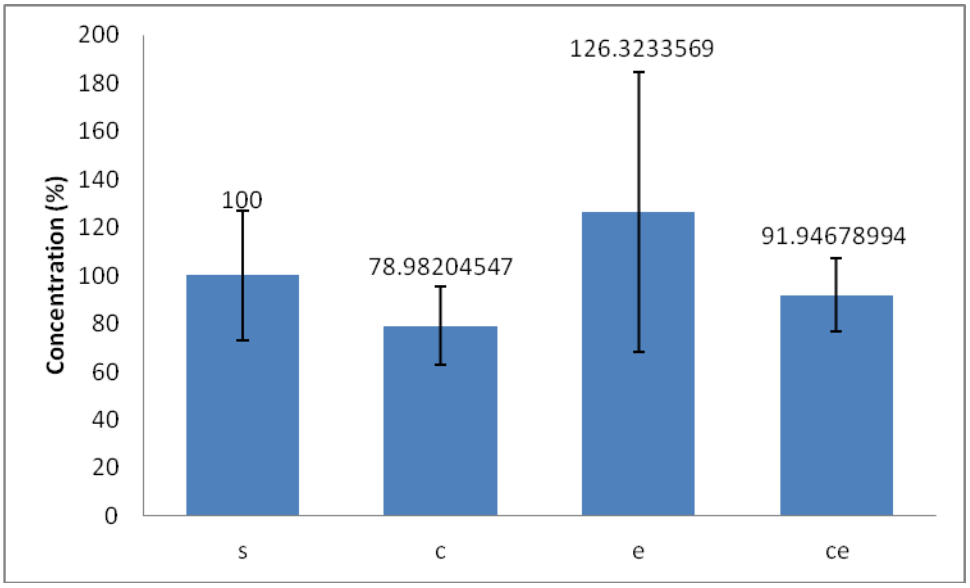
200
180
160
140
120
100
80
60
40
20
0
Concentration (%)
100
78.98204547
126.3233569
91.94678994
s
c
e
ce
Figure 11.