ORIGINAL ARTICLE

COMPARISON OF PROTECTIVE EFFECT OF GREEN TEA AND VITAMIN C AGAINST CYPERMETHRIN INDUCE NEPHROTOXICITY IN MICE

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Background Insecticide toxicity is the problem of every person in under developed countries. It is necessary to counteract its effect by natural and cheap remedies like green tea and vitamin C. In this manner common man can also enjoy blessings of life. The current research was performed to compare the protective function of green tea and vitamin C on experimental cypermethrin provoked nephrotoxicity **Method:** Forty healthy Balb/C mice purchased from National Institute of Health, Islamabad, Pakistan and divided in to four groups (10 each). Group a was control which received only normal diet. Group B, group C and group D were experimental groups which were given Cypermethrin, Cypermethrin with green tea and Cypermethrin with vitamin C respectively. These groups were also given normal diet. After 1 month blood was drawn by intra-cardiac method to assess renal parameters. **Results:** One month research showed increase in serum urea to 6.8±.48 m.mol/l (n=3.9±.44) while green tea and vitamin C normalize them to 4.0±.83m.mol/l and 3.4±.33m.mol/l respectively. Serum creatinine increased to 42.90±3.28m.mol/l (n=29.50±3.95) while green tea and vitamin C normalize them to 28.80±4.58m.mol/l and 22.60±2.06m.mol/l correspondingly. **Conclusion** The results showed that green tea and vitamin C neutralized toxicity induced by Cypermethrin in mice and their effect is comparable.

Keywords: Cypermethrin, serum urea, serum creatinine, green tea, vitamin C

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INTRODUCTION

Cypermethrin is a potent type two synthetic pyrethroid with low mammalian toxicity. It is effective both domestically and in agriculture. It has rapid absorption and excretion in human body.[1] In direct sunlight its half-life is 8–16 days. In soil and water its half-life is as long as 56 and 100 days respectively.[2]

The fable of low mammalian toxicity slowly but surely indicates adverse effect of this most commonly used insecticide which can be absorbed by oral and dermal route.[3] Intoxication can be measured by estimating its metabolites in urine and can be verified by measuring plasma levels of Cypermethrin.[4]

Cypermethrin is a broad-spectrum insecticide, which means that it not only kills desired insects but useful species also affected by it.[5] It is already reported in literature[1] that insecticides Pyrethroids have a repute to produce oxidative stress both biochemically and microscopically, this gives evidence that Cypermethrin toxicity is associated with loss of weight in kidneys along with perturbation of biochemical levels.[6]

Cypermethrin intoxication initiates free radical production and creates oxidative stress which is responsible of much pathology in the human body.[7] Oxidative stress is an injurious process that can produce damage to cell constituent in kidneys and other tissues.[8]

Mostly green tea is consumed by Chinese and Japanese nation. To prepare it, fresh leaves are first rolled and then heated which prevent oxidation by making polyphenol oxidase inactive.[9] The polyphenols in green tea include rutin, quercetin and kaempferol along with leucoanthocyanin, theanine and caffeine. Chemically it comprised of epigallocatechin' epicatechin-3gallate, epicatechin and epigallocatechin3-gallate.[10]

Green tea catechins have antioxidant properties against several pathological processes which are linked with free radical injury.[11] Catechins can trim down oxidative stress induced damage by their antioxidant effect.[12] Due to its antioxidant action, cadmium chloride toxicity also decreases and tissue gets rid of oxidative stress.[13] It slows down aging process by its antioxidant effect against aging induced oxidative stress.[14] Drinking green tea during chemical exposure can also reduce several parameters indicative of oxidative stress.[15]

Vitamin C (ascorbic acid) is a six-carbon lactone. In majority of mammals it is prepared from glucose in liver but due to deficiency of gluconolactone oxidase enzyme, human and some other species cannot synthesize it in body.[16] Vitamin C (ascorbic acid) is omnipresent and elemental in living cells, where it operates as a water-soluble

antioxidant and a crucial cofactor for numerous enzymes which take part in sundry metabolic reactions. Numerous studies have proved that vitamin C rich fruits and vegetables significantly dwindled menace of cerebrovascular accidents and cancer.[17]

Vitamin C is water soluble and it can reduce free radicals by not allowing chain reaction to commence and disseminate. In this manner it guards cells against oxidative stress.18 Kidneys are important to metabolize and eliminate toxic substances from body. Proteins metabolize to produce urea and creatinine as end products which solely eliminate by renal tissue.19

In view of the potential hazardous effect of Cypermethrin on health, this study was planned to determine the beneficial effects of ascorbic acid and green tea on oxidative stress in Cypermethrin exposed mice.

MATERIAL AND METHODS

In this laboratory based experimental study, forty female balb/C mice purchased from National Institute of Health with average weight of 30–40g were randomly segregated in to four groups. They were kept in iron cages (5/cage).Mice placed under standard laboratory conditions with exposure to 12 hours dark and 12 hours light at temperature 25±5 °C. In addition of it they had free access to water and standard pellet diet ad libitum. The distribution of four groups was as follows

Group A (control) mice had free access to water and standard diet. Group B (Cypermethrin) mice got 15 mg/kg Cypermethrin by oral gavage and fed normal diet with free access to water.[20]

Group C (green tea) mice received Cypermethrin and green tea extract. In order to make green tea extract, fifteen gram green tea leaves were added in one litre boiling distilled water for five minutes.[13] Then filtered and given to mice for whole day.

Group D (vitamin C) mice got Cypermethrin and vitamin C. One gram vitamin C dissolved in one litre of water was given to mice as sole supply of water for whole day.[21] All these substances were given for four weeks. After this duration mice anesthetized in chloroform chamber in groups and blood drawn by intra-cardiac method .The whole procedure completed with strict aseptic measures in National Institute of Health Islamabad. The blood samples immediately placed in gel separator tubes and allowed to clot at room temperature. Then centrifugation of samples done at 3000 rpm for 10 minutes at 30 °C. The clear supernatant serum separated and collected by pipette into dry clean tube. It kept frozen at -30°C in pathology department of Army Medical College Rawalpindi for biochemical tests. Serum urea and creatinine were measured by automated analyser Selectra E made by VITA LAB. By using Urea UV diagnostic kit and Creatinine-j jaffe Colorimetric Kinetic Kit. Data were presented as the mean± standard error of means and statistically analysed by SPSS version 15.Statistical analysis was performed by using one way analysis of variance (ANOVA) and post hoc Tukey test. The p value <0.05 was selected as a criteria for statistically significant value.

RESULTS

One month research showed that renal parameters increased due to nephrotoxicity produced by Cypermethrin while green tea and vitamin C restored them to normal levels. Our research demonstrated significant difference in serum urea in Cypermethrin group which raised to 6.8±0.48m.mol/l in comparison to control (3.9±0.44 m.mol/l). Serum urea of green tea group was 4.0±0.83 m.mol/l and vitamin C group was 3.4±0.33 m.mol/l which is near normal to control group. Serum creatinine also showed significant difference in Cypermethrin group which was raised to 42.9±3.2 µmol/l in comparison with control (29.5±3.9µmol/l). Serum creatinine of green tea group was 28.8±4.5 µmol/l and vitamin C group was 22.6±2.0 µmol/l.

Post hock Tuckey test illustrated significant difference, i.e., P value less than 0.05 in Cypermethrin group when compared with control. Similarly green tea and vitamin C also had significant difference with Cypermethrin, i.e., p value less than 0.05. Non-significant difference existed in group c and group d in comparison with control group i.e., P value greater than 0.05 as depicted in table-1.

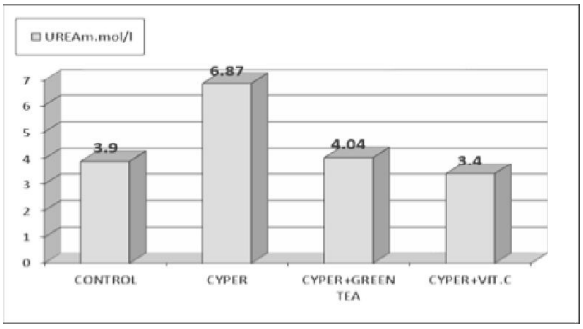

Figure-1: Comparison of serum urea in different groups

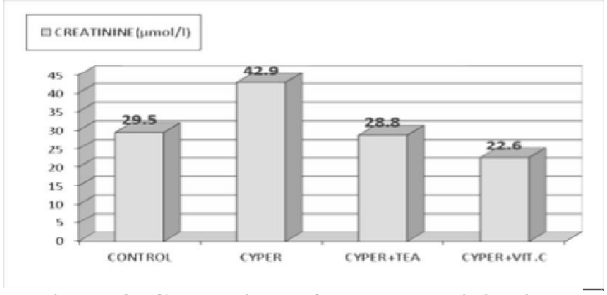

Figure-2: Comparison of serum creatinine in different groups

Table-1: Statistical difference of serum urea and serum creatinine between different pair of groups

Paired on post hock	*p* value	*p* value
GROUP a VS GROUP b	0.000*	0.000*
GROUP a VS GROUP c	0.943	0.972
GROUP a VS GROUP d	0.252	0.001*
GROUP b VS GROUP c	0.000*	0.000*
GROUP b VS GROUP d	0.000*	0.000*
GROUP c VS GROUP d	0.085	0.002*

*Significant difference (p<0.05), NS non-significant difference (p>0.05)

DISCUSSION

In this research oral administration of Cypermethrin outcome was amplification of serum urea and creatinine levels of mice. Serum urea and creatinine were used as markers of renal function and these results revealed damage to renal tissues.[22] Our body is a continuous source of free radical either due to normal metabolism or various harmful substances which are responsible for this event.[7] In a healthy person effect of oxygen free radicals is counteracted by antioxidants present in our body and there is a balance in production of free radicals and neutralization by anti-oxidant system. Oxidative stress is produced due to imbalance in this amicably architecture system.[23] It is the key perpetrator to produce hostile situation in the cell with existence threatening consequences. The free radicals attack produces oxidative damage to bio molecules which are part and parcel of our cells.[24] Our results revealed the effect of Cypermethrin exposure for 30 days in mice induced nephrotoxicity which was evidenced by biochemical parameters perturbations in kidney of mice. Since the oxidative damage is hub of pesticides toxicity it occurs due to production of free radicals, including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues.[25] The use of antioxidants to counteract the formed free radicals is the bedrock to minimize their deleterious effect. So, the main beneficial substances in green teas are tea catechins that have the most superior antioxidant action. Tea catechins are staunch free radical neutralizer as these have ability to reduce one electron.[26]

In renal cells damage there is increase in serum urea and creatinine levels. These became normal by green tea which evidenced burly quenching action of green tea polyphenols to free radical injury. These smother life threatening elements and harmful substances with healing of renal parenchyma and restore normal levels of serum urea and serum creatinine.[27]

Cypermethrin produced oxidative stress which increased serum urea and creatinine in group b but no significant change in group C in comparison with control. Group D also showed normal level of renal parameters in comparison with control. It means vitamin C and green tea had antioxidant properties while Cypermethrin has potential to produce oxidative stress. Monosodium glutamate induced injurious effects to kidneys of rats can be neutralized by vitamin C[28] which gave evidence about anti-oxidant property of vitamin C. Our study correlates with Sohini *et al.* 2007[29] who produced nephrotoxicity in wistar rats with 4 mg/100 g arsenic tri oxide and then observed vitamin C protective effect in dose of 25 mg/100 g. Analogous results were made public by Assayed *et al.* 2010[30] who showed reduction in cypermethrin induced teratogenicity by vitamin C. They gave 55.1 mg/kg body weight of Cypermethrin orally to wistar rats for 60 days while vitamin C in dose of 20 mg/kg body weight. They noticed a lot of deformities in young ones. Most of foetus born dead but vitamin C decrease reproductive toxicity efficiently.

Our study draws a parallel with research work of Sinan *et al*[31] who used 10 mg/kg/day Cypermethrin for swiss mice for four weeks and then observed that Cypermethrin induced free radical formation and lipid per oxidation which decreased as thymoquuinone dose increased. This research outcome exactly matches with Hussain *et al*[32] who used aflatoxin with Cypermethrin in Sprague – Dawley rats. They observed that rats who received aflatoxin alone had reduced food intake. Rats were depressed and lost body weight in comparison with control. When aflatoxin and Cypermethrin both introduced in rats toxicity level increased. This fact was indicated by further reduction in their body weight and even some rats died. Sekhar *et al*[33] selected 8.5 mg/kg body weight Cypermethrin and 5.6 mg/kg body weight sodium fluoride for albino mice in which Cypermethrin given with sodium fluoride enhance Cypermethrin induce oxidative stress in cells.

CONCLUSION

Cypermethrin induced nephrotoxicity in mice which evidenced by rise in serum urea and serum creatinine levels. Vitamin C and green tea protected kidneys and their effect was comparable.

AUTHOR'S CONTRIBUTION

SM: Conducted this study under supervision of KM. AKN: Directed in study design and data analysis

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