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Efectos del extracto de té verde sobre los daños oxidativos inducidos por cisplatina en riñones y testículos de ratas

Effect of green tea extract on cisplatin induced oxidative damage on kidney and testes of rats

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RESUMEN

Se administró extracto de té verde (Camellia sinensis) por vía oral a las ratas en dosis de 25, 50 y 100 mg/kg para investigar sus efectos sobre la nefrotoxicidad y la toxicidad testicular inducida por cisplatina (3mg/kg). El extracto de té verde restauró el nivel de creatinina, urea, nitrógeno ureico en sangre (NUS) y ácido úrico en el suero de animales tratados con cisplatina en comparación con los animales tratados sólo con cisplatina. Además, se observó que la administración de extracto de té verde restauró los niveles de enzimas antioxidantes como superóxido dismutasa (SOD), catalasa (CAT) y glutatión reducido (GSH), y enzimas ligadas a la membrana como Na+K+ATPasa, Ca2+ATPasa y Mg2+ATPasa y redujo la peroxidación lipídica (MDA) en los riñones y en los testículos de animales con alteraciones tras el tratamiento crónico con cisplatina. Por tanto, se puede concluir que el extracto de té verde posee actividad antioxidante y que, en virtud de esta acción, puede proteger los riñones y los testículos frente a los daños oxidativos inducidos por la cisplatina.

PALABRAS CLAVE: Antioxidante, Cisplatina, Radicales libres, Té verde, Nefrotoxicidad, Toxicidad testicular.

ABSTRACT

Green tea extract (Camellia sinensis) was administered orally to rats at the dose levels of 25, 50,100 mg/kg to investigate its effect on cisplatin (3mg/kg) induced nephrotoxicity and testicular toxicity. Green tea extract restored the level of creatinine, urea, blood urea nitrogen (BUN) and uric acid in serum of animals treated with cisplatin as compared to the animals treated with cisplatin alone. It was further found that administration of green tea extract restored the level of antioxidant enzymes such as, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), and membrane bound enzymes like Na+K+ATPase, Ca2+ATPase and Mg2+ATPase and decreased lipid peroxidation (MDA) in kidney and in testes of animals which were altered after chronic treatment with cisplatin. Thus it can be concluded that green tea extract possesses antioxidant activity and by virtue of this action it can protect the kidney and testes from cisplatin induced oxidative damage.

KEY WORDS: Antioxidant, Cisplatin, Free radicals, Green tea, Nephrotoxicity, Testicular toxicity.

1. INTRODUCCIÓN

La cisplatina (*cis*-diamina dicloroplatino II) es uno de los agentes antitumorales más potentes. Ha demostrado presentar actividad frente a diversos tumores, especialmente en cánceres de cabeza y cuello, de testículos, de ovarios, vejiga y cáncer pulmonar de células pequeñas[1]. Sin embargo, el uso de este agente está limitado por el desarrollo de nefrotoxicidad y ototoxicidad[2,3], que provocan alteraciones en los sistemas defensivos antioxidantes[4,5]. Se ha observado que la cisplatina también altera los niveles de hormona luteinizante (LH) y de la hormona estimulante del folículo (FSH), reduciendo la testosterona intratesticular y disminuyendo la motilidad y el recuento de los espermatozoides[6,7]. Las alteraciones de la función renal

1. INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum II) is one of the most potent antitumor agents. Activity has been demonstrated against a variety of tumors, particularly for head and neck, testicular, ovarian, bladder and small cell lung cancers[1]. However, the use of this agent is limited by the development of nephrotoxicity and ototoxicity[2,3], toxicities which involve alterations in the antioxidant defense systems[4,5]. Cisplatin has also been shown to alter the levels of luteinizing hormone (LH) and FSH, to reduce intratesticular testosterone, and to decrease sperm motility and count[6,7] The alterations in kidney function induced by cisplatin are characterized by signs of injury, such as changes in urine volume and in glutathione status. Cisplatin induced nephrotoxicity is closely associated with an increase in lipid peroxidation [8,9]. Cisplatin was able to generate reactive

inducidas por cisplatina se caracterizan por señales de daños, como cambios en el volumen de la orina y en el estado del glutatión. La nefrotoxicidad inducida por cisplatina está estrechamente relacionada con un aumento en la peroxidación lipídica [8,9]. La cisplatina pudo generar especies reactivas de oxígeno, como aniones de superóxido y radicales hidroxi[10,11,12], y también inhibió la actividad de enzimas antioxidantes en el tejido renal[9]. La quimioterapia con cisplatina indujo a una caída en los niveles de antioxidante en plasma, que pueden reflejar un fallo del mecanismo de defensa contra los daños oxidativos inducidos por fármacos antitumorales utilizados habitualmente[13].

El tratamiento previo con diversos antioxidantes como ebselen[14], vitamina C[15] y selenio[16, 17] ha demostrado ofrecer protección frente a la nefrotoxicidad inducida por cisplatina en humanos y en animales de laboratorio. El té verde es una excelente fuente de antioxidantes de polifenol, especialmente de los del grupo conocido como catecoles del té verde (GTC) [18]. Los polifenoles son metabolitos de las plantas presentes en gran número de alimentos vegetales que poseen excelentes propiedades antioxidantes y frente a los efectos de barrido de los radicales libres[19,20]. Los taninos del té verde también tienen un efecto protector frente a nefropatías inducidas por cisplatina, en células LLC-PK_1 y en ratas[21]. El té verde reduce la peroxidación lipídica inducida por el hierro en homogeneizados del cerebro y en astrocitos C6 cultivados y células pulmonares[22,23]. Además, también ha demostrado reducir la formación de aductos de espín de radicales hidroxilo y de la ruptura de cadenas de ADN inducida por radicales hidroxilo in vitro[24]. Se ha observado que el té verde también tiene efectos inhibidores de la tumorogénesis pulmonar inducida químicamente[25] También existen considerables pruebas epidemiológicas que sugieren que el consumo de té verde reduce el riesgo de incidencia de varios tipos de cáncer como resultado de sus mecanismos antioxidantes[26].

Hasta la fecha no se han realizado estudios sobre el efecto antioxidante del extracto de té verde en los daños oxidativos inducidos por cisplatina. Por tanto, la finalidad del presente estudio era investigar los efectos protectores del extracto de té verde en los daños oxidativos inducidos por cisplatina en riñones y testículos de ratas.

2. MATERIALES Y MÉTODOS

2.1. Productos químicos

El extracto de acetato de etilo de hojas de té verde (Camellia sinensis) estandarizado en polvo fue una muestra proporcionada gratuitamente por Cherain Chemicals, Baroda, India, con un contenido total en polifenoles del 35% La inyección de cisplatina fue una muestra proporcionada gratuitamente por la empresa Serum Institute of India Ltd., Pune, India. El resto de las sustancias químicas utilizadas fueron de grado analítico.

2 2. Animales

En el estudio se utilizaron ratas albinas macho adultas (de raza Wistar) con un peso de entre 175 y 225 g. Los animales se alimentaron ad libitum con dieta estándar de bolitas de pienso y tuvieron agua a su disposición en todo momento. Todos los experimentos y protocolos descritos en el presente informe fueron

oxygen species, such as superoxide anions and hydroxyl radicals[10,11,12], and it also inhibited the activity of antioxidant enzymes in renal tissue[9]. Cisplatin chemotherapy induced a fall in plasma antioxidant levels, which may reflect a failure of the antioxidant defense mechanism against oxidative damage induced by commonly used antitumor drugs[13].

The nephrotoxicity induced by cisplatin in human and experimental animal has been shown to be protected by the prior treatment of various antioxidans like ebselen[14] , vitamin C[15] and selenium [16, 17]. Green tea is an excellent source of polyphenol antioxidants, particularly of group known as green tea catechins (GTCs) [18] . Polyphenols are plant metabolites occurring widely in plant food and possess outstanding antioxidant and free radical scavenging properties[19,20].Green tea tannin protects cisplatin induced nephropathy in LLC-PK_1 cells and in rats[21] . Green tea reduces iron-induced lipid peroxidation in brain homogenates as well as in cultured C6 astrocytes and lung cells[22,23] . In addition, green tea has also been shown to reduce the formation of the spin-adducts of hydroxyl radicals and hydroxyl radical to induced DNA strand breakage in vitro[24] . Green tea has been found to have inhibitory effects on the chemical-induced lung tumorigenesis[25]. There is also considerable epidemiological evidence suggesting that the consumption of green tea lowers the risk of several types of cancer incidences as a result of these antioxidant mechanisms[26] .

So far, studies have not carried out on antioxidant effect of green tea extract on cisplatin induced oxidative damage. Therefore, the present study was aimed to investigate the protective effects of green tea extract on cisplatin induced oxidative damage on kidney and testes of rats.

2. MATERIAL AND METHODS

2 1. Chemicals

Standardized powdered, ethyl acetate extract of green tea leaves (Camellia sinensis) was gift sample from Cherain Chemicals, Baroda, India with total polyphenolic content 35% Cisplatin injection was gift sample from Serum Institute of India Ltd , Pune, India. All other chemicals used were of analytical grade.

2.2. Animals

Adult male albino rats (Wistar strain) weighing between 175 and 225 g were used for the study. The animals were fed ad libitum with standard pellet diet and had free access to water. All experiments and protocols described in present report were approved by the Institutional Animal Ethics Committee (IAEC) of M. S. University, Baroda and are in accordance with guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3. Experimental Procedure

The animals were divided into five groups each consisting of six rats and received following treatment.

Group I. Control group, received distilled water (3ml/kg

aprobados por el Comité de Ética Animal Institucional (Institutional Animal Ethics Committee, IAEC) de la universidad M. S University, Baroda, India, y cumplen las indicaciones del Comité de Control y Supervisión de Experimentos en Animales (Committee for the Purpose of Control and Supervision of Experiments on Animals, CPCSEA), del Ministerio de Justicia Social y Autorizaciones (Ministry of Social Justice and Empowerment) del Gobierno de India.

2.3. Procedimiento experimental

Los animales se dividieron en cinco grupos, cada uno formado por seis ratas, que recibieron los tratamientos siguientes

Grupo I: Grupo de control, recibió agua destilada (3ml/kg /día por vía oral durante 30 días) y agua esterilizada para inyección (3ml/kg, intraperitoneal) los días 1,7,14,21 y 28.

Grupo II: Recibió agua destilada (3ml/kg/día por vía oral durante 30 días) e inyecciones de cisplatina (3 mg/kg por vía intraperitoneal) en los días 1,7, 14, 21 y 28.

Grupo III: Extracto de té verde (25 mg/kg/día por vía oral durante 30 días) e inyecciones de cisplatina (3 mg/kg por vía intraperitoneal) en los días 1,7,14,21 y 28.

Grupo IV. Extracto de té verde (50 mg/kg/día por vía oral durante 30 días) e inyecciones de cisplatina (3 mg/kg por vía intraperitoneal) en los días 1,7,14,21 y 28.

Grupo V Extracto de té verde (100mg/kg/día por vía oral durante 30 días) e inyecciones de cisplatina (3 mg/kg por vía intraperitoneal) en los días 1,7,14,21 y 28.

Transcurridas 48 horas desde la última dosis de cisplatina, se extrajo una muestra de sangre mediante punción retroorbital y se separó el suero para las estimaciones de creatinina, urea, ácido úrico y nitrógeno ureico en sangre (NUS). Estos valores se determinaron mediante un kit de Span Diagnostics (India) Pvt Ltd., India. A continuación, se sacrificaron los animales y los riñones y los testículos se diseccionaron, pesaron y homogeneizaron en tampón Tris frío (10 mM, pH 7 4) con una concentración del 10% (p/v). Los homogenatos se centrifugaron a 10.000×g a una temperatura de 0 °C durante 20 minutos en una centrifugadora refrigerante de alta velocidad Remi C-24. El sobrenadante transparente se utilizó para los ensayos de las enzimas antioxidantes endógenas de la peroxidación lipídica (contenido de MDA)[27], superóxido dismutasa (SOD)[28], catalasa (CAT)[29] y glutatión reducido (GSH)[30]. El sedimento se volvió a introducir en suspensión en tampón Tris helado (10 mM, pH 7 4) para obtener una concentración final del 10% y se utilizó para la estimación de las distintas enzimas vinculadas a la membrana como la Na^+K^+ATPasa[31], Ca^{2+}ATPasa[32] y Mg^{2+}ATPasa[33] y las proteínas totales[34].

2.4. Análisis estadístico

Los resultados de todas las estimaciones anteriores se han indicado en términos de media ± E.S M. La diferencia entre los grupos se determinó estadísticamente por análisis de varianza (ANOVA) seguido de una prueba de comparación múltiple Tukey–Kramer con un nivel de significación de $P \leq 0,05$.

/day p.o. for 30 days) and sterile water for injection (3ml/kg, i.p.) on day 1,7,14,21,28.

Group II: Received distilled water (3ml/kg/day p o. for 30days) and cisplatin injection (3 mg/kg i p.) on day 1,7, 14, 21, 28

Groups III: Green tea extract (25 mg/kg/day p o.for 30 days) and cisplatin injection (3 mg/kg i p.) on day 1,7,14,21,28.

Group IV: Green tea extract (50 mg/kg/day p.o for 30 days) and cisplatin injection(3 mg/kg i p.) on day 1,7,14,21,28

Group V. Green tea extract (100mg/kg /day p o.for 30 days) and cisplatin injection(3 mg/kg i.p.) on day 1,7,14,21,28

After 48 hours of the last dose of cisplatin, blood sample was withdrawn by retroorbital puncture and serum was separated for estimations of creatinine, urea, blood urea nitrogen (BUN) and uric acid. These values were determined by using kit of Span Diagnostic Pvt. Ltd, India. The animal were then sacrificed and the kidney and testes were dissected out ,weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×g at 0° C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content)[27] endogenous antioxidant enzymes, superoxide dismutase (SOD)[28], catalase (CAT)[29]and reduced glutathione (GSH)[30] The sediment was resuspended in ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes such as Na^+K^+ATPase[31], Ca^{2+}ATPase[32]and Mg^{2+}ATPase[33]and Total proteins[34].

2.4 Statistical analisis

Results of all the above estimations have been indicated in terms of mean±S.E.M. Difference between the groups was statistically determined by analysis of variance (ANOVA) followed by Tukey –Kramer multiple Comparisons test with the level of significance set at $P \leq 0.05$.

Grupos *Groups*	Creatinina *Creatinine* (mg/dl)	*Urea* (mg/dl)	Ácido úrico *Uric acid* (mg/dl)	NUS *BUN* (mg/dl)
Control **(Group I)**	0 35±0 061	35 7±3 46	0 51±0 016	16 66±1 61
Cisplatin (3mg/kg.i.p) **(Group II)**	2 05±0 25***	107 81±16 32***	2 7±0 2***	50 34±7 62***
GTE(25mg/kg p o)+Cisplatin(3mg/kg i p.) **(Group III)**	0 93±0 11***	78.11±6 35NS	1.31±0 13***	36 47±2 96NS
GTE(50 mg/kg p.o) + Cisplatin (3mg/kg i p.) **(Group IV)**	0 73±0 10***	68 41±10 19NS	0.73±0 09***	31 94±4 76NS
GTE(100 mg/kg p.o)+Cisplatin (3mg/kg i p) **Group V)**	0 48±0.11***	55 25±10 48*	0.65±0 13***	25 79±4.89*
F value	22.20	6 77	44.07	6 77
P value	<0 0001	0 0077	<0 0001	0 0077

Los valores están expresados como media ± ESM (n = 6) El Grupo II se comparó con el Grupo I Los grupos III, IV y V se compararon con el Grupo II *P<0,05, **P <0,01, ***P <0,001, NS = No significativo
Values are expressed as mean ± SEM (n = 6) Group II was compared with Group I Group III, IV and V compared with Group II *P<0 05, **P <0 01, ***P <0 001, NS = Non significant

Tabla 1: Efecto del extracto de té verde (GTE)(30 días) junto con la administración crónica de cisplatina (3mg/kg por vía intraperitoneal) en los días 1, 7, 14, 21 y 28 en los niveles séricos de creatinina, urea, ácido úrico y NUS

***Table 1**: Effect of green tea extract(GTE)(30 days) along with chronic administration of cisplatin (3mg/kg i.p.) on day 1,7,14, 21and 28 on the serum levels of creatinine, urea, uric acid and BUN*

3. RESULTADOS

3.1. Nefrotoxicidad inducida por cisplatina

La administración de inyecciones de cisplatina sola (Grupo II) en los días 1, 7, 14, 21 y 28 provocaron un aumento significativo (P<0,001) de los niveles de creatinina, urea, ácido úrico y NUS, los marcadores del daño renal. La administración de extracto de té verde (Grupos III, IV y V) durante 30 días junto con cisplatina en los días 1, 7, 14, 21 y 28 disminuyó significativamente (P<0,001, P<0,05) los niveles de creatinina, ácido úrico, urea y NUS en comparación con los animales tratados con cisplatina sola (Grupo II). (Tabla 1)

Se observó una reducción significativa (P<0,001) de los niveles de SOD, CAT y GSH en los riñones de los animales tratados con cisplatina sola (en los días 1, 7, 14, 21 y 28) (Grupo II) en comparación con el grupo de control (Grupo I) y un aumento significativo (P<0,001) en la peroxidación lipídica (contenido de MDA) en los riñones de los animales tratados con cisplatina sola (Grupo II) en comparación con el grupo de control (Grupo I). Tras la administración de extracto de té verde en dosis de 100 mg/kg durante 30 días junto con inyecciones de cisplatina en los días 1, 7, 14, 21 y 28 se observó un aumento significativo (P<0,001 , P<0,01) en los niveles de GSH, CAT y SOD y una reducción significativa (P<0,001) en la peroxidación lipídica (contenido de MDA) en comparación con los animales a

3. RESULTS

3.1. Cisplatin induced nephrotoxicity

Administration of cisplatin injection alone (Group II) on 1st,7th,14th,21th,and 28th day resulted in a significant (P<0 001) elevation in the levels of creatinine, urea, uric acid and BUN, the markers of renal injury. Administration of green tea extract(Group III,IV,V) for 30 day along with cisplatin on 1st , 7th, 14th, 21th,and 28th day significantly (P<0.001,P<0.05)) decreased the levels of creatinine, uric acid, Urea and BUN as compared to animals treated with cisplatin alone (Group II). (Table 1)

There was a significant (P<0.001) reduction in levels of SOD, CAT and GSH was observed in kidney of animals treated with cisplatin alone (on 1st,7th,14th,21th,and 28th day) (Group II) as compared to control(Group I) and a significant (P<0.001) elevation in lipid peroxidation (MDA content) was observed in kidney of animals treated with cisplatin alone (Group II) as compared to control(Group I) Administration of green tea extract at dose of 100 mg/kg for 30 day along with cisplatin injection on 1st, 7th, 14th ,21th,and 28th day showed significant (P<0.001 , P<0 01) increase in GSH,CAT and SOD and significant(P<0 001) decrease in lipid peroxidation (MDA content) as compared to animals treated with cisplatin alone (Group II).(Table 2)

los que sólo se había administrado cisplatina (Grupo II).
(Tabla 2)

Grupos *Groups*	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (µg of GSH/mg protein)	Superoxide Dismutase (Units/mg protein	Catalase (µmoles of H_2O_2 consumed/min/mg protein)
Control **(Group I)**	2.0±0.08	4.25±0 54	2.95±0.49	3 82±0.15
Cisplatin (3mg/kgi.p.) **(Group II)**	3.36±0.31***	1.91±0.16***	0 71±0.11***	2.31±0.13***
GTE(25mg/kgp.o.) +Cisplatin(3mg/kg i.p) **(Group III)**	2.45±0.07**	2.92±0 4NS	1.01±0.13 NS	2 60±0 084 NS
GTE(50 mg/kg p.o.) + Cisplatin (3mg/kg i.p.) **(Group IV)**	2.24±0.11***	3.53±0 27*	2.15±0.31*	2.80±0.07 NS
GTE(100 mg/kg p.o.)+Cisplatin (3mg/kg i.p.) **Group V)**	1.90±0 08***	4.19±0.12***	2 58±0.46**	3 23±0.13***
F value	12.87	8.18	8.1	23 44
P value	<0 0001	0 0002	0.0002	<0.0001

Los valores están expresados como media ± ESM (n = 6) El Grupo II se comparó con elGrupo I. Los grupos III, IV y V se compararon con el Grupo II. *P<0,05, **P <0,01, ***P <0,001, NS = No-significativo
Values are expressed as mean ± SEM (n = 6) Group II was compared with Group I Group III, IV and V compared with Group II. *P<0 05, **P <0 01, ***P <0 001, NS = Non significant

Tabla 2· Efecto del extracto de té verde (GTE)(30 días) junto con la administración crónica de cisplatina (3 mg/kg i p) en los días 1, 7, 14, 21 y 28 sobre los niveles de peroxidación lipídica (contenido de MDA), el glutatión reducido, la superóxido dismutasa y la catalasa en los riñones de las ratas
Table 2: *Effect of green tea extract(GTE)(30 days) along with chronic administration of cisplatin(3 mg/kg i.p.) on day 1,7,14,21,and 28 on the levels of lipid peroxidation (MDA content), reduced glutathione, superoxide dismutase and catalase in kidney of rat*

Las ratas tratadas con cisplatina (Grupo II) en los días 1, 7, 14, 21 y 28 mostraron un descenso significativo (P<0,01, P<0,05, P<0,001) en la actividad de las enzimas Na^+K^+ATPasa, Ca^{2+}ATPasa y Mg^{2+}ATPasa en comparación con el grupo de control (Grupo I). La administración de extracto de té verde durante 30 días junto con cisplatina en los días 1, 7, 14, 21 y 28 aumentó significativamente (P<0,01, P<0,05, P<0,001) la actividad de las enzimas Na^+K^+ATPasa, Ca^{2+}ATPasa y Mg^{2+}ATPasa en comparación con los animales a los que sólo se les administró cisplatina (Grupo II). (Tabla 3)

3.2. Toxicidad testicular inducida por cisplatina

Las ratas a las que se administró cisplatina (Grupo II) en los días 1, 7, 14, 21 y 28 mostraron una reducción significativa (P<0,001, P<0,001) en los niveles de SOD, CAT y GSH y un aumento significativo (P<0,001) de la peroxidación lipídica en los testículos, en comparación con el grupo de control (Grupo I). La administración de extracto de té verde (Grupos III, IV, V) durante 30 días junto con cisplatina en los días 1, 7, 14, 21 y 28 provocó un aumento significativo (P<0,01, P<0,001) en los niveles de SOD, GSH y CAT y una reducción significativa (P<0,001) de la peroxidación lipídica en los testículos en comparación con los animales a los que sólo se administró cisplatina (Grupo II), lo que implica un efecto antioxidante del extracto de té verde. (Tabla 4)

Cisplatin treated rats(Group II) on 1st,7th,14th,21th,and 28th day showed a significant (P<0 01,P<0.05,P<0.001) decrease in the activities of Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase enzymes as compared to control (Group I). Administration of green tea extract for 30 day along with cisplatin on 1st,7th,14th,21th,and 28th day significantly (P<0.01,P<0.05,P<0 001) increased the activities of Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase enzymes as compared to animal treated with cisplatin alone(Group II) (Table 3)

3.2. Cisplatin induced testicular toxicity

Cisplatin treated rats (Group II) on 1st,7th,14th,21th,and 28th day showed a significant (P<0.001,P<0.001) decrease in the levels of SOD,CAT and GSH and significant (P<0.001) increase in lipid peroxidation in testes, as compared to control (Group I) Administration of green tea extract (Group III,IV,V) for 30 day along with cisplatin on 1st , 7th, 14th, 21th,and 28th day caused a significant (P<0.01,P<0 001) increase in SOD, GSH and CAT and a significant(P<0.001) decrease in lipid peroxidation in testes as compared to animals treated with cisplatin alone(Group II) implying an antioxidant effect of green tea extract (Table 4)

Grupos *Groups*	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/mg protein	Ca^{2+}ATPase(µmoles of inorganic phosphorus liberated/min/mg protein	Mg^{2+}ATPase(µmoles of inorganic phosphorus liberated/min/mg protein
Control **(Group I)**	12 67±0 61	6 98±0 24	9 98±0 38
Cisplatin (3mg/kgı.p) **(Group II)**	8 22±1.08**	5.34±0 54*	4 25±0 24***
GTE(25mg/kgp.o) +Cısplatin(3mg/kg i.p.) **(Group III)**	10 10±0 89 NS	6.02±0.26 NS	5 58±0 41 NS
GTE(50 mg/kg p o.) + Cisplatin (3mg/kg i.p) **(Group IV)**	11 44±0.61*	6.84±0.25 NS	7 02±0 26***
GTE(100 mg/kg p o.)+Cisplatin (3mg/kg i.p.) **Group V)**	12.12±0.21**	7.07±0 5*	7.32±0.41***
F value	5.66	3.68	37.25
P value	0.0022	0 0172	<0.0001

Los valores están expresados como media ± ESM (n = 6) El Grupo II se comparó con el Grupo I Los grupos III, IV y V se compararon con el Grupo II *P<0,05, **P <0,01, ***P <0,001, NS = No significativo

Values are expressed as mean ± SEM (n = 6) Group II was compared with Group I Group III, IV and V compared with Group II *P<0 05, **P <0.01, ***P <0 001, NS = Non significant

Tabla 3· Efecto del extracto de té verde (GTE)(30 días) junto con la administración crónica de cisplatina (3 mg/kg por vía intraperitoneal) en los días 1, 7, 14, 21 y 28 en los niveles de Na^+K^+ATPasa, Ca^{2+}ATPasa y Mg^{2+}ATPasa en los riñones de ratas

Table 3. *Effect of green tea extract(GTE) (30 days) along with chronic administration of cisplatin (3 mg/kg i p) on day 1,7,14,21,and 28 on the levels of Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in kidney of rat*

Grupos *Groups*	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (µg of GSH/mg protein	Superoxide Dismutase (Units/mg protein	Catalase (µmoles of H_2O_2 consumed/min /mg protein)
Control **(Group I)**	1.22±0 043	4.15±0.32	4 92±0.97	7.29±0.77
Cisplatin (3mg/kgi p) **(Group II)**	2 61±0 11***	2.50±0.25**	0.59±0.12***	2.78±0.49***
GTE(25mg/kgp o) +Cisplatin(3mg/kg i p) **(Group III)**	2.28±0.14NS	3 08±0.28 NS	0.99±0 07 NS	3.55±0.62 NS
GTE(50 mg/kg p o.) + Cisplatin (3mg/kg i p) **(Group IV)**	2.05±0.18*	3 88±0.23*	2.79±0.42*	5.29±0.26*
GTE(100 mg/kg p o)+Cisplatin (3mg/kg i.p.) **Group V)**	1.60±0.1***	4.05±0.30**	3.44±0.32**	6.95±0.66***
F value	18 65	6 47	12.64	11.41
P value	<0 0001	0.001	<0.0001	<0.0001

Values are expressed as mean ± SEM(n = 6) Group II was compared with Group I Group III, IV and V compared with Group II *P<0.05, **P <0.01, ***P <0 001, NS = Non significant

Los valores están expresados como media ± ESM (n = 6). El Grupo II se comparó con el Grupo I. Los grupos III, IV y V se compararon con el Grupo II *P<0,05, **P <0,01, ***P <0,001, NS = No significativo

Tabla 4 Efecto del extracto de té verde (30 días) junto con la administración crónica de cisplatina (3 mg/kg i p) en los días 1, 7, 14, 21 y 28 sobre los niveles de peroxidación lipídica (contenido de MDA), el glutatión reducido, la superóxido dismutasa y la catalasa en los testículos de las ratas

Table 4: *Effect of green tea extract(30 days) along with chronic administration of cisplatin(3 mg/kg i p) on day 1,7,14,21,and 28 on the levels of lipid peroxidation (MDA content), reduced glutathione, superoxide dismutase and catalase in testes of rat*

Se observó un aumento significativo (P<0,01, P<0,05, P<0,001) en la actividad de las enzimas Na^+K^+ATPasa, Ca^{2+}ATPasa y Mg^{2+}ATPasa en los animales a los que sólo se administró cisplatina (Grupo II) en los días 1, 7, 14, 21 y 28, en comparación con el grupo de control (Grupo I). La administración de extracto de té verde (Grupos III, IV y V) durante 30 días junto con inyecciones de cisplatina en los días 1, 7, 14, 21 y 28 aumentó significativamente (P<0,01, P<0,05, P<0,001) la actividad de las enzimas Na^+K^+ATPasa, Ca^{2+}ATPasa y Mg^{2+}ATPasa en los testículos en comparación con los animales a los que sólo se les administró cisplatina (Grupo II). (Tabla 5)

A significant (P<0 01,P<0.05,P<0 001) decrease in activities of Na^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase enzymes was observed in animals treated with cisplatin alone(Group II) on 1st,7th,14th,21th,and 28th day as compared to control (Group I) Administration of green tea extract (Group III,IV,V) for 30 day along with cisplatin injection on 1st,7th,14th,21th,and 28th day significantly (P<0 01,P<0.05)increased activities of Na^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase enzymes in testes as compared to animals treated with cisplatin alone(Group II).(Table 5)

Grupos *Groups*	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/mg protein	Ca^{2+}ATPase(µmoles of inorganic phosphorus liberated/min/mg protein	Mg^{2+}ATPase(µmoles of inorganic phosphorus liberated/min/mg protein
Control **(Group I)**	8 5±0.34	4.24±0 57	6 56±0.40
Cisplatin (3mg/kgi.p.) **(Group II)**	5.77±0.44**	2.80±0.22*	3.26±0.31***
GTE(25mg/kgp.o.) +Cisplatin(3mg/kg i p.) **(Group III)**	6.78±0.38NS	3.32±0.23 NS	3.69±0 17 NS
GTE(50 mg/kg p.o) + Cisplatin (3mg/kg i.p) **(Group IV)**	7 80±0 49*	3 77±0.26 NS	4.51±0 51 NS
GTE(100 mg/kg p o.)+Cisplatin (3mg/kg i p) **Group V)**	8.43±0 51**	4 30±0 20*	5.48±0.45**
F value	6 95	3.62	11 67
P value	0 0006	0.018	<0.0001

Values are expressed as mean ± SEM (n = 6) Group II was compared with Group I Group III, IV and V compared with Group II .
*P<0 05, **P <0.01, ***P <0 001, NS = Non significant
Los valores están expresados como media ± ESM (n = 6) El Grupo II se comparó con el Grupo I Los grupos III, IV y V se compararon con el Grupo II *P<0,05, **P <0,01, ***P <0,001, NS = No significativo

Tabla 5 Efecto del extracto de té verde (GTE)(30 días) junto con la administración crónica de cisplatina (3 mg/kg por vía intrapentoneal) en los días 1, 7, 14, 21 y 28 en los niveles de Na^+K^+ATPasa, Ca^{2+}ATPasa y Mg^{2+}ATPasa en los testículos de ratas

***Table 5.** Effect of green tea extract(GTE)(30 days) along with chronic administration of cisplatin (3 mg/kg i.p.)on day 1,7,14,21,and 28 on the levels of Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in testes of rat.*

4. DISCUSIÓN

La cisplatina genera especies reactivas de oxígeno, como radicales hidroxilo y aniones superóxido, y estimula la peroxidación lipídica[35,36]. Aunque no se conoce bien el mecanismo exacto de la nefrotoxicidad inducida por cisplatina, varios estudios sugieren que está relacionado con la formación de radicales libres. Se acepta que ambos están relacionados con el estrés oxidativo y provocan un desequilibrio entre la formación de radicales derivados del oxígeno y el potencial antioxidante del organismo Varios estudios han demostrado que la administración de cisplatina está asociada a un aumento en la formación de radicales libres y con un fuerte estrés oxidativo[15,17]. Como resultado del incremento en la formación de radicales libres en la toxicidad inducida por cisplatina, el equilibrio

4. DISCUSSION

Cisplatin generates reactive oxygen species such as superoxide anion and hydroxyl radicals, and stimulates lipid peroxidation[35,36]. Although the exact mechanism of cisplatin-induced nephrotoxicity is not well understood, several studies have suggested the involvement of free radical formation It is accepted that both correlate to oxidative stress and cause an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential. It has been shown in various studies that cisplatin administrations are associated with increased formation of free radicals, and with heavy oxidative stress[15,17] As a result of an increase in the formation of free radicals in cisplatin-induced toxicity, the balance normally present in cells between radical formation and protection against them is disturbed[37,38]. This will lead to oxidative damage of cell components, e g. proteins, lipids,

entre la formación de radicales y la protección frente a ellos existente en las células en condiciones normales se ve alterado[37,38]. Esto provoca daños oxidativos en componentes de las células como proteínas, lípidos y ácidos nucleicos[39] Existen evidencias de que la cisplatina causa un profundo deterioro en el sistema reproductor y en el potencial de fertilidad de las ratas[6,49]. La cisplatina inhibe la actividad de las enzimas antioxidantes (SOD, CAT y GSH) y se produce una reducción de los tioles celulares[40] en los riñones y testículos de las ratas que sugiere que la citotoxicidad de la cisplatina se deriva de la formación de especies reactivas de oxígeno (ROS)[9] Los resultados obtenidos en este estudio concuerdan con estos informes previos, que indican que el aumento de la peroxidación lipídica y la disminución del glutatión reducido y de las enzimas antioxidantes (SOD y catalasa) contribuyen a los daños oxidativos inducidos por cisplatina. Como resultado, se alteran los niveles séricos de creatinina, urea, nitrógeno ureico en sangre (NUS) y ácido úrico, que son indicadores para el diagnóstico de la nefrotoxicidad.

En línea con nuestro estudio, se ha averiguado que diversos antioxidantes como el hidroxianisol butilado (BHA) y el glutatión (GSH) previenen la peroxidación lipídica inducida por cisplatina y la reducción del glutatión[41] y las enzimas antioxidantes El extracto de té verde contiene galocatequina (GC), epigalocatequina (EGC), epicatequina (EC), galato de epigalocatequina (EGCg) y galato de epicatequina (ECg). Los componentes del té tienen efectos antioxidantes, antimutagénicos y anticarcinogénicos que podrían proteger a los humanos frente al riesgo de cáncer ocasionado por agentes medioambientales[42]. Sano et al[43] estudiaron los efectos inhibidores de las hojas de té verde en la peroxidación lipídica inducida por tert-butil y observaron un efecto antioxidante similar tras la administración oral de los principales polifenoles del té. Shim et al[44] estudiaron el efecto quimiopreventivo del té verde en los fumadores de cigarrillos y observaron que bloqueaba el aumento inducido por los cigarrillos de la frecuencia de intercambio de cromátidas hermanas. El efecto antihiperglucémico del té negro ya había sido comunicado anteriormente por Gomes et al[45]. Las ratas con diabetes por estreptozotocina presentaron una mayor sensibilidad a la agregación de plaquetas y a la trombosis, lo que se podía mejorar mediante la administración de catequinas de té verde en la dieta[46,47]. Los polifenoles del té verde tienen propiedades antioxidantes derivadas de su capacidad para secuestrar iones metálicos y barrer especies reactivas de oxígeno[48].

En el presente estudio, la administración de extracto de té verde ofreció una protección significativa frente a los daños oxidativos inducidos por cisplatina en riñones y testículos. La administración de extracto de té verde redujo significativamente la peroxidación lipídica y aumentó los niveles de glutatión, catalasa y SOD en los daños oxidativos inducidos por cisplatina La reducción en los niveles séricos de creatinina, urea, nitrógeno ureico en sangre (NUS) y ácido úrico son indicativos del efecto protector del extracto de té verde. La Na^+K^+ATPasa, la Ca^{2+}ATPasa y la Mg^{2+}ATPasa son las enzimas ligadas a la membrana, y los niveles de Na^+K^+ ATPasa, Ca^{++} ATPasa y Mg^{++} ATPasa se redujeron en los riñones y testículos de las ratas tratadas con cisplatina. Como estas enzimas ligadas a la membrana son grupos tioles que contienen enzimas dependientes

and nucleic acids[39] .There are evidences that cisplatin has a profound deletenous effect on the reproductive system and fertility potential in rat[6,49].Cisplatin inhibits activities of antioxidant enzymes (SOD CAT and GSH) and there is depletion of cellular thiols[40] in rat kidney and testes suggesting that cisplatin cytotoxicity results from generation of reactive oxygen species (ROS)[9] . The results obtained in this study is in agreement with these previous reports, that the enhancement in lipid peroxidation, and decrease in reduced glutathione and antioxidant enzymes (SOD and catalase) contributes to cisplatin induced oxidative damage. This has resulted in the altered levels of serum creatinine, urea, blood urea nitrogen (BUN) and uric acid which are the diagnostic indicator of nephrotoxicity

In line with our study, it has been found that several antioxidants like butylated hydroxyl anisole (BHA) and glutathione (GSH) prevented the cisplatin induced lipid peroxidation and depletion of glutathione[41] and antioxidant enzymes Green tea extract contains gallocatechin (GC), epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCg) and epicatechin gallate (ECg). Tea components possess antioxidant, antimutagenic and anticarcinogenic effects and could protect humans against the risk of cancer by environmental agents[42]. Sano et al[43] studied the inhibitory effects of green tea leaves against tert-butyl hydroperoxide induced lipid peroxidation and a similar antioxidant effect on the kidney was observed after oral administration of a major tea polyphenols. Shim et al[44] studied the chemopreventive effect of green tea among cigarette smokers and found it block the cigarette-induced increase in sisterchromatid exchange frequency Anti-hyperglycemic effect of black tea has been reported earlier by Gomes et al[45]. Streptozotocin diabetic rats showed increased sensitivity to platelet aggregation and thrombosis and this abnormality could be improved by dietary catechin of green tea[46,47] Green tea polyphenols has the antioxidant properties that result from their ability to sequester metal ions and to scavenge reactive oxygen species[48].

In the present study, treatment with green tea extract offered a significant protection from the cisplatin induced oxidative damage to kidney and testes. Green tea extract treatment significantly reduced lipid peroxidation and increased the levels of glutathione, catalase and SOD in cisplatin induced oxidative damage. The reduction in serum levels of creatinine, urea, blood urea nitrogen (BUN) and uric acid indicates protective effect of green tea extract. Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase are the membrane bound enzymes and the levels of Na^+K^+ ATPase, Ca^{++} ATPase and Mg^{++} ATPase were reduced in kidney and in testes of cisplatin treated rats. Since these membranes bound enzymes are thiol group containing enzymes, that are lipid dependant[50] and hence the restoration of the activities of ATPase enzymes suggest the ability of green tea extract to protect the thiol group from oxidative damage through inhibition of lipid peroxidation.

In conclusion, green tea extract administration during cisplatin therapy reduces the risk of cisplatin induced oxidative damage and the protection can be attributed to a decrease in lipid peroxidation, restoration of activities of antioxidant and membrane bound enzymes.

de los lípidos[50], la recuperación de la actividad de las enzimas ATPasa sugiere la capacidad del extracto de té verde para proteger al grupo de tioles frente a los daños oxidativos mediante la inhibición de la peroxidación lipídica.

En conclusión, la administración de extracto de té verde durante el tratamiento con cisplatina reduce el riesgo de daños oxidativos inducidos por cisplatina, protección que se puede atribuir a un descenso en la peroxidación lipídica y a la recuperación de la actividad de las enzimas ligadas a la membrana y antioxidantes.

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Protective effect of green tea extract on doxorubicin induced cardiotoxicity in rats

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SUMMARY

Doxorubicin induces oxidative stress leading to cardiotoxicity causing ECG abnormalities and increases in biomarkers associated with toxicity. Green tea extract (GTE) is reported to possess antioxidant activity mainly via its polyphenolic constituent, catechins. This study was intended to determine the effect of various doses of GTE (25, 50 and100 mg/kg/day p.o. for 30 days) on doxorubicin -induced electrocardiographic and biochemical changes in rat heart The latter included lactate dehydrogenase (LDH), creatine kinase (CK), and glutamic oxaloacetate transaminase (GOT) in serum and superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), as well as membrane bound enzymes like Na^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and decreased lipid peroxidation (LP) in heart tissue Results demonstrated that rats which received GTE were less susceptible to such changes indicating protection afforded by GTE.

Keywords: Doxorubicin, Green tea, Antioxidant; Catechins, Electrocardiogram

INTRODUCTION

It has been widely reported that doxorubicin, an anthracycline antibiotic for cancer treatment, causes cardiotoxicity, primarily due to the production of free radicals (Doroshow and Locker, 1982; Doroshow, 1983; Myers, 1982; Olson *et al.*, 1981). The clinical effectiveness of doxorubicin treatment for several cancers is affected by the dose-limiting side effect of cardiotoxicity (Lefrak *et al*, 1973). Several studies have concluded that antioxidants like á-tocopherol (αTC) (Myers *et al.*, 1977) and a-phenyl-tert-butyl-nitrone (Paracchini *et al.*, 1993) afforded protection from doxorubicin- induced myocardial injury without affecting its antineoplastic activity.

Polyphenols are plant metabolites occurring widely in foods of plant origin and possess outstanding antioxidant and free radical scavenging properties (Harbone, 1989; Scott *et al* , 1993).Green tea is an excellent source of polyphenol antioxidants, particularly of a group known as green tea catechins (GTCs) (Zhu *et al.*, 1997). Green tea reduces iron-induced lipid peroxidation in brain homogenates as well as in cultured C6 astrocytes and lung cells (Lin *et al.*, 1998; Mazzio *et al.*, 1998). In addition, green tea has also been shown to reduce the formation of the spin-adducts of hydroxyl radicals and hydroxyl radical - DNA strand breakage *in vitro* (Hiramoto *et al.*, 1996). Green tea has been found to have inhibitory effects on chemical-induced lung tumorigenesis (Xu *et al.*, 1992). There is also considerable epidemiological evidence suggesting that the consumption of green tea lowers the risk of

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heart disease as well as several types of cancer incidences as a result of these antioxidant mechanisms (Ahmad and Mukhtar, 1999).

However, to the best of our knowledge, the effect of GTE on doxorubicin-induced cardiovascular abnormalities in rat heart have not been previously explored. Therefore, the aim of the present study was to investigate the effects of green tea extract on doxorubicin-induced cardiovascular abnormalities in a rat model.

MATERIALS AND METHODS

Chemicals

Standardized powdered, ethyl acetate extract of green tea leaves (*Camellia sinensis*) was donated by Cherain Chemicals, Baroda, India. Total polyphenolic content was 35%. Doxorubicin injection was donated by the Serum Institute of India Ltd., Pune. Epinephrine hydrochloride, super oxide dismutase (SOD), malondialdehyde and catalase, were purchased from Sigma Aldrich; USA. Reduced glutathione, 5, 5'dithiobis (-2 nitrobenzoic acid) (DTNB) and thiobarbituric acid (TBA) were purchased from Hi Media; India. All other chemicals were of analytical grade.

Animals

Adult albino rats of either sex (Wistar strain) weighing between 200 and 250 g were used for the study. The animals were fed ad libitum with standard pellet diet and had free access to water. All experiments and protocols described were approved by the Institutional Animal Ethics Committee (IAEC) of M. S. University, Baroda, India.

Experimental Protocol

Chemical analysis of green tea extract

TLC fingerprint profile of the extract was established using HPTLC. For development of the TLC fingerprint, 500 mg of powdered green tea extract was extracted three times with 25ml of methanol. Extracts were pooled, filtered and concentrated to 25 ml. Suitably diluted stock solution of methanolic extract with gallic acid standard solution and catechin were spotted on a pre-coated Silica gel G60 F254 TLC plate (E.Merck) using CAMAG Linomat IV Automatic Sample Spotter and the plate was developed in the solvent system of toluene: ethyl acetate: formic acid (6: 6: 1). The plate was dried at room temperature and scanned using CAMAG TLC Scanner 3 at UV 254 nm and R_f values, and peak area of the resolved bands were recorded. Relative percentage area of each band was calculated from peak areas. The TLC plate was developed by spraying with 5% methanolic ferric chloride solution for the detection of phenolic compounds.

Groups and Treatment Schedule

Powdered green tea extract was reconstituted in distilled water. Doxorubicin injection was dissolved in sterile water for injection. The animals were divided into five groups each consisting of six rats and received following treatment

Doxorubicin- induced acute cardiotoxicity

Group I: Control group, received distilled water (3 ml/kg/day p.o. for 30 days) followed by sterile water for injection (1 ml/kg, i.v.) on 30th day.

Group II: Received distilled water (3 ml/kg/day p.o. for 30 days) followed by doxorubicin injection (10 mg/kg i.v.) on 30th day.

Groups III: Green tea extract (25 mg/kg/day p.o.for 30 days) followed by doxorubicin injection (10 mg/kg i.v.) on 30th day.

Group IV: Green tea extract (50 mg/kg/day p.o. for 30 days) followed by doxorubicin injection (10 mg/kg i.v.) on 30th day.

Group V: Green tea extract (100 mg/kg/day p.o. for 30 days) followed by doxorubicin injection (10 mg/kg i.v.) on 30th day.

After 48 hours of the injection of either doxorubicin or vehicle, electrocardiographic changes were recorded and serum markers were studied after removal of blood and the heart was excised under euthanasia

in chilled Tris buffer (10 mM pH 7.4) for measurement of tissue markers of oxidative stress.

Electrocardiography

Electrocardiograms were recorded under mild ether anesthesia through needle electrodes (Lead II) using Biopac MP30 data acquisition system (Biopac Systems, Santa Barbara, CA). The changes in heart rate, QT interval and ST interval were determined from the ECG.

Biochemical Parameters

Serum markers: Serum levels of lactate dehydrogenase (LDH) and serum creatine kinase (CK), were determined by using standard kits of Reckon Diagnostic Ltd, India while glutamic oxaloacetate transaminase(SGOT) was estimated by using the standard kit of Span Diagnostic Pvt Ltd, India.

Biomarkers of the oxidative stress: The excised heart was then weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×*g* at 0°C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of malondialdehyde content as indicator of lipid peroxidation (LP) (Slater and Sawyer, 1971), endogenous antioxidant enzymes, superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) (Colowick *et al.*, 1984) and reduced glutathione (GSH) (Moron *et al.*, 1979).

Membrane bound enzymes: The sediment after centrifugation of tissue homogenate was resuspended in ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes such as Na^+K^+ATPase (Bonting, 1970), Ca^{2+}ATPase (Hjerten and Pan, 1983) and Mg^{2+}ATPase(Ohnishi *et al.*, 1982) and total proteins(Lowry *et al.*, 1975).

Statistical analysis

Results of all the above estimations have been indicated in terms of mean±S.E.M. Difference between the groups was statistically determined by analysis of variance (ANOVA) followed by Tukey –Kramer multiple comparisons test with the level of significance set at $P \leq 0.05$.

RESULTS

Chemical Analysis: The fingerprint chromatograms are shown in Fig. 1. Details of the fingerprint analysis are given in Table 1.

Electrocardiographic Changes: The ECG changes in all the groups are summarized in Table 2. The doxorubicin administration significantly increases ST and QT interval while heart rate was significantly decreased as compared to control rats. The administration of GTE significantly restores ECG changes towards normalcy in a dose-dependent manner.

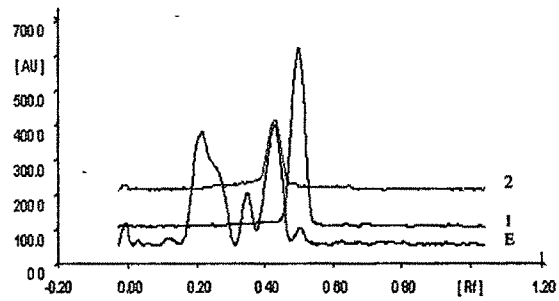

Fig. 1. TLC densitometric chromatogram of methanolic extract of Green tea with gallic acid standard and catechin standard solution. E: Extract, 1: gallic acid, 2: catechin standard solution.

Table 1. Details of fingerprint chromatograms of green tea extract after scanning at 254 nm

Extract	Solvent system	No. of spots
Methanolic extract	Toluene: Ethyl acetate: Formic acid (6 : 6 : 1).	8
Rf values	0.03, 0.12, 0.22, 0.35, 0.43, 0.50, 0.63, 0.68	
Relative %	3.30, 1.84, 33.03, 15.11, 35.09, 4.99, 1.27, 1.05	

Table 2. Effect of green tea extract (30 days) followed by acute administration of doxorubicin (10 mg/kg i v.) on 30th day on ECG changes

Groups	ST interval (msec)	QT interval (msec)	Heart rate (bpm)
Group I	34.16 ± 2.71	70.83 ± 3 0	412.5 ± 14.32
Group II	65.83 ± 4.9***	95.83 ± 3.51***	252.33 ± 14.74***
Group III	47.5 ± 2 14**	83.33 ± 2.1**	278.5 ± 16.91NS
Group IV	40.0 ± 1.82***	70.0 ± 2 88***	293.83 ± 15.01 NS
Group V	33.33 ± 2.78***	70.0 ± 3 16***	329.16 ± 18.72*
F value	18.98	14.88	14.98
P value	P<0.0001	P<0.0001	P<0.0001

Values are expressed as mean ±SEM. Group II was compared with Group I. Group III,IV and V were compared with Group II. *P<0.05, **P <0.01, ***P <0.001, **NS** = Non significant

Table 3. Effect of green tea extract (30 days) followed by acute administration of doxorubicin (10 mg/kg i.v.) on 30th day on the serum levels of lactate dehydrogenase, creatine kinase, and GOT

Groups	Lactate Dehydrogenage (U/L)	Creatine Kinase (U/L)	SGOT (U/ml)
Group I	169.83 ± 4.62	231.16 ±12.68	32.33 ± 2.0
Group II	525.5 ±10.63***	456 83 ± 43.71***	154.18 ± 9.68***
Group III	412.83 ± 32.53**	425.83 ± 32.28NS	71.98 ± 8.47***
Group IV	384.5 ± 11.94***	311.66 ± 25.86*	56.1 ± 7.47***
Group V	278.0 ± 20.85***	269.5 ±16 04***	46.09 ± 9.91***
F value	51 88	11.94	35.72
P value	<0.0001	<0 0001	<0 0001

Values are expressed as mean ± SEM.Group II was compared with Group I. Group III, IV and V compared with Group II. *P<0.05, **P <0 01, ***P <0.001, **NS** = Non significant

Biochemical Parameters

Serum markers: The levels of serum marker enzymes in all the groups are given in Table 3. Doxorubicin administration significantly increases serum level of CK, LDH and GOT as compared to control rats. The administration of GTE significantly restores marker levels towards normalcy in a dose-dependent manner.

Biomarkers of the oxidative stress: The levels of biomarkers of oxidative stress enzymes in all the groups are presented in Table 4. Doxorubicin administration significantly increases LP while there was significant decrease in GSH, SOD and CAT levels as compared to control rats. The administration of GTE significantly improves GSH, SOD and CAT levels after doxorubicin administration while LP level changes towards normalcy in a dose-dependent manner.

Membrane bound enzymes: Doxorubicin damages cell membrane as evident from significant decrease in levels of membrane bound enzymes like Na^+K^+ ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase as compared to control. GTE fails to prevent damage at lower doses while significant improvement was observed at 100 mg/kg dose (Table 5).

DISCUSSION

The results indicate that doxorubicin induces pathological changes in both the ECG and biochemical markers indicative of cardiotoxicity, predominantly due to an increase in free radical production. These results were consistent with earlier studies (Neri *et al.*, 1997; Deatley *et al.*, 1999; Gewirtz, 1999). The results further suggest that administration of GTE improved the ECG and biochemical marker levels indicating decrease in

Table 4. Effect of green tea extract (30 days) followed by acute administration of doxorubicin (10 mg/kg i.v.) on 30th day on the levels of lipid peroxidation (MDA content) , reduced glutathione, superoxide dismutase and catalase in heart of rat

Groups	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (µg of GSH/mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/min/mg protein)
Group I	3.06 ± 0.16	9.45 ± 1.21	2.33 ± 0.36	4.02 ± 0 32
Group II	4.61 ± 0.09***	4.17 ± 0.28***	0.53 ± 0.09***	1.82 ± 0.09***
Group III	3.57 ± 0.17***	5.94 ± 0.5NS	1.23 ± 0.1 NS	1.96 ± 0.15 NS
Group IV	3.49 ± 0.05***	6.73 ± 0.15*	1 38 ± 0.11NS	2.95 ± 0.11**
Group V	3.07 ± 0.09***	7.34 ± 0.17**	2.07 ± 0.33***	4.0 ± 0.17***
F value	25.82	9.95	9.26	30.54
P value	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Values are expressed as mean ±SEM. Group II was compared with Group I. Group III, IV and V were compared with Group II. *P<0.05, **P <0.01, ***P <0.001, NS = Non significant.

Table 5. Effect of green tea extract (30 days) followed by acute administration of doxorubicin (10 mg/kg i.v.) on 30th day on the levels of Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in heart of rat

Groups	Na^+K^+ATPase (µmoles of inorganic phosphrous liberated/min/mg protein	Ca^{2+}ATPase (µmoles of inorganic phosphrous liberated/min/mg protein	Mg^{2+}ATPase (µmoles of inorganic phosphrous liberated/min/mg protein
Group I	7.0 ± 0.2	3.86 ± 0 17	3.01 ± 0.17
Group II	4.04 ± 0.45**	2.09 ± 0.17***	2.04 ± 0 22*
Group III	4.23 ± 0.4 NS	2.06 ± 0.19 NS	2 39 ± 0.21 NS
Group IV	4.94 ± 0.37 NS	2.47 ± 0.12 NS	3.01 ± 0.23*
Group V	5.68 ± 0.71 NS	3.15 ± 0.13***	3.26 ± 0.21**
F value	6.87	22.49	5.68
P value	P=0.0007	P<0.0001	P=0.0021

Values are expressed as mean ±SEM. Group II was compared with Group I. Group III, IV and V were compared with Group II. *P<0.05, **P <0.01, ***P <0.001, NS = Non significant

oxidative stress as evident by increased levels of GSH, SOD and CAT with decreased production of LP. The restoration of membrane bound enzymes like Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in GTE treated rats suggests a membrane-stabilizing protective effect of GTE. These protective effects were also supported by the restoration of serum marker enzymes.

It has been reported that catechins are important constituents of green tea that are responsible for much of the antioxidant and protective effects. We verified the catechin content of the extract using ethyl acetate as medium and found that it contains 35% catechins. In one of the study involving doxorubicin-induced fatty acid composition modification in cardiomyocytes, it was revealed that only one of the GTEs which was rich in catechin contents was able to counteract the detrimental changes and elevation of conjugated dienes (Hrelia *et al.*, 2002). It seems that antioxidant agents can protect the heart from doxorubicin-induced injury as confirmed by several studies (Quiles *et al.*, 2000). Further, it is also reported that GTE exhibits more potent antioxidant activity than other conventional antioxidants (such as vitamin E and C). At the same time, GTE also shows anti cancer action (Ahmad and Mukhtar, 1999). Thus, GTE could be a better option for ameliorating doxorubicin-induced pathological changes. We conclude that the GTE was able to prevent the electrocardiographic abnormalities and

pathological changes in biochemical markers, which were otherwise induced by doxorubicin. This protection may be due to the catechin content of GTE, which is found be a more potent antioxidant than many counterparts (Ahmad and Mukhtar, 1999).

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