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Research Article

ISOLATION AND NEPHROPROTECTIVE ACTIVITY OF BUTRIN FROM ALCOHOLIC FLOWER EXTRACT OF *BUTEA MONOSPERMA*

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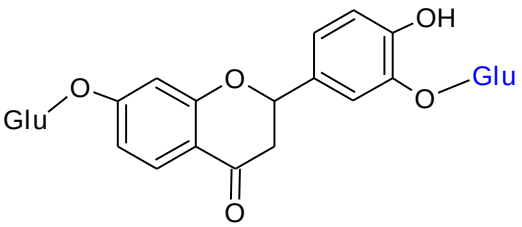
Abstract: Butea monosperma (fabaceae) is use ayurveda for treatment of inflammation, liver disorders, epilepsy , wound healing, diarrhea, diabetic activities. It is also known as modugu in telugu. The presenr study was under taken to evaluate the nephro protective activity of butrin alcoholic flower extract Butea monosperma using gentamicin , cisplatin ,cyclosporine induced methods and reperfusion induced ischemia of rat kidney methods. The comparative histopatholigcal study of kidney exhibited almost normal architecture as compared to control group. Action showed by Butrin (10 mg/kg, 20 mg/kg) was by dose dependent manner that BT 20 mg /kg was more significant in normalizing the toxicity caused by gentamicin, cisplatin and cyclosporine and I/R. The result obtained show that the alcoholic extract of Butea monosperma posses significant nephroprotective activity.
Keywords: Butea monosperma, butrin, nephroprotective activity

Introduction:

Butea monosperma (fabaceae), is a medium-sized deciduous tree belongs to family Fabaceae. This tree is also called "Flame of the Forest" and Bastard Teak. It grows throughout the Indian subcontinent, especially in Indo-Gangetic plains. It loses its leaves as the flowers develop, January -March. The trunk becomes twisted and gnarled by the wind, making it a conversation piece.[1] Butrin (BT) was isolated from Butea monosperma flowers.[2] The important active principles of butea monosperma is butrin.[3] It has been reported that Butrin was proved as well-known antihepatotoxic principles of B. monosperma (Wagner et al.,1986)[4], antioxidant.[5] Butea monosperma has been proved as anticonvulsant[6],antiinflammatory[7], antidiarrhoeal[8], antiestrogenic effects[9], cutaneous wound healing[10], anti-implantation and anti-ovulatory activity[11], anthelminthic[12], nootropic activity[13], bactericidal and fungicidal influence[14], antidiabetic[15], diuretic and antistress activities. Since a nephroprotective activity has not been reported on Butrin, an attempt was made to study this activity.

The plant as been investigated phyto chemically for cadenolides, alkaloids. The main constituent of flower is butrin (BT) (1.5 %) besides butein (0.37 %) and butin (0.04 %). Also contains flavonoids and steroids. Other than these in flowers, coreopsin, isocoreopsin,sulphurein (glycoside) and other two with monospermoside and isomonospermoside structures .



Structure of butrin (BT)

The methanolic extract of *Butea monosperma* reported for anti inflammatory, analgesic activity. The present study was under taken to evaluate the nephro protective activity of butrin alcoholic flower extract Butea monosperma using gentamicin , cisplatin ,cyclosporine induced methods and reperfusion induced ischemia of rat kidney methods.[1]

MATERIALS AND METHODS:

Plant material:

The flowers of *Butea monosperma* were collected from the gardens of Ongole. The plant material was taxonomically identified by prof. k. siddappa, Botanist. Sree Siddaganga college for boys, Tumkur, Karnataka, India

Preparation of extract:

The dried B. monosperma flowers were coarse powdered and macerated successively with n hexane, ethyl acetate and methanol. The hexane, ethyl acetate extracts were evaporated under reduced pressure to give the crude extract. The methanol extract upon standing white solid separated out, which was recrystalized from methanol and dichloromethane.[6]

Phytochemical analysis: [2]

Phytochemical analysis of different extracts was carried out by successive solvent extraction. Weighed quantity of air dried powdered flowers was extracted in soxhlet apparatus successively with n hexane, ethyl acetate and methanol. The methanol extract upon standing white solid separated out, which was recrystalized from methanol and dichloromethane. It is subjected to initial phytochemical screening for detection of phyto constituents.

Experimental animals:

Healthy Wistar rats (200-250 g) and albino mice (25-30 g) of either sex were used. The animals were obtained from GSN pharmaceuticals, kukatpally [Ref: 769/2010/CPCSEA]. Studies were performed in accordance with the CPCSEA

guidelines. The animals were fed with commercially available rat pelleted diet. Water was allowed ad libitum under strict hygienic conditions. The animals were housed at room temperature in a well ventilated animal house under 12 h light/dark cycles.

Stastical analysis:

All the values were stastically analyzed by one-way analysis of variance (ANOVA) followed by tukey-kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant .all values are expressed as Mean± SEM.

Acute toxicity studies: [16]

The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal dose of drug. Healthy Wistar rats (200-250 g) and albino mice (25-30 g) of either sex were used. Two set of healthy female rats (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 h deprived from food, water withdrawn before 4 h of the dosing, body weights were noted before and after dosing with BT (2000 mg/kg) p.o. individually animals were observed for 4 h to see any clinical symptoms, any change in behaviour or mortality. 6 h, post dosing, again body weights were recorded. Form the next day onwards, each day 1 h, the behavioural change, clinical symptoms or mortality rates were observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

Screening models for nephroprotective activity

Model 1 – Gentamicine (GENT) induced nephrotoxicty:[17,18]

Male albino rats weighing 200-250 g were used for the study. The dose of BT is lower 10 mg/kg, higher dose 20 mg/kg was used. The animals were divided into five group six animals each. Group I: Control which receives only normal saline Group II: GENT (80 mg/kg, i.p.) for eight days. Group III: BT higher dose 20 mg/kg for eleven days. Group IV: GENT (80 mg/kg, i.p.) for eight days + BT lower dose 10 mg/kg which is started prior to GENT injection and continued with the eight days GENT treatment. Group V: GENT (80 mg/kg, i.p.) for eight days + BT higher dose 20 mg/kg which is started prior to GENT injection and continued with the eight days GENT treatment.

Table 1: Effect of BT on urine creatinine, urea, serum creatinine, urea, lipid peroxidation, creatinine and urea clearance on GENT induced nephrotoxicity in rats.

Parameters	GI Control	GII BT	GIII GENT	GIV BT 10 mg/kg	GV BT 20 mg/kg
Urinary glucose (mg/day)	Nil	Nil	19.850±2.856^{c}	13.950±0.953^{d}	5.70±0.976^{f}
Urinary Sodium (meq/ day)	0.547±0.2140	0.3117±0.02949	0.8510±0.079^{c}	0.403±0.030^{f}	0.5317±0.056^{f}
Urinary potassium (meq/day)	0.5475±0.021	0.851±0.079	0.311±0.029^{a}	0.403±0.030	0.5317±0.0565^{f}
Urine creatinine (mg/dl)	1.107±0.163	1.429±0.299	2.699±0.5913^{b}	0.355±0.098^{d}	1.973±0.278^{e}
Serum creatinine (mg/dl)	0.8484±0.045	0.832±0.055	1.248±0.075^{b}	1.054±0.811	0.899±0.090^{d}
Serum urea (mg/dl)	35.77±1.916	59.97±4.410	128.7±8.889^{c}	101.9±7.239^{d}	61.32±3.906^{f}
Lipid peroxidation	36.55±3.132	32.62±2.898	148.2±12.38^{c}	116.3±9.043^{d}	55.62±8.433^{f}

Groups II and III were compared with Group I, a= P < 0.05, b= P < 0.01 and c= P < 0.001, Group IV and V compared with Group III, d= P <0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group.

Model 2 – Cisplatin (CP) induced nephrotoxicty:[19,20,21]

Male albino mice weighing 25-30 g were used for the study. The dose of BT is lower 10 mg/kg, higher dose 20 mg/kg was used. The animals were divided into five groups six animals each. Group I: Vehicle (normal saline) as normal. Group II: A single dose of cisplatin (12 mg/kg, i.p.) was kept as negative control. Group III: Animal receives BT high dose 20 mg/kg alone. Group IV: BT lower dose 10 mg/ kg along with cisplatin treatment. Group V: BT higher dose 20 mg/kg along with cisplatin treatment. The extract was administered 1 h before and at 24 h and 48 h after CP inj 72 h after the cisplatin injection, animals were sacrified using ether-anesthesia, blood samples was collected by heart puncture for measuring serum urea and serum creatinine levels. Kidneys were quickly removed and washed with ice-cold normal saline and homogenates (10 % w/v) was prepared in PBS. A part of the homogenate was used for the estimation of reduced glutathione (GSH) and lipid peroxidation. The remaining homogenate was centrifuged at 5000 rpm for 10 min at 4^{0}C, after removal of the cell debris, supernatant was used for the assay of catalase (CAT), glutathione etc.

Table 2: The effect of BT on serum creatinine, urea, lipidperoxidation, GSH, catalase on CP induced nephrotoxicity.

Parameters	GI- Control	GII- BT	GIII- CP	GIV-BT 10 mg/kg	GV-BT 20 mg/kg
Serum creatinine (mg/dl)	0.8923 ± 0.0423	0.9142± 0.0368	1.254± 0.07985[a]	1.013 ± 0.03724[d]	0.9673 ± 0.04266[e]
Serum urea (mg/dl)	54.38 ± 1.702	58.57 ± 4.145	99.62 ± 3.029[c]	70.86 ± 2.181[f]	62.20 ± 2.150[f]
Lipidperoxidation (nM/mg protein)	1.948 ± 0.2542	2.058 ± 0.2900	10.17 ± 0.7806[c]	6.907 ± 0.3900[e]	4.302 ± 0.6928[f]
GSH (µg/mg protein)	42.24 ± 2.808	41.10 ± 3.614	22.61 ± 1.499[c]	33.98 ± 1.912[d]	37.51 ± 1.803[e]
Catalase (units/mg protein)	30.37 ± 3.154	35.27 ± 2.654	11.36 ± 0.9730[c]	25.90 ± 1.619[e]	28.36 ± 2.513[f]

Groups II and III were compared with Group I, a= P < 0.05, b= P < 0.01 and c= P < 0.001, Group IV and V compared with Group III, d= P < 0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Tukey.

Model 3 – Cisplatin(CsA) induced nephrotoxicty:[22]

Male albino rats weighing 200-250 g were used for the study. The dose of BT is lower 10 mg/kg, higher dose 20 mg/kg were used. The animals were divided into five groups six animals each Group I: Vehicle (normal saline) as normal. Group II: CsA (50 mg/kg, p.o.) for 21 days was kept as negative control, Group III: BT high dose 20 mg/kg for 21 days Group IV: BT lower dose 10 mg/kg along with CsA treatment. Group V: BT higher dose 20 mg/kg along with CsA treatment. Twenty-four hours after the last treatment, animals were sacrificed and blood was collected from orbital sinus route and analysed for blood urea nitrogen (BUN) and serum creatinine (SCr), and kidney samples was analysed for lipidperoxidation, GSH, catalase and histopathological examination.

Table 3: The effect of BT on serum creatinine, urea, lipidperoxidation, GSH, catalase on CsA induced nephrotoxicity.

Parameters	GI Control	GII BT	GIII CsA	GIV BT 10mg/kg	GV BT 20 mg/kg
Serum creatinine (mg/dl)	0.909 ± 0.062	0.774 ± 0.062	1.204 ± 0.086[a]	1.190 ± 0.073	1.042 ± 0.039[d]
Serum urea (mg/dl)	42.38 ± 2.429	42.34 ± 2.369	106.1 ± 4.527[c]	91.62 ± 4.287	62.40 ± 4.741[f]
Lipidperoxidation (µ mol MDA /mg protein)	0.433 ± 0.047	0.4739 ± 0.779	1.227 ± 0.115[c]	0.7797± 0.069[e]	0.7160 ± 0.037[f]
GSH (µg/mg protein)	12.78 ± 0.493	11.34 ± 6.844	3.278 ± 0.363[b]	6.844 ± 0.517[f]	7.181 ± 0.471[f]
Catalase (units/mg protein)	19.09 ± 0.609	18.01 ± 0.884	8.740 ± 0.474[b]	13.78 ± 0.893[e]	14.07 ± 1.179[f]

Group II and II were compared with group I, b= P<0.01 and c=p< 0.001 Group IV and V compared with Group III d= P < 0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Tukey

Method 4: Reperfusion-induced (I/R) ischemia of rat kidney:[23]

Male albino rats weighing 150-200 g were used for the study. The dose of BT is lower 10 mg/kg, higher dose 20 mg/kg was used. . The animals were divided into five groups six animals each. Rats were anesthetized with thiopental sodium 40 mg/kg, i.p. and the abdominal region was shaved with a safety razor and sterilized with povidone iodine solution. A midline incision will be made and both the kidneys was isolated. Renal ischemia was be instituted by occluding both the renal pedicles for 45 min followed by reperfusion for 24 h. After the surgical procedures, the midline incision was sutured back with the local applications of povidone and neosporin. The animals was allowed to recover from anesthesia. At the end of reperfusion period, the blood samples was collected and used for the measurement of renal function (blood urea nitrogen,

creatinine). The abdomen was reentered and bilateral nephrectomies was carried out. The left kidney was used for further enzymatic analysis (glutathione, catalase, lipid peroxidation), whereas the right kidney was stored in 10 % formalin for histological examination.

Table 4: The effect of BT on serum creatinine, urea, lipidperoxidation, GSH and catalase on reperfusion- induced ischemia.

Parameters	GI- SHAM	GII- BT	GIII- I/R	GIV I/R+BT 10mg/kg	GV I/R+BT 20mg/kg
Serum creatinine (mg/dl)	0.904 ± 0.034	0.975 ± 0.109	1.238 ± 0.071[b]	1.149 ± 0.028[d]	1.015 ± 0.056[e]
Serum urea (mg/dl)	44.89 ± 4.029	42.92 ± 1.926	117.2 ± 6.592[c]	91.11 ± 6.284[e]	65.58 ± 2.935[f]
Lipidperoxidation (n mol/mg protein)	0.9904 ± 0.08	1.072 ± 0.16	3.004 ± 0.28[c]	1.834 ± 0.19[e]	1.651 ± 0.18[f]
GSH (µg/mg protein)	10.36 ± 0.65	11.06 ± 0.53	1.302 ± 0.15[c]	4.096 ± 0.36[e]	4.887 ± 0.44[f]
Catalase (units/mg protein)	31.01 ± 1.62	30.58 ± 1.78	15.02 ± 0.45[c]	21.19 ± 0.51[d]	23.09 ± 0.70[f]

Groups II and III were compared with Group I, a= P < 0.05, b= P < 0.01 and c= P < 0.001, Group IV and V compared with Group III, d= P < 0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Tukey.

Discussion:

Gentamicin, CP, CsA group and I/R shown significant increase in lipidperoxidation level which causes necrosis or damage of kidney, this was prevented by the co administration of BT. The BT (20 mg/kg) showed more significant effect than BT(10 mg/kg). The glutathione, SOD and catalase this are the protective antioxidants which protects from damage from ROS were significant decrease in I/R, CP and CsA group. The coadministration BT showed significant increase in antioxidant level The gentamicin, CP, I/R and CsA group showed necrosis but prior coadministration of BT showed the protection against the cell damage.

Conclusion:

Gentamicin, CP, CsA, I/R treatment, urinary creatinine, urinary urea, and increased urinary glucose significantly. The lipidperoxidation level was increased and the glutathione, superoxide dismutase and catalase levels were decreased with treatment with gentamicin, CP, CsA and I/R. Treatment with BT has reduced the gentamicin, CP, CsA and I/R induced elevated levels of serum creatinine, serum urea, urinary glucose lipidperoxidation. Treatment with BT has prevented the increased levels of lipidperoxidation and decreased levels of glutathione, SOD and catalase due to gentamicin / CP / CsA/ I/R challenge. Treatment with BT extract has improved the nephroprotective of after gentamicin/ CP/ CsA/ I/R challenge.

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