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Effects of Hydroethanolic Leaf Extract of *Ipomoea asarifolia* (Convolvulaceae) in Doxorubicin and Isoproterenol-Induced Toxicity in Rats

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ABSTRACT

This study investigated the effects of the hydroethanolic leaf extract of *Ipomoea asarifolia* on doxorubicin and isoproterenol-induced toxicities in rats. Rats were randomly assigned into groups- Group 1: distilled water (10 mL/kg orally), Group 2: toxicant, Groups 3-5: *I. asarifolia* (100, 200 and 400 mg/kg orally, respectively) and toxicant, and Group 6: gallic acid (15 mg/kg orally) and toxicant. Treatment lasted for 21 days in the doxorubicin (DOX) model and DOX (5 mg/kg intraperitoneally) was administered on days 7, 14 and 21. Treatment was carried out for 30 days in the isoproterenol (ISO) model and ISO (85 mg/kg subcutaneously) was administered on days 28-30. A day after the treatment, blood samples were collected for biochemical analysis and animals were sacrificed. Vital organs were harvested for *in-vivo* antioxidants assay. In the DOX model, *I. asarifolia* (100-400 mg/kg) produced significant ($p < 0.01$) reduction in levels of lactate dehydrogenase (LDH) and triglycerides (TG) relative to the DOX group. The extract reversed the diminution in heart reduced glutathione (GSH) level caused by DOX. ISO significantly increased ($p < 0.001$) levels of LDH and TG, but these effects were reversed by *I. asarifolia* (100 and 400 mg/kg). The extract (400 mg/kg) significantly reversed ($p < 0.01$) the increase in kidney MDA level elicited by ISO. *I. asarifolia* (200 and 400 mg/kg) significantly ($p < 0.001$) reversed the increase in liver MDA and reduction in CAT levels elicited by ISO. These findings suggest that the extract possesses cardioprotective effects against doxorubicin and isoproterenol-induced toxicities.

Keywords: *Ipomoea asarifolia*, Convolvulaceae, Doxorubicin, Isoproterenol, Biochemical parameters, Antioxidant indices.

Introduction

For decades, remedies derived from plants have been used in the prevention, management and treatment of various disease conditions based on their richness in secondary metabolites[1] which are responsible for diverse pharmacological activities. This is the case especially in the rural regions of the world where dwellers have no access to orthodox drugs or cannot afford them. It has been reported that plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating reactive oxygen species (ROS)-induced oxidative damage which has been identified as the root cause of the development and progression of several diseases.[2] Active components of plants have also been harnessed over the past years as new drugs and/or lead compounds. The search for new drugs from plants has been on the increase in recent times based on the need to provide more therapeutic options in the treatment of various diseases. Another germane motivation is the discovery and development of new drugs and standardized phytomedicines with minimal side effects and better efficacy, and which are affordable, compared to currently available orthodox drugs.

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Doxorubicin (DOX) is an anthracycline glycoside used in cancer therapy. However, its clinical use very often becomes a limiting factor in anticancer therapy due to high irreversible cardiotoxicity.[3] Oxidative stress, lipid peroxidation and mitochondrial dysfunction have been associated with DOX-induced cardiomyopathy.[4] Doxorubicin accumulates mainly in the kidney and to a lesser extent in the liver, heart and small intestine.[5] The drug has been reported to cause varied toxicities including cardiac, pulmonary, hepatic, renal, haematological and testicular toxicity, which restricts its chemotherapeutic use.[6-8]

Isoproterenol (ISO) is a catecholamine which is used therapeutically in patients with bradycardia or heart block. Isoproterenol elicits a reduction of total peripheral resistance with simultaneous direct ionotropic and chronotropic effects on the heart; it produces severe stress in the myocardium and myocardial infarction at supra-maximal doses, an effect that results from the generation of highly cytotoxic free radicals (including oxygen free radicals) through the autoxidation of catecholamines.[9]

Ipomoea asarifolia (Desr.) Roem. and Schult. (Convolvulaceae), commonly called "Morning glory", is widely distributed in West Africa, including Nigeria.[10] In Nigeria, the plant is locally known by diverse names, depending on the part of the country; "*Ogbo oro ayaba*", "*Ewe gboro*" (Yoruba, South West-Nigeria) and "*Duman kadaa*" (Hausa, Northern Nigeria). Extracts of the plant have been reported to be used in traditional medicine for the treatment of various ailments, including inflammation and painful conditions,[11] liver diseases,[12] gastrointestinal disorders and diabetes.[13] Extract of the plant has been reported to possess *in-vitro* antioxidant activity.[13] As stated earlier, both doxorubicin and isoproterenol-induced toxicity have been linked to free radicals. In view of the foregoing facts, this study was designed to investigate the effect of the hydroethanolic leaf extract of *Ipomoea asarifolia* in drug (doxorubicin and isoproterenol) induced toxicity in rat.

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Materials and Methods

Chemicals

Doxorubicin (Celon Laboratories Ltd., Andhra Pradesh, India) and isoproterenol (Hritlk Chemicals Corp., Mumbai, India). The other chemicals employed for biochemical estimations were of analytical standard grades.

Plant Collection and Identification

Fresh leaves of *Ipomoea asarifolia* were sourced from Akinmorin Village, Afijio Local Government, Oyo State, Nigeria, in the month of April 2013. The plant was identified and authenticated in the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria by Mr. T.K. Odewo. A voucher specimen (LUH 5546) was deposited in the institutional herbarium for reference purpose.

Plant Extraction

The collected plant leaves were washed with distilled water and air-dried until a constant weight was obtained. The dried materials were powdered using an electric blender and extracted (100 g/L) using hydroethanol (1:1). Maceration of the plant material was done 3 times for 48 h each time to ensure exhaustive extraction. The extract was decanted and filtered using Whatman filter paper no. 4 and the combined filtrate was evaporated to dryness under reduced pressure at 40ºC. A solid chocolate brown extract was obtained with a yield of 21.21%. The dried extract was reconstituted in distilled water, to obtain desired concentrations, just before administration to experimental animals.

Animals

Adult Swiss mice (15-20 g) and Sprague Dawley rats (150-250 g) of both sexes used for this study were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. The animals were maintained under standard environmental conditions (23-25ºC, 12 h/12 h light/dark cycle) and fed with standard rodent diet (Livestock Feeds Plc., Lagos, Nigeria) and water *ad libitum*. The rats were housed in polypropylene cages, grouped into six animals per cage. Animals were acclimatized for 3 weeks before the commencement of the experiment.

The conduct of this study was guided by the provisions of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.[14]

Phytochemical Screening

Qualitative phytochemical screening of the hydroethanolic leaf extract of *I. asarifolia* was carried out using established procedures.[15-17]

Acute Toxicity Test

Fasted mice (12 h) were allotted to eight groups of five animals each. Six different groups were separately administered *I. asarifolia* extract (250, 500, 1000, 2000, and 4000 mg/kg) and distilled water (10 mL/kg) given intraperitoneally (i.p.). The 2 remaining groups were independently given *I. asarifolia* extract (5000 mg/kg) and distilled water orally (p.o.). Mice were observed for 2 h post-treatment for behavioural changes. Twenty-four hours post-administration, mortality in each group was noted, as applicable, and the median lethal dose (LD_{50}) was derived using the log dose- probit analysis method.[18] Mice that survived in each group were further observed for 14 days for possible signs of delayed toxicity.

Experimental Protocol

A total of 72 rats were divided into 2 pools of 36 rats each for each of the two models employed in this study. For each model, 36 rats were divided into 6 groups consisting of 6 rats each. The ratio of male: female rats was equal across the different groups.

Doxorubicin-Induced Toxicity Model

Group 1: Distilled water 10 mL/kg p.o. (control)
Group 2: DOX
Group 3: *I. asarifolia* extract 100 mg/kg + DOX
Group 4: *I. asarifolia* extract 200 mg/kg + DOX
Group 5: *I. asarifolia* extract 400 mg/kg + DOX
Group 6: Gallic acid 15 mg/kg + DOX

DOX was administered at a dose of 5 mg/kg i.p. on the 7th, 14th and 21st days making a cumulative dose of 15 mg/kg.[3] *I. asarifolia* extract and gallic acid were given p.o. for 21 days. Rats were weighed before the commencement of treatment and at 7 days intervals throughout the course of the experiment. Twenty-four hours after the last treatment, rats were anaesthetized by i.p. administration of 5 mL/kg of a solution of 1% chloralose in 25% urethane (w/v) and blood samples were collected into plain sample bottles through the retro-orbital plexus vein. Thereafter, rats were sacrificed by cervical dislocation. The heart, liver and kidneys were harvested from each rat and weighed. These vital organs, rinsed in phosphate buffered saline, were used for estimation of antioxidant indices.

Isoproterenol-Induced Toxicity Model

The grouping was the same as in the doxorubicin-induced toxicity model, except that *I. asarifolia* extract and gallic acid were administered p.o. for 30 days, and ISO was given subcutaneously (s.c.) at the dose of 85 mg/kg on the 29th and 30th day[19] in place of DOX. Twelve hours after the second injection of ISO, procedures carried out in the doxorubicin-induced toxicity model were repeated.

Biochemical Parameters

Blood samples collected into plain sample bottles were allowed to coagulate. Serum was collected after centrifugation at 3000 rpm for 5 min. for estimation of biochemical parameters, including lactate dehydrogenase (LDH), triglycerides (TG), high-density lipoprotein (HDL), creatinine, and urea using Roche and Cobas commercial kits and the Roche Hitachi automated 912 analyser.[20]

Antioxidant Indices

The supernatants derived from the homogenates of the isolated heart, liver and kidney tissues were used for the estimation of antioxidant indices, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPx), using established procedures.[20,21]

Statistical Analysis

Results obtained in this study are expressed as mean ± S.E.M. The data were analyzed using One-way analysis of variance (ANOVA) with Tukey's post-hoc test using GraphPad Prism 6 Software (GraphPad Software Inc., CA, USA). Results were considered significant at $p < 0.05$.

Results and Discussion

As shown in Table 1, DOX caused significant increase ($p < 0.001$) in the level of LDH compared with control. The DOX-induced increase in LDH was significantly reversed ($p < 0.001$) by *I. asarifolia* (200 and 400 mg/kg) and gallic acid (15 mg/kg), with *I. asarifolia* at the dose of 200 mg/kg producing the greatest reversal. DOX caused significant increase in the levels of creatinine ($p < 0.001$) and urea ($p < 0.01$) relative to control. Values for creatinine in *I. asarifolia* intervention groups (100, 200 and 400 mg/kg) were comparable and not significantly different from that of DOX ($p > 0.05$) while gallic acid (15 mg/kg) caused significant reduction ($p < 0.01$) in the level of creatinine compared with the DOX group. Significant elevation ($p < 0.01$, 0.001) in the level of urea was observed in the DOX and *I. asarifolia* 200 and 400 mg/kg intervention groups compared with control while values in the *I. asarifolia* 100 mg/kg and gallic acid 15 mg/kg were comparable and not significantly different ($p > 0.05$) from control (Table 1). DOX elicited significant reduction ($p < 0.001$) in the level of HDL relative to control. The same trend of the result (significant reduction in the level of HDL) was observed in the *I. asarifolia* (100, 200 and 400 mg/kg) and gallic acid 15 mg/kg intervention groups. However, in respect of TG, DOX caused significant increase ($p < 0.001$) in the level of this parameter. *I. asarifolia* interventions (100, 200 and 400 mg/kg) significantly reversed ($p < 0.001$) the effect of DOX on TG with the greatest reversal elicited at the dose of 100 mg/kg. Gallic acid did not significantly alter ($p > 0.05$) the effect of DOX on the level of TG (Table 1). It has been reported that doxorubicin-induced free radical generation triggers membrane degradation and disruption of cardiac myocytes which can lead to elevations of LDH.[22,23] The results obtained in this study showed an increase in levels of LDH when doxorubicin was administered alone to rats compared with control. This indicates cardiotoxicity which may be as a result of generation of free radicals which extensively damages the myocardium. This damage results in increased membrane permeability thereby leading to leakage of LDH[24] and increased serum concentration. During cardiotoxicity, there is interference in the metabolism and biosynthesis of lipids thereby increasing TG levels and decreasing the levels of HDL in the serum.[25] In this study, doxorubicin

administered alone to rats elicited a significant increase in TG and reduction in HDL levels in the serum compared with control, indicating cardiotoxicity. The ameliorative effect of the hydroethanolic leaf extract of *I. asarifolia* was observed at doses of 100, 200 and 400 mg/kg based on observed significant reduction in the levels of LDH and TG relative to the doxorubicin group. This suggests that the extract possesses cardioprotective activity. In this study, doxorubicin also caused significant increases in the levels of creatinine and urea compared to the control group. Elevations in the serum levels of urea and creatinine are indicative of renotoxicity.[26] This confirms the ability of doxorubicin to cause toxicity in respect of the kidneys. The increase in the serum levels of urea and creatinine was not reversed by co-administration of the extract at various doses and gallic acid with doxorubicin.

As shown in Table 2, ISO significantly increased ($p < 0.001$) the level of LDH relative to control, with *I. asarifolia* at the dose of 200 mg/kg and gallic acid (15 mg/kg) not reversing this effect ($p > 0.05$). However, *I. asarifolia* interventions at doses of 100 and 400 mg/kg significantly reversed ($p < 0.001$) the elevation in the level of LDH caused by ISO. Also, ISO and the various interventions (*I. asarifolia* 100, 200 and 400 mg/kg, and gallic acid 15 mg/kg) did not generally cause significant alteration ($p > 0.05$) in the levels of creatinine and urea compared with control and ISO (Table 2). ISO caused a significant reduction ($p < 0.05$) in the level of HDL compared with the control group. This effect of ISO was not reversed by the various interventions (*I. asarifolia* 100, 200 and 400 mg/kg, and gallic acid 15 mg/kg). ISO significantly increased ($p < 0.001$) the level of TG relative to the control group. This effect of ISO was significantly reversed *I. asarifolia* 100 and 400 mg/kg ($p < 0.001$) and gallic acid 15 mg/kg ($p < 0.01$) (Table 2). In this study, significant increases in the levels of LDH and TG, and significant reduction in the level of HDL were observed with the administration of isoproterenol alone to rats compared with control. This agrees with previous research findings where significant increases in LDH and TG, and significant reduction in HDL levels were observed in the serum of isoproterenol-treated rats.[27,28] *I. asarifolia* exhibited ameliorative effect on isoproterenol-induced cardiotoxicity at doses of 100 and 400 mg/kg based on observation of significant reductions in the levels of LDH and TG relative to the toxicant group. Isoproterenol showed no tendency to cause renotoxicity as the levels of urea and creatinine were not significantly altered relative to the control group. The extract at various doses and gallic acid co-administered with isoproterenol did not significantly alter urea and creatinine levels compared to the control and toxicant groups. Gallic acid (3,4,5-trihydrobenzoic acid) is a phenolic substance with documented antioxidant and hepatoprotective properties.[29] It has also been reported to have antioxidative and anticarcinogenic properties; it inhibits lipid peroxidation, reduces oxidative stress and scavenges free radicals produced in oxidative stress condition.[30] In respect of serum biochemical parameters, gallic acid significantly reduced the levels of LDH and creatinine relative to the DOX group. Also, it significantly reduced the level of TG compared with the ISO group. These observations suggest cardio- and reno-protective effects for gallic acid.

As shown in Table 3, DOX caused significant reduction in the heart level of GSH ($p < 0.001$), CAT ($p < 0.01$) and GPx ($p < 0.05$), and non-significant reduction in the level of SOD. The effect of DOX on GSH was significantly reversed by *I. asarifolia* 200 mg/kg and gallic acid 15 mg/kg ($p < 0.01$). Only gallic acid 15 mg/kg significantly reversed the effect of DOX on SOD, CAT and GPx ($p < 0.05$, 0.001 and 0.05 respectively). DOX significantly reduced the level of MDA ($p < 0.01$) compared to control with *I. asarifolia* 400 mg/kg and gallic acid 15 mg/kg eliciting significant reversals ($p < 0.05$, 0.01 respectively). Oxidative stress is a pathologic condition resulting from either increased production of free radicals or decreased levels of antioxidants like GSH, CAT, GPx, and SOD.[31] Malondialdehyde, end-product of lipid peroxidation, is further elevated by overproduction of free radicals which in turn reduces the antioxidant enzymes and increases disturbance of calcium influx induced by toxic agents.[3] In this study, doxorubicin significantly reduced heart levels GSH, CAT and GPx relative to control, thereby indicating cardiotoxicity. This is in accordance with previous research findings by Saad *et al*.[32] that documented decreases in GSH, GPx and CAT levels. Doxorubicin is enzymatically converted to a semiquinone radical which directly transfers its electron to molecular oxygen thereby generating free radicals, namely superoxide and hydrogen peroxide.[33] The ameliorative effect of the extract in reversing the cardiotoxicity associated with the use of doxorubicin is indicated by the fact that the extract at the dose of 200 mg/kg significantly increased the level of GSH relative to the toxicant group. Also, the administration of the standard drug (gallic acid) in combination with doxorubicin confirmed the antioxidant potential of the drug in ameliorating the cardiotoxic effect of doxorubicin as justified by significant increases in the level of GSH, SOD, CAT and GPx compared with the doxorubicin only group.

In respect of the heart, as shown in Table 4, ISO caused a significant reduction in the level of GSH, SOD, CAT and GPx ($p < 0.05$, 0.001, 0.05 and 0.001 respectively). The various interventions (*I. asarifolia* 100, 200 and 400 mg/kg, and gallic acid 15 mg/kg) did not significantly alter the effects of ISO on these *in-vivo* antioxidants. No significant change in the level of MDA was observed in all the treatment groups relative to control or the ISO group. Isoproterenol has been reported to cause severe oxidative stress in the myocardium.[34] In this study, isoproterenol significantly reduced the heart levels of all four antioxidants (GSH, CAT, GPx and SOD) with a concomitant non-significant increase in the level of MDA compared with control. This observation is in accordance with previous research reports that documented significant increases in the levels of diagnostic marker enzymes in the plasma and a decline in antioxidants, including GSH, following injections of isoproterenol.[35] However, the extract at various doses and gallic acid co-administered with isoproterenol did not reverse the effects of ISO on antioxidant parameters in the heart. This suggests that the cardioprotective effect of the extract as deduced from the results of the biochemical parameters assay, in respect of the ISO model, is not associated with antioxidant activity in the heart.

As shown in Table 5, DOX did not cause significant change ($p > 0.05$) in the kidney level of all the antioxidant indices relative to the control group. Compared to the control and DOX groups, *I. asarifolia* 100 mg/kg caused significant increase in the level of CAT ($p < 0.001$, 0.01 respectively). In respect of the kidneys, doxorubicin did not significantly alter the levels of the *in-vivo* antioxidants and MDA compared with the control group. However, the extract at doses of 100 and 400 mg/kg co-administered with the toxicant increased the levels of CAT, SOD and GPx relative to the doxorubicin group. This possibly suggests a tendency to increase renal *in-vivo* antioxidant activity in pathologic conditions.

In respect of the kidneys, relative to the control group, ISO did not significantly alter ($p > 0.05$) the level of GSH and CAT. However, ISO significantly reduced ($p < 0.001$) the level of SOD and GPx, and significantly increased ($p < 0.05$) the level of MDA compared with the control group. Only gallic acid 15 mg/kg significantly reversed ($p < 0.01$, 0.001) the effect of ISO on SOD and GPx. However, *I. asarifolia* 400 mg/kg caused significantly reduction ($p<0.05$) in the level of MDA relative to the ISO group (Table 6). Concerning isoproterenol-induced toxicity, the toxicant significantly reduced the kidney levels SOD and GPx, and concurrently significantly increased the level of MDA relative to the control group. *I. asarifolia* at the dose of 400 mg/kg reversed the effect of isoproterenol on MDA while gallic acid acted likewise in respect of SOD and GPx. Conservatively, these results suggest some beneficial effect for gallic acid and the extract at 400 mg/kg against pro-oxidative tendencies in the kidney.

In respect of the liver, as shown in Table 7, DOX did not elicit any significant change ($p > 0.05$) in the level of all the antioxidant indices compared with the control group. *I. asarifolia* 100 mg/kg and gallic acid 15 mg/kg caused significant reduction in the levels of GSH ($p < 0.05$, 0.01) relative to the control and DOX groups. *I. asarifolia* 200 and 400 mg/kg, and gallic acid 15 mg/kg caused significant reduction in the level of SOD and GPx ($p < 0.05$, 0.01) relative to the DOX group. A significant increase ($p < 0.05$) was elicited by gallic acid in respect of the level of MDA compared with the DOX group. Concerning the liver, doxorubicin did not significantly alter the levels of antioxidant indices relative to the control group. Compared to the toxicant group, *I. asarifolia* at various doses and gallic acid did not beneficially change the values of the antioxidant indices. Unlike the case with doxorubicin, isoproterenol caused significant diminution in the levels of SOD, CAT and GPx, with accompanying increase in the level of MDA. The extract at 200 and 400 mg/kg respectively elicited beneficial effects in reducing the levels of MDA and increasing the level of CAT relative to the toxicant group.

In respect of the liver, ISO significantly reduced the levels of SOD, CAT and GPx ($p<0.01$, 0.05 and 0.01 respectively) relative to the control. ISO also significantly increased ($p<0.001$) the level of MDA. *I. asarifolia* 200 and 400 mg/kg significantly reversed the effects of ISO on MDA ($p<0.001$) and CAT ($p<0.01$). ISO and the interventions did not significantly ($p > 0.05$) alter the level of GSH (Table 8). The results, in this case, suggest that ISO-induced oxidative stress in the liver. The reversal of the effects of ISO on hepatic levels of MDA and CAT by the extract is suggestive of potential ameliorative effect on hepatotoxicity.

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Table 1: Effect of *I. asarifolia* extract on biochemical parameters in doxorubicin-induced toxicity test.

Treatment group	Dose (mg/kg)	LDH (IU/L)	Creatinine (mg/dL)	Urea (mg/dL)	HDL (mg/dL)	TG (mg/dL)
Distilled water	10 (ml/kg)	1685.26 ± 9.71	43.98 ± 0.44	5.38 ± 0.14	1.66 ± 0.03	0.57 ± 0.02
DOX	5	1983.08 ± 15.81	54.35 ± 0.16^{a}	7.68 ± 0.20^{b}	1.28 ± 0.03^{b}	1.49 ± 0.03^{c}
DOX + *I. asarifolia*	5/100	2065.56 ± 60.33	51.05 ± 0.60	6.70 ± 0.07	1.02 ± 0.10^{c}	0.85 ± 0.02$^{c,\alpha}$
DOX + *I. asarifolia*	5/200	1035.43 ± 35.58$^{b,\alpha}$	52.82 ± 3.37	8.00 ± 0.46^{b}	1.23 ± 0.03^{b}	1.24 ± 0.02$^{c,\alpha}$
DOX + *I. asarifolia*	5/400	1185.98 ± 19.50$^{a,\alpha}$	51.53 ± 3.07	7.50 ± 0.80^{b}	1.28 ± 0.03^{b}	0.88 ± 0.05$^{c,\alpha}$
DOX + Gallic acid	5/15	1620.02 ± 53.93	45.56 ± 0.37	6.74 ± 0.02	1.40 ± 0.03^{a}	1.39 ± 0.02^{c,**}
I. asarifolia	400	1786.30 ± 212.54*	54.71 ± 3.36^{b}	6.24 ± 0.43	1.32 ± 0.08^{b}	0.80 ± 0.05$^{c,\alpha}$

Values are mean ± S.E.M. (n = 3-5). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. distilled water; $^{\alpha}p < 0.001$ vs. DOX; *p < 0.01, **p < 0.001 vs. DOX + *I. asarifolia* 400 mg/kg (one-way ANOVA with Tukey's post-hoc test).

Table 2: Effect of *I. asarifolia* extract on biochemical parameters in isoproterenol-induced toxicity test.

Treatment group	Dose (mg/dL)	LDH (IU/L)	Creatinine (mg/dL)	Urea (mg/dL)	HDL (mg/dL)	TG (mg/dL)
Distilled water	10 (ml/kg)	1685.26 ± 30.16	43.98 ± 2.19	5.38 ± 0.35	1.66 ± 0.08	0.57 ± 0.01
ISO	85	2440.58 ± 3.00^{c}	46.26 ± 3.54	5.95 ± 0.79	1.18 ± 0.08^{a}	0.67 ± 0.01^{c}
ISO + *I. asarifolia*	85/100	1690.58 ± 32.27$^{\beta}$	46.03 ± 6.07	7.08 ± 2.05	1.18 ± 0.09^{a}	0.56 ± 0.01$^{\beta}$
ISO + *I. asarifolia*	85/200	2353.00 ± 7.22^{b}	45.84 ± 3.33	6.60 ± 0.29	1.10 ± 0.00^{a}	0.70 ± 0.01^{c}
ISO + *I. asarifolia*	85/400	1910.95 ± 33.22$^{\alpha}$	43.67 ± 2.77	4.83 ± 0.30	1.18 ± 0.14^{a}	0.57 ± 0.01$^{\beta}$
ISO + Gallic acid	85/15	2464.74±104.41^{c,**}	47.98 ± 3.35	6.16 ± 0.29	1.30 ± 0.11	0.62 ± 0.00^{a,*}
I. asarifolia	400	2049.94 ± 170.38	56.37 ± 3.74	6.44 ± 0.87	1.60 ± 0.09	0.82±0.02$^{c,\beta,***}$

Values are mean ± S.E.M. (n = 3-5). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. distilled water; $^{\alpha}p < 0.05$, $^{\beta}p < 0.001$ vs. ISO; *p < 0.05, **p < 0.01, ***p < 0.001 vs. ISO + *I. asarifolia* 400 mg/kg (one-way ANOVA with Tukey's post-hoc test).

Table 3: Effect of *I. asarifolia* extract on heart antioxidant indices in doxorubicin-induced toxicity test.

Treatment group	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10 (ml/kg)	0.48 ± 0.03	4.47 ± 0.39	31.24 ± 0.82	1.36 ± 0.12	0.11 ± 0.01
DOX	5	0.06 ±0.01^{c}	3.15 ±0.21^{a}	19.19±1.38^{b}	0.95 ± 0.06^{a}	0.06 ± 0.00^{b}
DOX + *I. asarifolia*	5/100	0.15 ±0.02^{c}	3.71 ± 0.06	21.29 ±0.19^{a}	1.12 ± 0.02	0.08 ± 0.01
DOX + *I. asarifolia*	5/200	0.26 ± 0.06$^{a,\alpha}$	2.43 ± 0.19^{c}	20.82 ± 1.54^{b}	0.73 ± 0.06^{c}	0.08 ± 0.01
DOX + *I. asarifolia*	5/400	0.11 ±0.01^{c}	3.11 ±0.45^{a}	25.18 ± 2.86	0.94 ± 0.14^{a}	0.10 ± 0.01$^{\alpha}$
DOX + Gallic acid	5/15	0.24 ±0.025^{a}	4.67 ± 0.36$^{\alpha,*}$	33.13 ± 3.12$^{\gamma}$	1.41 ± 0.11$^{\alpha,*}$	0.12 ± 0.01$^{\beta}$
I. asarifolia	400	0.30 ± 0.09$^{\beta,*}$	5.01 ± 0.17$^{\beta,**}$	32.36 ± 0.95$^{\gamma}$	1.51 ± 0.05$^{\beta,**}$	0.11 ± 0.01$^{\beta}$

Values are mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. distilled water; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ vs. DOX; *p < 0.05, **p < 0.01 vs. DOX + *I. asarifolia* 400 mg/kg (one-way ANOVA with Tukey's post-hoc test).

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Table 4: Effect of *I. asarifolia* extract on heart antioxidant indices in isoproterenol-induced toxicity test.

Treatment group	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10 (ml/kg)	0.48 ± 0.03	4.47 ± 0.39	31.24 ± 0.82	1.36 ± 0.12	0.11 ± 0.01
ISO	85	0.28 ± 0.04^{a}	1.88 ± 0.15^{c}	22.56 ± 1.42^{a}	0.57 ± 0.04^{c}	0.20 ± 0.03
ISO + *I. asarifolia*	85/100	0.37 ± 0.07	2.26 ± 0.21^{c}	26.08 ± 1.86	0.68 ± 0.07^{c}	0.22 ± 0.06
ISO + *I. asarifolia*	85/200	0.16 ± 0.01^{c}	1.67 ± 0.08^{c}	15.20 ± 0.83$^{c,\alpha}$	0.50 ± 0.02^{c}	0.12 ± 0.00
ISO + *I. asarifolia*	85/400	0.21 ± 0.02^{b}	1.95 ± 0.15^{c}	16.86 ± 0.55^{c}	0.58 ± 0.00^{a}	0.10 ± 0.02
ISO + Gallic acid	85/15	0.36 ± 0.05	2.14 ± 0.27^{c}	22.16 ± 3.16^{b}	0.65 ± 0.08^{c}	0.16 ± 0.05
I. asarifolia	400	0.27 ± 0.04^{a}	2.22 ± 0.17^{c}	16.55 ± 1.03^{c}	0.68 ± 0.05^{c}	0.09 ± 0.01

Values are mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. distilled water; $^{\alpha}p < 0.05$ vs. ISO (One-way ANOVA with Tukey's post-hoc test).

Table 5: Effect of *I. asarifolia* hydroethanolic leaf extract on kidney antioxidant indices in doxorubicin-induced toxicity test.

Treatment Group	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10 (mL/kg)	0.24 ± 0.00	3.05 ± 0.07	18.35 ± 1.13	2.15 ± 0.02	0.11 ± 0.01
DOX	5	0.25 ± 0.02	4.38 ± 0.38	20.82 ± 1.81	1.29 ± 0.11	0.12 ± 0.03
DOX + *I. asarifolia*	5/100	0.32 ± 0.05	5.86 ± 0.68^{c}	34.13 ± 3.49$^{c,\beta}$	1.73 ± 0.20^{c}	0.19 ± 0.05^{b}
DOX + *I. asarifolia*	5/200	0.27 ± 0.03	3.19 ± 0.17	20.98 ± 0.27	0.95 ± 0.05	0.12 ± 0.01
DOX + *I. asarifolia*	5/400	0.14 ± 0.02	1.76 ± 0.06$^{\beta}$	16.20 ± 0.38	0.53 ± 0.02$^{\beta}$	0.09 ± 0.00
DOX + Gallic acid	5/15	0.35 ± 0.06*	3.58 ± 0.63**	23.22 ± 3.06	1.06 ± 0.18	0.14 ± 0.05
I. asarifolia	400	0.44 ± 0.06$^{a,\alpha,}$**	4.79 ± 0.34	31.73 ± 2.16$^{b,\alpha,}$**	1.45 ± 0.10^{a,}**	0.11 ± 0.02

Values are mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. distilled water; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$ vs. DOX; *p < 0.05, **p < 0.001 vs. DOX + *I. asarifolia* 400 mg/kg (one-way ANOVA with Tukey's post-hoc test).

Table 6: Effect of *I. asarifolia* hydroethanolic leaf extract on kidney antioxidant indices in isoproterenol-induced toxicity test.

Treatment group	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10 (ml/kg)	0.24 ± 0.00	3.05 ± 0.07	18.35 ± 1.13	0.90 ± 0.02	0.11 ± 0.01
ISO	85	0.18 ± 0.01	1.39 ± 0.20^{c}	20.05 ± 2.26	0.41 ± 0.06^{c}	0.23 ± 0.04^{a}
ISO + *I. asarifolia*	85/100	0.26 ± 0.06	1.48 ± 0.08^{c}	19.39 ± 1.44	0.40 ± 0.03^{c}	0.29 ± 0.03^{c}
ISO + *I. asarifolia*	85/200	0.24 ± 0.02	1.41 ± 0.05^{c}	15.20 ± 0.05	0.42 ± 0.01^{c}	0.19 ± 0.00
ISO + *I. asarifolia*	85/400	0.22 ± 0.01	1.39 ± 0.18^{c}	12.41 ± 0.13$^{a,\beta}$	0.42 ± 0.05^{c}	0.11 ± 0.03$^{\beta}$
ISO + Gallic acid	85/15	0.24 ± 0.02	2.46 ± 0.27$^{\beta,}$*	15.27 ± 1.03	0.73 ± 0.08$^{\beta,}$*	0.18 ± 0.02
I. asarifolia	400	0.20 ± 0.04	1.29 ± 0.19^{c}	14.05 ± 0.87	0.38 ± 0.06^{c}	0.16 ± 0.03

Values are mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{c}p < 0.001$ vs. distilled water; $^{\beta}p < 0.01$ vs. ISO; *p < 0.01 vs. ISO + *I. asarifolia* 400 mg/kg (one-way ANOVA with Tukey's post-hoc test).

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Table 7: Effect of *I. asarifolia* hydroethanolic leaf extract on liver antioxidant indices in doxorubicin-induced toxicity test.

Treatment group	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10 (ml/kg)	0.28 ± 0.03	3.67 ± 0.38	19.90 ± 1.78	1.09 ± 0.12	0.08 ± 0.01
DOX	5	0.27 ± 0.03	4.66 ± 0.11	17.75 ± 0.68	1.41 ± 0.03	0.05 ± 0.00
DOX + *I. asarifolia*	5/100	0.13 ± 0.01$^{b,\alpha}$	3.56 ± 0.41	15.79 ± 0.51	1.08 ± 0.13	0.08 ± 0.00
DOX + *I. asarifolia*	5/200	0.20 ± 0.05	3.28 ± 0.32$^{\alpha}$	15.89 ± 1.18	0.984 ± 0.10$^{\alpha}$	0.08 ± 0.01
DOX + *I. asarifolia*	5/400	0.17 ± 0.02	2.72 ± 0.10$^{\gamma}$	19.89 ± 1.75	0.81 ± 0.03$^{\gamma}$	0.08 ± 0.01
DOX + Gallic acid	5/15	0.10 ± 0.01$^{b,\beta}$	2.92 ± 0.29$^{\beta}$	18.57 ± 3.52	0.86 ± 0.08$^{\beta}$	0.09 ± 0.02$^{\alpha}$
I. asarifolia	400	0.15 ± 0.01^{a}	3.06 ± 0.06$^{\beta}$	16.46 ± 0.18	0.91 ± 0.02$^{\beta}$	0.08 ± 0.01

Values are mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{b}p < 0.01$ vs. distilled water; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ vs. DOX (one-way ANOVA with Tukey's post-hoc test).

Table 8: Effect of *I. asarifolia* hydroethanolic leaf extract on liver antioxidant indices in isoproterenol-induced toxicity test.

Treatment group	Dose (mg/kg)	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10 (ml/kg)	0.28 ± 0.02	3.67 ± 0.38	19.90 ± 1.78	1.09 ± 0.12	0.08 ± 0.01
ISO	85	0.21 ± 0.01	2.29 ± 0.03^{b}	12.73 ± 0.92^{a}	0.67 ± 0.01^{b}	0.23 ± 0.03^{c}
ISO + *I. asarifolia*	85/100	0.30 ± 0.03	2.25 ± 0.06^{b}	14.70 ± 1.18	0.66 ± 0.03^{b}	0.24 ± 0.01^{c}
ISO + *I. asarifolia*	85/200	0.19 ± 0.01	1.37 ± 0.08^{c}	13.17 ± 0.60^{a}	0.41 ± 0.02^{c}	0.06 ± 0.00$^{\gamma}$
ISO + *I. asarifolia*	85/400	0.25 ± 0.03^{a}	2.28 ± 0.27^{b}	22.27 ± 2.56$^{\gamma}$	0.69 ± 0.08^{b}	0.19 ± 0.05^{a}
ISO + Gallic acid	85/15	0.24 ± 0.01	1.86 ± 0.35^{c}	13.81 ± 0.84**	0.56 ± 0.11^{c}	0.14 ± 0.01^{1}
I. asarifolia	400	0.18 ± 0.01	1.16 ± 0.16$^{c,\alpha,*}$	10.54 ± 0.83^{b,***}	0.35 ± 0.05$^{c,\alpha,*}$	0.08 ± 0.01$^{\gamma,*}$

Values are mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. distilled water; $^{\alpha}p < 0.05$, $^{\gamma}p < 0.001$ vs. ISO; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ vs. ISO + *I. asarifolia* 400 mg/kg (one-way ANOVA with Tukey's post-hoc test).

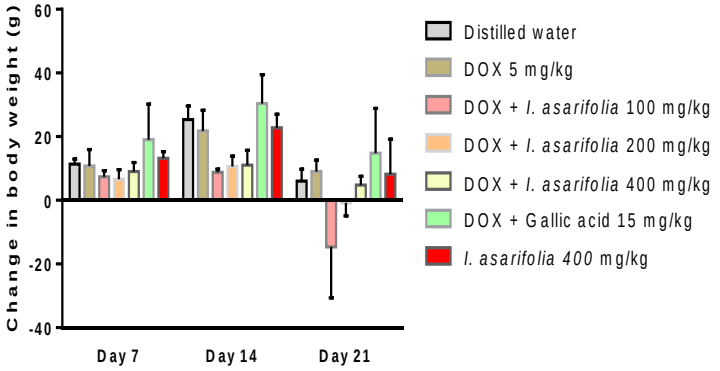

Figure 1: Effect of *I. asarifolia* extract on change in body weight in doxorubicin-induced toxicity test. Bars are mean ± S.E.M. (n=3-5). $p > 0.05$ (one-way ANOVA with Tukey's post-hoc test).

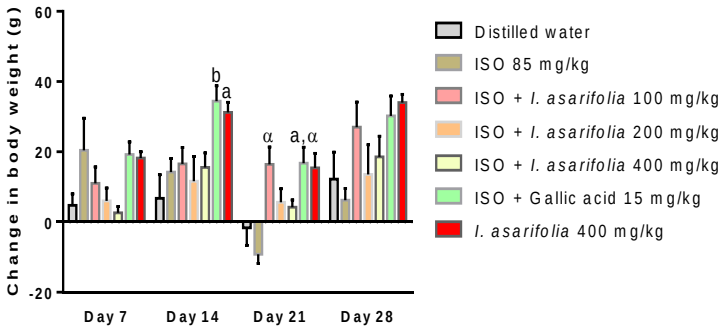

Figure 2: Effect of *I. asarifolia* extract on change in body weight in isoproterenol-induced toxicity test. Bars are mean ± S.E.M. (n=3-5). $^{a}p < 0.05$, $^{b}p < 0.01$ vs. distilled water; $^{\alpha}p < 0.001$ vs. DOX (one-way ANOVA with Tukey's post-hoc test).

As shown in Figure 1, there were no significant differences ($p > 0.05$) in the change in body weight of rats across the treatment groups on days 7, 14 and 21 compared to the control group in the DOX model. In respect of the ISO model, on days 7 and 28 there were no significant differences ($p > 0.05$) in the change in body weight of rats comparing *I. asarifolia* extract and gallic acid treated groups with the control and ISO groups. On day 14, gallic acid intervention in ISO treated rats caused significant increase ($p < 0.05, 0.01$) in change in body weight of rats compared to the control group, with no significant difference ($p > 0.05$) relative to the ISO group. Gallic acid intervention in ISO-treated rats also caused significant increase ($p < 0.05$) in change in body weight of rats on day 21 compared to the control and ISO groups. *I. asarifolia* extract 100 mg/kg intervention in ISO-treated rats also caused a significant increase ($p < 0.05$) in change in body weight of rats compared to the ISO group at this interval, with value being comparable and not significantly different ($p > 0.05$) relative to the control group (Figure 2). Tan *et al*.[36] reported that reductions in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances. In this study, DOX and ISO did not cause significant alteration in change in body weight within the treatment period compared with control. The combination with the extract at various doses presented a similar trend of result while the ISO plus gallic acid group showed significant increase in change in weight gain from mid to the end of the treatment period.

I. asarifolia extract did not produce any mortality when administered p.o. at the dose of 5000 mg/kg. In respect of the i.p. route, mortality was 0 and 100% respectively at doses of 250 and 4000 mg/kg. The i.p. LD_{50} value was obtained to be 1000 mg/kg with a confidence interval of 482.68-1517.32 mg/kg. It has been documented that no dose-related toxicity should be considered above 5000 mg/kg body weight.[37] Hence, the hydroethanolic leaf extract of *I. asarifolia* can be considered relatively non-toxic. The results of the oral acute toxicity test and the i.p. LD_{50} value corresponds to what was reported by Akindele *et al*.[38]

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine.[39] Phytochemical screening of the

hydroethanolic leaf extract of *I. asarifolia* revealed the presence of saponins, tannins, flavonoids, phenols, reducing sugars, glycosides, terpenoids, steroids and phlobatannins. Flavonoids and tannins are well known polyphenolic natural antioxidants.[3] Phenols have been found to be very effective in preventing the build-up of damaging cholesterol.[40]

Conclusion

The results obtained in this study suggest that the hydroethanolic leaf extract of *Ipomoea asarifolia* possesses cardioprotective effects against doxorubicin and isoproterenol induced toxicities. The effect of the extract in respect of the DOX model is associated with enhancement of GSH levels. The extract also reduced the extent of lipid peroxidation induced by ISO in the kidneys and liver by enrichment of CAT level.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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