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Full Length Research Paper

Tissue level antioxidant activity of leaf extract of *Syzygium jambos* linn. In paracetamol intoxicated Wistar rats

Nataraja Thamizh Selvam[1]*, Venkatakrishnan V[2] and Damodar Kumar S[3]

[1]Research Scholar, Pachaiyappa's College, Chennai, Tamil Nadu.
[2]Assistant Professor, Central University, Pondicherry,
[3]Professor and Head, Department of Chemistry, Pachaiyappa's College, Chennai, Tamil Nadu.

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In recent years, oxidative stress and free radicals have been implicated in various diseases. *Syzygium jambos*, is a plant used by traditional physicians in Kerala, India for various ailments including wounds, ulcers and dermatopahty. The present study was undertaken to assess the antioxidant activity of methanolic extract of *S. jambos* leaf in paracetamol intoxicated Wistar albino rats. The rats were divided in to five groups of six animals each, categorized as healthy control group, disease control, standard group and two test groups. The test extract was administered in two difference doses as 100 and 200 mg/kg/ b.wt. The biochemical parameters SGOT, SGPT levels in blood and antioxidant profiles including of superoxide dismutase, catalase and glutathione levels in blood and different tissues (liver, kidney, heart) were evaluated. The results showed elevated levels of antioxidant enzymes in blood and tissues in treatment groups suggesting significant antioxidant activity.

Key words: *Syzygium jambos*, antioxidant activity, liver intoxication, oxidative stress, herbal medicine.

INTRODUCTION

Herbal medicine is one of the main branches of medicine. In recent years herbal medicine is gaining popularity in day-to-day life. Herbal medicine is cheap, easily available and has rare or less side effects (Etherton et al., 2002; Kakegawa et al., 1985; Agarwal et al., 2000; Wada et al., 2002). The plant-derived compounds are showing promise in the treatment of cancer, HIV and diabetes. The oxidative stress is one of the main phenomena which are associated with various disease conditions and as such controlling and management of oxidative stress is directly contributing for the management and cure of disease (Aroma, 1998). All living organism contain antioxidant enzymes and chemicals. Anti oxidant systems either prevent these reactive species or free radicals from being formed or remove them before they can damage vital components of the cell. Enzymatic free radical system in body comprises of Superoxide Dismutase (SOD), Catalase, Gpx (Glutathione peroxidase), and Glutathione reductase etc. These enzymes convert the toxic reactive species into non-toxic species by the interactive network of antioxidant enzymes (Vilet et al., 1991).

There are many studies reporting antioxidant potential of various plants and plant products.

The present study is executed scientifically to evaluate the antioxidant activity of methanolic extract of *Syzygium jambos* leaf in the paracetamol intoxicated animal model system.

S. jambos is belongs to the family of Myrtaceae. It is a large shrub or small tree with spreading branches. The leaves are simple, opposite, lanceolate, narrowed into short perioles.

Flowers greenish white and pinkish white and fruits are pale yellow to pinkish white and they are distributed throught out India especially in hill region of up to 1350mt height.

The parts including leaves, bark, fruits are used by traditional users for dermatopathy, diarrhea, colic helminthiasis, wounds and ulcers (Warrier et al., 2002).

*Corresponding author. Email: nthamizhselvam@gmail.com

Table 1. Phyto-constituents of *S. jambos* leaf extract.

PhytochemicalAnalysis	Extract of *Syzigium jambos*
Carbohydrate	+++
Phenols	+
Flavonoids	+++
Tannins	+
Steroids	++
Terpenoids	++
Alkaloids	+
Glycosides	–
Saponins	+
Aminoacids	++

+++ Very strongly present ++ Strongly present + Present - Absent

MATERIALS AND METHODS

Plant Material

The *S. jambos* leaves were collected from western ghat region of Kerala and it was authenticated by the taxonomist, Kerala Forest Research Institute, Peechi, Thrissur. The voucher specimen is maintained in the Pachiyappa's College, Chennai.

Preparation of Extract

The shade dried leaves (100 gm) were extracted by soxhlet extraction apparatus using methanol as solvent at its boiling point 70- 80^{0}C. The hot extraction was carried out for continuous 10 hours. The extract was then filtered and the filtrates were evaporated using rotary evaporator under reduced pressure until drying. Then the extract was dissolved in distilled water before administration to experimental rats.

Phytochemical Studies

The phytochemical analysis of the test extract was carried out as per the standard protocol (Maluventhan and Murugesan, 2010; Hassan et al., 2006; EL- Olemyl 1994; Trease GE 1978)) and the details have been presented in Table 1.

Animal Study

The animal studies were carried with the technical support of Madras Veterinary College, Chennai. The study was carried out as per CPCSEA guidelines and the protocol was approved by the animal ethical committee (IAEC: 832/06/a/2012-13). Six to seven months old Wistar albino rats weighing 150-200 gm were used. The animals were fed laboratory pellet chow and given water ad libitum. All rats were clinically healthy. The animals were randomly divided into five groups of six animals each and the standard protocol was used (Gupta A, 2006; Thamizh Selvam et al., 2013).

The methanolic extracts of *S. jambos*, was administered at two different concentrations (100 and 200 mg/kg body weight) as lower dose (Group IV) and higher dose (Group V) through orally for the period of ten days in test groups. The disease group (Group II) and test group received single dose of paracetamol at 2.5 gm/kg body weight. The control group (Group I) received distilled water throughout the experiment and standard group (Group III) received silymarin 100 mg/kg body weight for the experimental period. The animals were fasted for 24 hrs on 10th day of the experiment and blood sample was collected. The biochemical parameters like SGOT, SGPT levels and *in vivo* antioxidant status including SOD, glutathione peroxidase and catalase were assessed in the blood samples of the test groups, control groups and disease groups. At the end of the experiment, the animals were sacrificed under anesthesia using diethyl ether and the tissue samples of liver, kidney and heart were collected for evaluation of antioxidant levels in tissues.

Statistical Analysis

The data were expressed as mean ± SEM and statistically analyzed by one way ANOVA.

RESULTS

The extraction efficiency (extract yield) of the *S. jambos* leaf was 14.52 gm %. The phytochemical analysis of the

Table 2. Effect of test extracts on liver function tests in paracetamol intoxicated Wistar albino rats.

S.No	Groups	Biochemical parameters	
		SGOT	SGPT
1	Healthy Control	98.35 ± 2.29	48.24 ± 2.61
2	Disease Control	362.44 ± 3.74	254.96 ± 4.20
3	Silymarin Treated	153.26 ± 2.76*	68.49 ± 2.55**
4	*S. jambos* L.D (100mg/kg)	183.67 ± 3.54**	133.48 ± 4.11**
5	*S. jambos* H.D.(200mg/kg)	162.46 ± 4.95*	89.70 ± 4.20*

Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control

Table 3. Effect of methanolic extract of *S. jambos* on blood antioxidant enzyme levels in rat subjected to paracetamol induced toxicity.

Parameters	SOD (Units/mg protein)	GPx (n moles of glutathione oxidized/min/mg protein)	Catalase (nM H_2O_2 decomposed/min/mg protein)
Healthy Control	11.13 ± 0.69	12.47 ± 0.79	18.34 ± 2.48
Disease Control	5.41 ± 0.62*	5.86 ± 0.38**	6.68 ± 1.27*
Positive Control	9.68 ± 0.44*	11.40 ± 0.59*	16.16 ± 2.45*
S. jambos L.D	7.42±1.13*	8.97±0.36**	13.24±2.61*
S. jambos H.D	10.86±1.33**	11.62±0.25*	15.50±3.27*

Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control.

crude extract showed the presence of carbohydrates, flavonoids, steroids, terpenoids and amino acids (Table 1).

The liver marker enzymes SGOT and SGPT levels found to be increased in the disease control group and the same have been significantly decreased in the extract treated groups (Table 2). The *in vivo* antioxidant activity of methanolic extract of *S. jambos*, was evaluated in Wister albino rats intoxicated with paracetamol. The antioxidant enzymes superoxide dismutase, catalase and glutathione levels were measured in the tissue samples of liver, kidney and heart tissues of the experimental animal groups. The report showed that the antioxidant status have been significantly (p<0.05 and <p0.01) improved in the test extracts administered groups when compared with the disease control group (Table 3-6). *S. jambos* treated group showed highest SOD activity in the liver followed by kidney and heart tissue and the result was comparable with the standard silymarin. The overall experiment exhibited the significantly improved antioxidant status in the tissue samples of *S. jambos* treated groups and the efficacy was comparable with standard drug silymarin (Table 3 and Figure 1-3). The antioxidant enzyme levels in the blood samples of the experimental animals also revealed the significant improvement in the test extract administered groups.

DISCUSSION

The present study proved the antioxidant activity of the methanolic extract of *S. jambos*, in the *in vivo* system. The antioxidant system is comprised of different types of functional components classified as first line, second line, third line and fourth line defenses. The first line defense preventive antioxidants are which act by quenching of $O_2^{\cdot-}$, decomposition of H_2O_2 and sequestration of metal ions. The antioxidants belonging to this category are enzymes, like superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and non-enzymatic molecules like minerals and some proteins. Super oxide dismutase mainly acts by quenching of super oxide radical, produced in different aerobic metabolism. Catalase is a tetrameric enzyme, present in most of the

Figure 1. Effect of test extracts on Catalase enzyme level in liver, kidney and heart tissues.

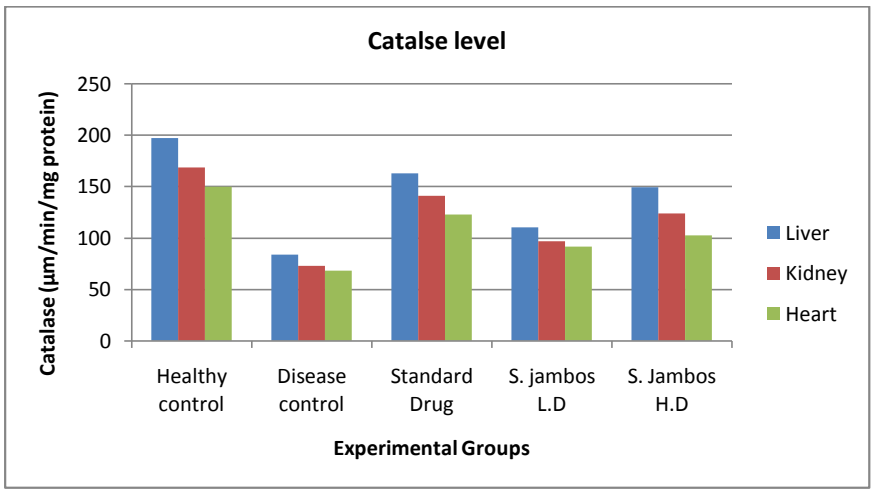


Figure 2. Effect of test extracts on Glutathione level in liver, kidney and heart tissues.

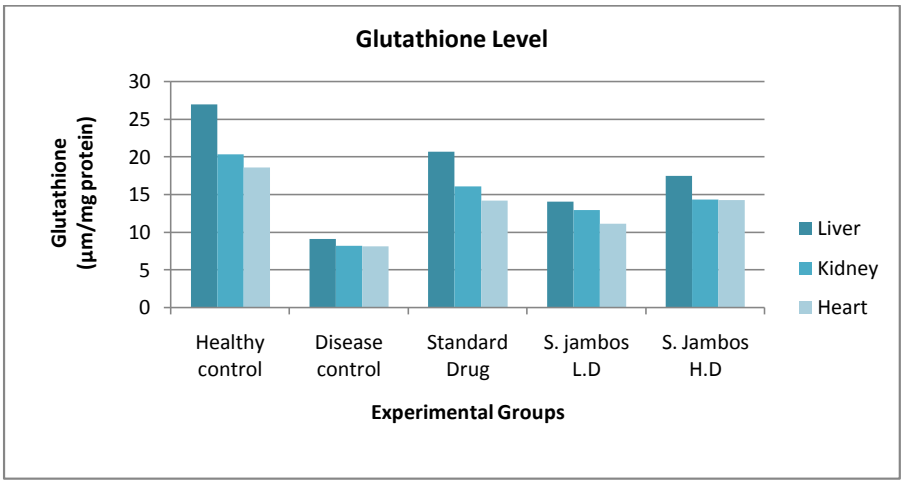
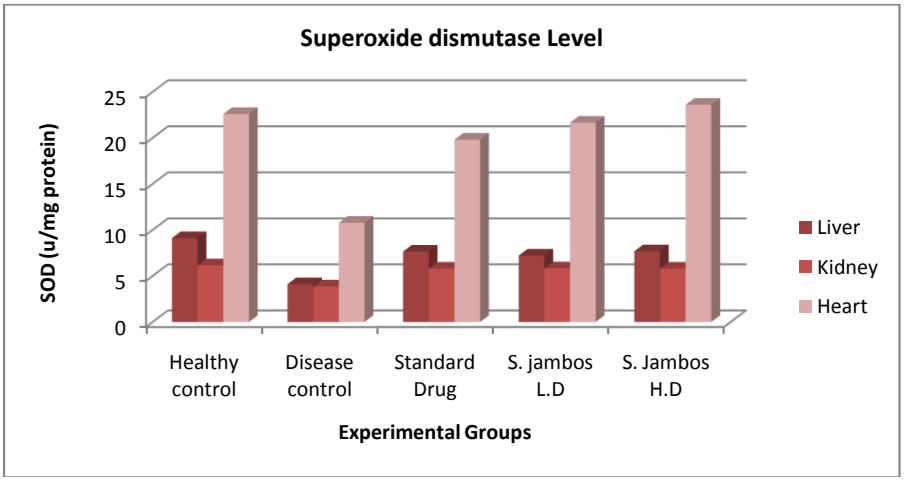


Figure 3. Effect of test extracts on Superoxide dismutase level in liver, kidney and heart tissues.



cells and acts by catalyzing the decomposition of H_2O_2 to water and oxygen. Glutathione peroxidase is a selenium containing enzyme which catalyses the reduction of H_2O_2 and lipid hydroperoxides, generated during lipid peroxidation, to water and oxygen (Dandagi *et al*., 2008; Girish *et al*., 2009).

In the present study, the catalase, superoxide dismutase and glutathione levels were significantly increased in the test extract treated animal groups both in blood and tissues (liver, kidney and heart). The efficacy of the extract was found to be significant and dose dependent. Recent studies on various plants and herbal formulations also showing the similar effect (Girish *et al*., 2009; Ye *et al*., 2009; (Habbu *et al*., 2008; Lin *et al*., 2008; Ye *et al*., 2009). The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years in a well defined experimental systems (Vidyashankar *et al*., 2010) and concluded that ROS and lipid peroxidation may play a role in pathogenesis of hepatic fibrosis with loss of normal liver architecture (Schmidt, 2005; Campion *et al.*, 2008). The results obtained thus indicate that the methanolic extracts of *S. jambos*, has potent antioxidant activity and it may be the due to the synergistic effect of the major phytoconstitutents like flavonoids, phenols and lignans. The further research is highly required in the aspect of isolation and characterization of potential compounds and their validation for new drug discovery.

CONFLICT OF INTEREST

Authors report no conflict of interest.

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