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HEPATOPROTECTIVE ACTIVITY OF BERRY EXTRACTS OF *DIOSPYROS KAKI* LINN. AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE MODEL

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Keywords:

Diospyros kaki, Serum glutamate oxaloacetate, Serum glutamate pyruvate transaminase, Total bilirubin, Alkaline phosphatise

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ABSTRACT: Currently, nutrition and health linkages focused on the emerging strategy of diet based regimen to combat various physiological threats including cardiovascular disorders, oxidative stress, diabetes mellitus, *etc*. Berry / Fruit part of *Diospyros kaki* Linn. was extracted using petroleum ether, chloroform, ethanol consecutively and the obtained extracts were screened for hepatoprotective activity using CCl_4 induced liver damage model. The activity was assessed by comparing the serum enzyme levels such as serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, total bilirubin, alkaline phosphatase of fruit extracts treated group with carbon tetrachloride treated animals and results showed dose-dependent activity, ethanolic extract treated group showed highly significant activity, whereas chloroform extract treated group has shown the significant action but less compare to ethanolic extract, petroleum ether treated group showed moderate action and petroleum ether extract at a dose of 100 mg/kg b.w showed least significant action. The results were further supported by histopathological studies.

INTRODUCTION: The liver is the largest organ of the body. It is located between the portal and the general circulation, between the organs of the gastrointestinal tract and the heart. The main function of the liver is to take up nutrients, to store them, and to provide nutrients to the other organs [1]. The liver is not only an important power and sewage treatment plant of the body.

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The liver is probably the best example of a cheap recycling system. The function of the liver as a clearance organ, however, harbors the danger that the substances that should be degraded and eliminated lead to tissue damage [2]. Thus, effective defense mechanisms are necessary. During the process of elimination, there is a chance of accumulation of different kinds of toxic materials inside the hepatocytes, and there is a chance of liver infection, and hepatic disorders such as hepatitis. Amongst, persimmon (*Diospyros kaki* L.) is one of these nutritious fruits bestowed with strong antioxidant activity [3, 4]. Persimmon is fleshy fibrous tropical, deciduous fruit belonging to *Ebenaceae* family.

It is commonly cultivated in warm regions of the world including China, Korea, Japan, Brazil, Turkey, and Italy [5, 6]. In 2007, the global production of persimmon reached over 3.3 million tons, with 70.0 % from China, 10.0 % from Korea and 7.0 % from Japan. The persimmon is not so popular in European Communities, but its demand is increasing owing to consumer's awareness regarding its hidden health-promoting potential.

Generally, over 400 species of persimmon are planted globally. Among these, *Diospyros kaki, Diospyros virginiana, Diospyros oleifera,* and *Diospyros lotus* [7] are of significant importance. It is interesting for the readers that *D. kaki* (Japanese persimmon) is the most promising species. Fruit is used to treat Ischemia stroke, angina, internal hemorrhage, hypertension, atherosclerosis, and some infectious diseases. Folklore usage of *D. kaki* revealed to be used as an anti-diarrhoeal, improve eye movement, aid digestion, lower B.P, bleeding hemorrhoids, reduce cholesterol level, strengthen bones, boosts cognitive function, fight intestinal disorders and boosts body's immune system, bitter astringent, antiviral, antibacterial, astringent, styptic, treat various respiratory abnormalities such as Influenza, cold, cough [8]. Even though different kind of allopathic molecules are available in the market all of them are suffering from some are the other toxic effect, so an urgent need of developing a herbal medicine which has got both liver protecting and nutritional value is required hence an attempt has been made to screen the hepatoprotective activity of fruit extracts of *Diospyros kaki* Linn.

MATERIALS AND METHODS:

Plant Materials: *D. kaki* berries were collected in October 2016 from the market of Missouri and were authenticated by Prof. D. Ramakanth Raju retired botanist Acharya Nagarjuna University, a voucher specimen (Snvl/jntu/2017-05) has been deposited in the Viswabharati College of Pharmacy, Guntur, Andhra Pradesh.

Preparation of Plant Extracts: Obtained plant material has been dried under shade and made into coarse powder passed though sieve# 20 and has been successively soxhelated using solvents like petroleum ether, chloroform, and ethanol for 72 h. Obtained extracts were made solvent free using rota evaporator and stored in a vacuum desiccator. Yield was found to be 0.28%, 0.3142%, 3.62% respectively. Obtained extracts were tested for preliminary phytochemical screening [9]. Oral suspensions of the extracts were prepared at a dose of 200 mg/ml and 100 mg/ml using 5% aqueous gum acacia [10].

Acute Toxicity Studies: Adult Swiss albino mice 20-25gm were taken for acute toxicity tests. The mice were divided into control and test groups containing 6 animals each. The control group received vehicle (5% of normal saline's), and the test group receives graded doses of extracts. The animals were observed up to 4 h then occasionally up to 48 h for the sign of any behavioral changes and motility, and LD_{50} values were calculated [11.]

Determination of Hepatoprotective Activity: The institutional animal ethical committee approved the experimental protocol (Reg. no. 1963/po/Re/s/17/ CPCSEA).

Selection of Animals: Wister albino rats about 150-200g were chosen for study. The animals were fed with a balanced diet and tap water *ad libitum*. The animals were maintained at room temperature and 40 - 70% RH with 12 h light / 12 h dark cycle. They were allowed free access to a standard dry pellet diet. The food was withdrawn 18 h before the experiment but allowed free access to water.

Group I received vehicle 5% aqueous gum acacia, Group II to Group IX received CCl_4 for 7 days at a dose of 0.25 ml/100gm [9], Group II serves as toxic group receives only CCl_4, Group III serves as standard receives silymarin 25 mg/kg b.w and group IV received *D. kaki* petroleum ether extract (D.K.P.E) at a dose of 100 mg/kg b.w, group V received *D. kaki* petroleum ether extract (D.K.P.E) at a dose of 200 mg/kg b.w, Group VI received *D. kaki* chloroform (D.K.C.E) at a dose of 100 mg/kg b.w, Group VII received *D. kaki* chloroform extract (D.K.C.E) at a dose of 200 mg/kg b.w, Group VIII received *D. kaki* ethanolic extract (V.M.E.E) at a dose of 100 mg/kg b.w, and group IX received *D. kaki* ethanolic extract (V.M.E.E) at a dose of 200 mg/kg b.w. Each group consisting of 6 animals. The vehicle and the test samples were administered orally for 7 days, and the liver damage was induced in rats on the 7th day after 6 h of administration of a

drug, by giving a single oral dose of CCl_4 in olive oil (1:1 ratio). On the 8th day, the blood samples were withdrawn by puncturing retro-orbital plexus [8]. The blood samples were allowed to clot for 30 min, and serum was separated by centrifuging at 2500 rpm for 10 min.

Assessment of Liver Function: Assessment of liver function was done by studying changes in biochemical parameters *viz* Reitman and Frankel method 12 estimated serum glutamic oxaloacetate transaminase (SGOT)/ (AST) and serum glutamic pyruvic transaminase (SGPT)/ (ALT). Total bilirubin [13], Alkaline phosphatase was also estimated [14].

Statistical Analysis: [15] The results are expressed as mean ± S.E.M and the statistical significance of the difference between groups was analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests. $P<0.05$ was considered significant. The percentage reduction was calculated by considering the difference between the mean values of toxicant and control as 100% reduction.

Histological Study: For the histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique [16]. Sections (5 μm) of livers stained with hematoxylin and eosin, were observed microscopically for histopathological studies.

TABLE 1: RESULTS OF PRELIMINARY PHYTOCHEMICAL TESTS OF THE *D. KAKI* BERRY EXTRACTS

S. no.	Tests	*D. kaki*		
		P. E	C. E	E. E
1	Alkaloids	-	+	-
2	Aminoacids	+	+	+
3	Carbohydrates	-	-	+
4	Flavonoids	-	+	+
5	Mucilage	-	+	+
6	Proteins	-	+	+
7	Starch	-	-	+
8	Steroids & triterpenoids	+	-	-
9	Glycosides	-	-	+

TABLE: 2: EFFECTS OF *D. KAKI* BERRY EXTRACTS ON SERUM BIOLOGICAL PARAMETERS IN CCl_4 INDUCED LIVER DAMAGE MODEL

S. no.	Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dl)
1	Control Group -I	124.17±6.77	110.76±4.78	194.84±6.99	1.62±0.30
2	Toxic control Group-II	354.49±23.16**	401.02±17.54**	444.50±14.76**	8.71±0.61**
3	Standard Group-III (25mg/kg)	128.23± 06.28*	126.24±06.17*	190.18±11.12*	6.05±0.29*
4	D.K.P.E, Group-IV, (100mg/kg)	364.74±13.02	395.11±05.22	430.22±15.22	7.94±0.16
5	D.K.P.E, Group-V (200mg/kg)	344.12±08.01	360.21±17.11	411.14±05.12	7.16±0.23
8	D.K.C.E Group-VII (100mg/kg)	309.43±10.26*	315.83±10.14*	403.26±08.21*	7.01±0.44*
9	D.K.C.E Group-VI (200mg/kg)	295.50±19.03*	264.57±06.71*	288.16±09.99*	6.76±0.64*
6	D.K.E.E Group-VI (100mg/kg)	208.50±07.21*	205.14±09.29*	213.17±15.57*	6.43±0.23*
7	D.K.E.E, Group-V (200mg/kg)	154.21±06.18*	140.24±17.22*	201.19±11.21*	6.31±0.62*

Values are mean ±SEM n=6, **P<0.01 When compared with the control, *P<0.01 when compared with the toxic control

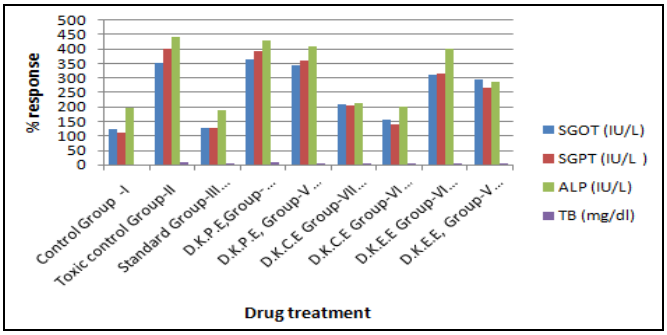
TABLE 3: PERCENTAGE DECREASE IN BIOCHEMICAL PARAMETERS DUE TO TREATMENT WITH *D. KAKI* BERRY EXTRACTS

Groups	% Decrease in levels of			
	SGOT	SGPT	AKLP	Total bilirubin
Standard Silymarin (25 mg/kg)	99.42%	95.68%	99.04%	86.02%
D.K.P.E (100 mg/kg)	6.88%	11.05	8.62%	24.61%
D.K.P.E (200 mg/kg)	11.86%	15.22%	14.25%	49.54%
D.K.C.E (100 mg/kg)	18.6%	27.89%	18.47%	53.34%
D.K.C.E (200 mg/kg)	26.74%	47.64%	63.67%	61.33%
D.K.E.E (100 mg/kg)	66.69%	67.88%	93.80%	74.88%
D.K.E.E (200 mg/kg)	88.2%	88.81%	98.62%	77.71%

RESULTS AND DISCUSSION: This work is an attempt made for the validation of rational usage of *D. kaki* berry as a hepatoprotective agent in liver infections. In acute toxicity study, no mortality was found up to 2000 mg/kg p.o of *D. kaki* berry extracts treated animal group. The LD_{50} was not determined, and $1/10^{th}$ of the tested proven safe concentration is taken as our experimental dose.

CCl_4 is a hepatoxin commonly used for the production of experimental liver toxicity [17]. The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and a high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue and requires less effort than that required for histological analysis, moreover without sacrifice of the animal. From the results, it was observed that there is a significant increase in the levels of SGOT, SGPT & total bilirubin in the toxicant group. Pretreatment with plant extracts and silymarin in test groups and standard group respectively daily for seven days showed significant ($p<0.01$) protective effect against CCl_4 induced hepatotoxicity when compared to the toxicant group.

From the results, it was observed that the percentage reduction in silymarin pretreated group in the biochemical parameters, SGOT, SGPT, ALKP, TB were found to be 99.42, 95.68, 99.04, and 86.02 respectively, whereas ethanolic extract treated group was showing a highly significant reduction of biochemical parameters at a dose of 200 mg/kg b.w whereas chloroform extract has shown moderated activity and petroleum ether extract showed the least significant hepato-protective activity



GRAPH 1: GRAPHICAL REPRESENTATION OF THE % RESPONSE OF BIOCHEMICAL PARAMETERS WITH *D. KAKI* BERRY EXTRACTS TREATMENT

Hence the ethanolic extract, chloroform extract of *D. kaki* at the dose of 200 mg/kg and 100 mg/kg were found to have significant hepatoprotective activity. The hepatoprotective activity of *D. kaki* could be due to the presence of alkaloids [17], proteins, and mucilage in case of chloroform extract. Whereas ethanolic extract possesses amino-acids, carbohydrates, flavonoids, and mucilage [18] which also are reported to have hepatoprotective and anti-oxidant properties.

Histopathological Sections of the Liver in Rats: Results of histopathological studies provided supportive evidence for biochemical analysis.

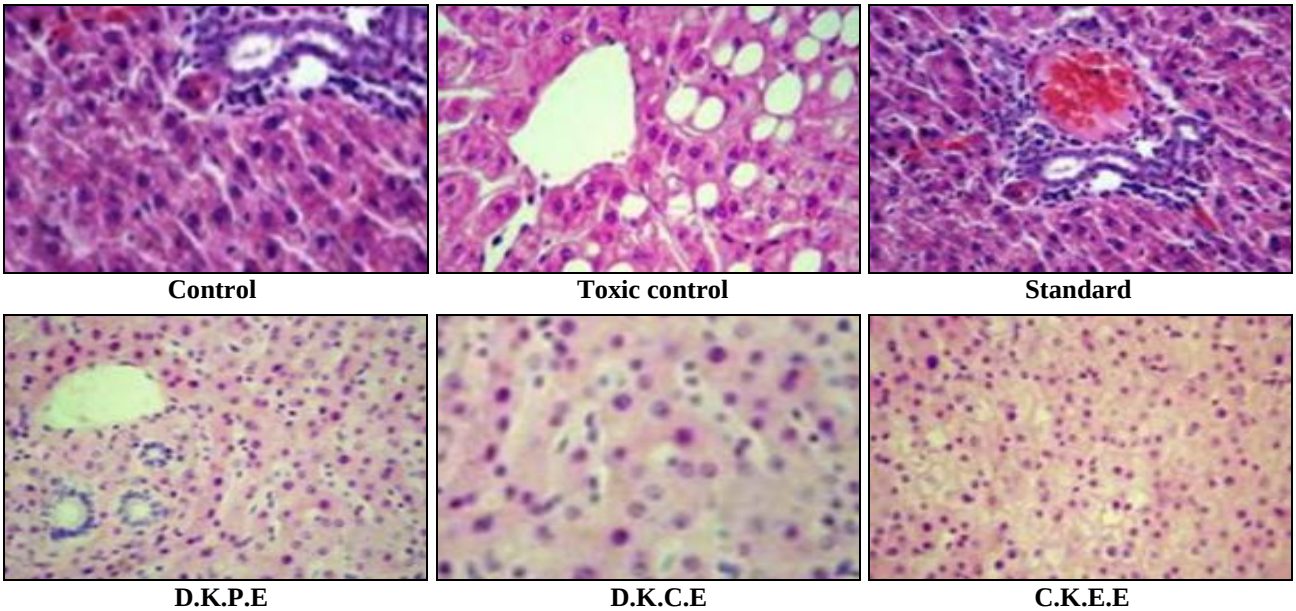


FIG. 1: HISTOPATHOLOGICAL SECTIONS OF THE LIVER IN RATS

Histology of liver section of normal control animal exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus, and nucleolus and well brought out central vein whereas that of CCl_4 intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia [20] crowding of central vein and apoptosis. Treatment with petroleum extract of *D. kaki* at a dose of 200 mg/kg b.w. showed moderate to weak activity in protecting the liver cells from CCl_4-injury. Among the plant extract, treatment with chloroform extract returned the injured liver to quite normal. And in the case of animal treated with ethanolic extract almost, it is equivalent to the standard group liver. Now, it could be decided that the hepatoprotective activity was dose and time-dependent. Out of three different extracts, the ethanol extract of *D. kaki* had shown very high significant potential hepatoprotective activity at a dose of 200 mg/kg. b.w. Even chloroform extract had shown significant protection against ccl_4 induced liver toxicity.

Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus, and nucleolus and well brought out central vein whereas that of CCl_4 intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia [20] crowding of central vein and apoptosis. Treatment with petroleum extract of *D. kaki* at a dose of 200 mg/kg b.w. showed moderate to weak activity in protecting the liver cells from CCl_4-injury. Among the plant extract, treatment with chloroform extract returned the injured liver to quite normal. And in the case of animal treated with ethanolic extract almost, it is equivalent to the standard group liver. Now, it could be decided that the hepatoprotective activity was dose and time-dependent. Out of three different extracts, the ethanol extract of *D. kaki* had shown very high significant potential hepatoprotective activity at a dose of 200 mg/kg. b.w. Even chloroform extract had shown significant protection against Ccl_4 induced liver toxicity.

CONCLUSION: From this work, it can conclude that the folklore usage of *D. kaki* as a hepatoprotective drug has been validated, it is useful in treating different liver infections and diseases.

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CONFLICT OF INTEREST: The Authors declare no conflicts of interest

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