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HEPATOPROTECTIVE ACTIVITY OF *UVARIA NARUM* IN PARACETAMOL-INDUCED HEPATIC DAMAGE IN RATS: A BIOCHEMICAL AND HISTOPATHOLOGICAL EVALUATION

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Keywords:

Uvaria narum, Paracetamol, Hepatoprotective effect, Serum enzymes, Catalase, Hepatocytes

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ABSTRACT: *Uvaria narum* Wall (Annonaceae) is described as a hepatoprotective in Ayurveda, the Indian system of medicine. However, there are no scientific reports in the modern literature regarding its usefulness as a hepatoprotective agent. The present study was carried out to evaluate the hepatoprotective activity of alcoholic (UNAL) and aqueous (UNAQ) extracts of leaves of *Uvaria narum* Wall in paracetamol-induced hepatotoxicity in rats. Alcoholic and aqueous extracts at doses of 200 and 400 mg/kg were administered orally once daily for 7 days. The hepatoprotective activity was assessed using various biochemical paradigms related to oxidative stress like increased liver lipid peroxidation, elevation of serum marker enzymes (AST, ALT, ALP, and LDH), decreased non-enzymatic (glutathione) and enzymatic antioxidants (CAT and SOD) activity and reduction of total proteins in liver homogenate along with histopathological studies of liver tissue. Treatment with UNAL and UNAQ extracts prevented the elevation of serum biomarkers AST, ALT, ALP, LDH, TB, and depletion of TP dose-dependently. The depletion of antioxidants GSH, SOD, and CAT and increased MDA formation in liver tissue by paracetamol was reversed by UNAQ and UNAL extracts dose-dependently. UNAQ and UNAL extracts also improved histoarchitecture alterations of hepatocytes. The hepatoprotective activity of UNAQ and UNAL extracts (200 mg/kg) was almost comparable to that of silymarin (100 mg/kg) treated group. Phytochemical analysis of UNAQ and UNAL extracts revealed the presence of quercetin as polyphenolic flavonoid compounds, which have been known for their hepatoprotective activities. The present findings demonstrate UNAL and UNAQ extracts of *Uvaria narum* could protect the liver cells from paracetamol-induced liver damage perhaps, by its antioxidant effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites of paracetamol which may be attributed to the individual or combined action of phytoconstituents present in it.

INTRODUCTION: *Uvaria narum* (Dunall) Wall; belonging to family Annonaceae is commonly found in mainly Kerala of southern India. It is known as Kariballi in Kanada, Nilavalli in Sanskrit and Pulliccan in Tamil [1-5]. In Ayurveda, it is widely used in the treatment of ailments such as eczema, jaundice, and swelling. The leaves are useful in vitiated conditions of vata and pitta [6]. Buchbauer, G and his co-workers have been analyzed the essential oil of *Uvaria narum* leaves by GC, GC/MS, GC/FTIR, and GC-sniffing techniques. The sample was found to be rich in beta-caryophyllene (9.99%), benzoic acid (9.75%), benzyl benzoate (6.23%), alpha-bulnesene (5.74%),

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beta-elemene (5.61%), alpha-copaene (5.39%), 3-hexenoic acid (4.46%) and germacrene-D (4.26%) [7]. However, to best of our knowledge, there is no scientific report is available in support of the hepatoprotective activity of alcoholic and aqueous extracts of *Uvaria narum* and its tentative mechanism (s) are still unknown. Hence, the study was undertaken to evaluate its socially claimed hepatoprotective effect using paracetamol-induced hepatic damage in rat model.

METHODS AND MATERIALS:

Plant Material: The plant *Uvaria narum* (Dunall) Wall; belonging to family Annonaceae was collected from the vicinity of Lonavala, Pune, Maharashtra, India in June. The plant herbarium was taxonomically identified by the taxonomist of Botanical Survey of India (BSI), Pune. A voucher specimen (BSI/WC/Tech/2008/354-RRW/UN-3.) has been deposited in Pharmacognosy museum at K.L.E University's College of Pharmacy, Belgaum for future reference.

Drugs and Chemicals: Paracetamol and Silymarin were obtained as a gift sample from Torent Research Centre, Ahmedabad, and Cadila Pharma Ltd. India, respectively. Thiobarbituric acid (TBA), reduced glutathione, oxidized glutathione and nicotinamide adenine dinucleotide (NADPH) were obtained from Himedia Laboratories, Mumbai, India, 5, 5-dithiobis (2-nitrobenzoic acid)-(DTNB) and epinephrine were purchased from Sigma Chemical Co, St. Louis, MO, USA. Standard reagents and kits for determination of AST, ALT, ALP, LDH, total proteins and total bilirubin were purchased from Span Diagnostics, Surat, India and Ranbaxy laboratories, Delhi, India. All other chemicals were obtained from local sources and were of analytical grade.

Preparation of Extracts: The powdered plant material (500 gm) was subjected to successive solvent extraction with the solvents in the order of increasing polarity *i.e.* petroleum ether (40-60 ºC), followed by chloroform and alcohol. The aqueous extract was prepared by cold maceration process. The extracts were dried under reduced pressure using rotary evaporator afforded semi-solid extracts [8]. The alcoholic and aqueous extracts were then used for hepatoprotective and *in-vivo* antioxidant activity.

Phytochemical Analysis: The preliminary phytochemical analysis of UNAQ and UNAL was carried out by documented method [9]: alkaloid (Hager's, Mayer's, Wagner's and Dragendroffs test), flavonoids (Shinoda test), tannins and polyphenolic compounds (Lead acetate test, ferric chloride test, potassium permanganate test) and steroids (Salkowskis's and Libermann-Burchard test). Spectral studies of the TLAQ and TLAL were carried out using FTIR (Perkin Elmer, USA) to analyze its major phytoconstituents.

HPTLC Analysis: The aqueous and alcoholic extracts of *Uvaria narum* were dissolved in respective HPTLC grade ethanol and water which were used for sample application on precoated silica gel GF 254 aluminium sheet (Made- Merck, Germany). The samples (5 µL) were spotted in the form of bands of width 6 mm with a 100 µL sample using a Hamilton syringe on silica gel which was pre-coated on aluminium plate GF-254 plates (20 cm × 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

The linear ascending development of chromatogram was carried out in a (20 cm × 10 cm) twin trough glass chamber saturated with the mobile phase (Ethyl acetate: formic acid: glacial acetic acid: Water (99:12:11:27 v/v/v) for isolation of Quercetin. The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid and 5 % Ferric chloride as spray reagent and dried at 100 ºC in a hot air oven for 3 min. The plate was kept in the photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 214 nm, respectively. The R_f values and fingerprint data were recorded by WIN CATS software. Quercetin was used as reference standard for comparison.

Experimental Animals: Wistar rats of either sex (150-200 g) were procured from National Toxicology Centre, Pune. Rats, 6 in a group were housed in clean propylene cages and maintained under standard conditions: humidity (50 ± 5%), temperature (25 ± 2 ºC) and light (12 h light: 12 h dark cycle) and fed with a standard diet (Amrut lab Animal feed Pune, India) and water *ad libitum*.

Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee KLE University's College of Pharmacy, Belgaum, Karnataka (IAEC Reg. No.: 627/02/a/ CPCSEA).

Acute Oral Toxicity Studies: Acute toxicity study for aqueous and alcoholic extract was carried out on rats according to OECD-423 guidelines. The extracts at various doses were administered up to 2000 mg/kg, p.o. Animals were observed for toxic effect, behavioral changes and mortality, if any for a period of 72 h. The doses of extracts were selected by findings of pilot experiments [10].

Paracetamol- Induced Hepatotoxicity in Rats (Acute Model): Paracetamol (Acetaminophen; Torrent Research Centre, Ahmedabad) was suspended in 1% CMC and administered p.o; at a dose of 2 g/kg. This dose is known to cause hepatotoxicity in rats. Animals, after acclimatization (6-7 days) in the animal quarters, were fasted overnight and randomly divided into seven groups of six animals each and treated in the following way.

Group, I served as normal (vehicle) control and fed orally with normal saline (5 ml/kg b.w; p.o) daily for seven days. Group II rats were similarly treated as group I. Animals of Group III were treated with the standard drug, silymarin (100 mg.kg b.w; p.o) daily for seven days. Group IV and V rats were treated with (200 mg and 400 mg /kg b.w) of the aqueous extract of *Uvaria narum* (UNAQ) and Group VI and VII rats were treated with (200 mg and 400 mg /kg b.w) of the alcoholic extract of Uvaria narum (UNAL) daily for seven days respectively. On the seventh day, paracetamol suspension was given by oral route, in a dose of (2 gm/kg) in 1% CMC to all rats except the rats in group I [11].

Assayof Liver Biomarkers: On the 8th day, under light ether anesthesia blood was withdrawn directly from the heart and after that rats were sacrificed by euthanasia. The liver tissues were removed, washed with normal cold saline and preserved at -20 ºC. The serum was separated by centrifugation at 1200 rpm (Remi, USA) below 30 °C for 15 min and used for the assay of liver marker *viz*; Serum alanine aminotransferase (ALT) [12], serum aspartate aminotransferase (AST) [12], alkaline phosphate (ALP) [13], lactic dehydrogenase (LDH) [14], total bilirubin (TB) [15] and total proteins (TP) [16] were determined by standard methods using enzyme assay kits.

Antioxidant Assays: After blood withdrawal, animals were sacrificed by euthanasia, and 10 % liver homogenate was prepared in Triss buffer at (pH-7.4) and then centrifuged (Remi, Pvt. Ltd.) at 6000 rpm for 15 min and the supernatants used for the measurement of antioxidant marker enzymes *viz.* the lipid peroxidation was assayed in homogenate by determining the formation of MDA [17], catalase (CAT) [18], superoxide dismutase (SOD) [19], and reduced glutathione (GSH) [20, 21].

Histopathological Studies: The liver tissue preserved in 10% w/v formalin was embedded in paraffin wax and cut into 3-4 µm thick section on microtome and sections were stained using hematoxylin and eosin and observed under a microscope for histoarchitecture alterations.

Statistical Analysis: The data were analyzed and expressed as the mean ± standard Error Mean (SEM). Student's t-test was used to determine the significance of the findings. Dunnet test followed by ANOVA analyzed experimental data. Differences were considered statistically significant when $P<0.05$.

RESULTS:

Preliminary Phytochemical Analysis: The preliminary phytochemical analysis of UNAQ showed the presence of flavonoids, tannins, and polyphenolic compounds whereas, UNAL showed the presence of flavonoids, tannins, saponins and alkaloids. Physicochemical properties of UNAQ and UNAL are outlined in **Table 1**. The FTIR spectral peaks of the isolated flavonoids (Quercetin) from both the extracts were summarised in **Table 2**.

HPTLC Analysis: The HPTLC chromatogram at 214 nm showed the presence of quercetin and exhibited blackish (visible) band in the R_f range of 0.47 to 0.52. According to the literature of flavonoids, the R_f range is found to be 0.4 to 0.6. Therefore, theChromatogram fingerprint suggests the presence of quercitin in the aqueous and alcoholic extract of *U. narum* **Fig. 1**, **2**, **3** and **4**.

TABLE 1: PRELIMINARY PHYSICOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF AQUEOUS (UNAQ) AND ALCOHOLIC (UNAL) EXTRACTS OF LEAF OF *UVARIA NARUM*

Test	Observations	
	UNAQ extract	**UNAL extract**
Nature and appearance	Semisolid, dark green with an aromatic odor	Sticky, Semisolid green with an aromatic odor
Solubility	Soluble in water	Soluble in alcohol, toluene, acetone
Phytochemical present	flavonoids, tannins and polyphenolic compounds	flavonoids, tannins, saponins, and alkaloids

TABLE 2: FTIR SPECTRAL PEAKS OF ISOLATED FLAVONOID (QUERCITIN) FROM AQUEOUS (UNAQ) AND ALCOHOLIC (UNAL) EXTRACTS OF LEAF OF *UVARIA NARUM*

Spectral Peaks cm^{-1}	Molecular Nature
2851.33	C-H Stretching alkane
1593.72	Saturated cyclic five-member ring
1350.24	C=O stretching
1424.66	CH_3 bending alkene
766.24	CH_3 bending alkane

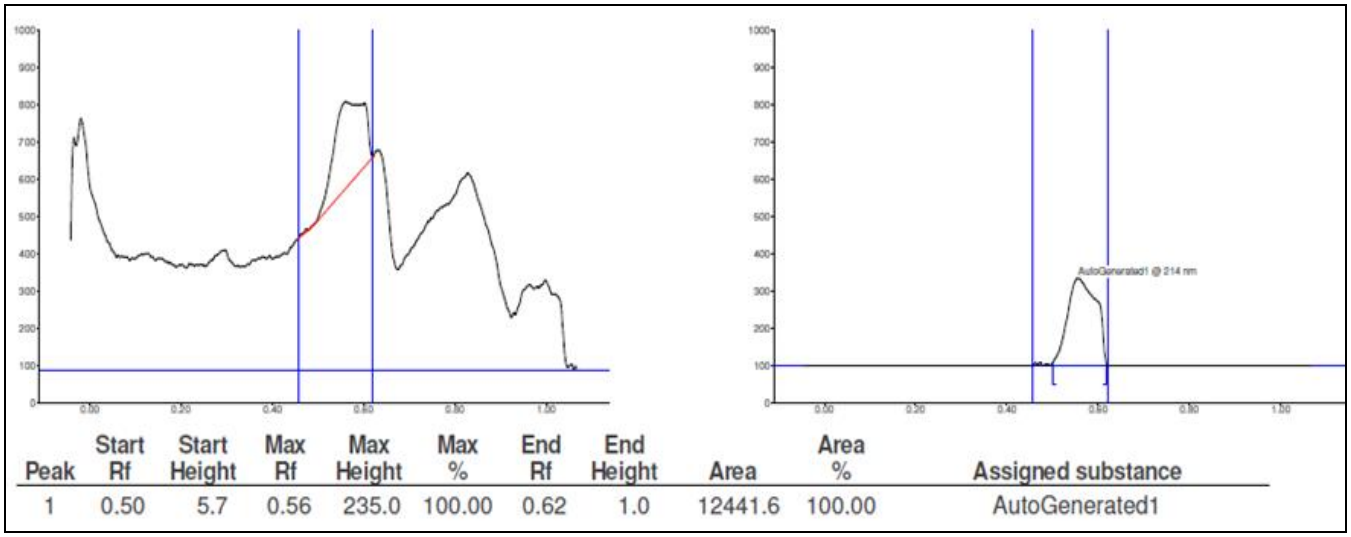


FIG. 1: CHROMATOGRAM OF ALCOHOLIC EXTRACT OF *UVARIA NARM*

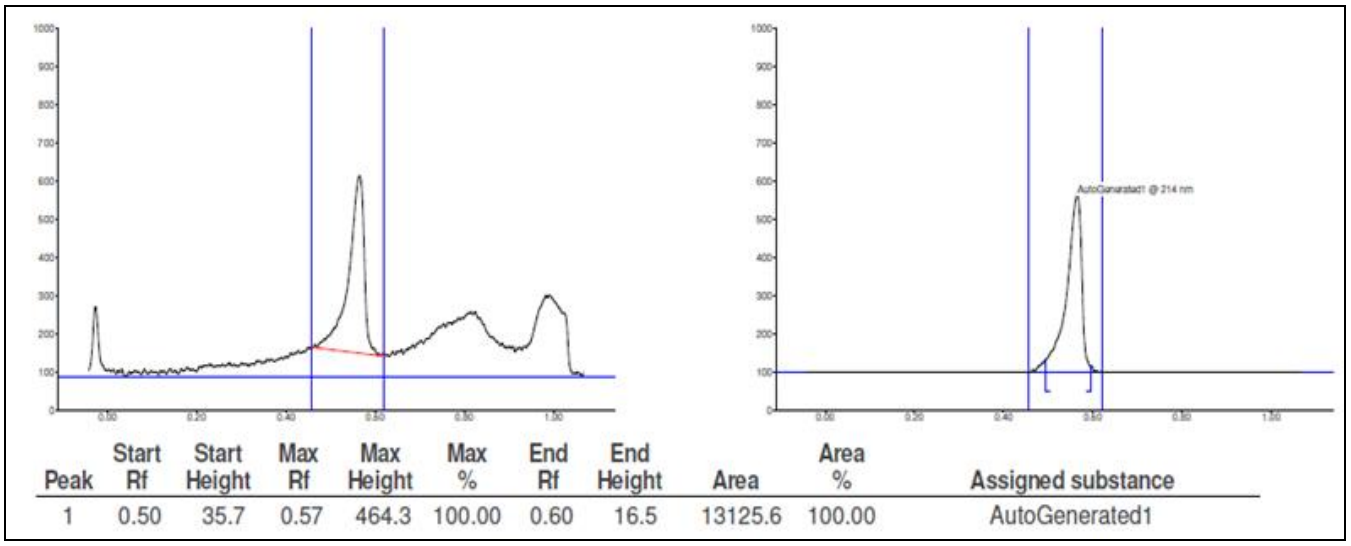
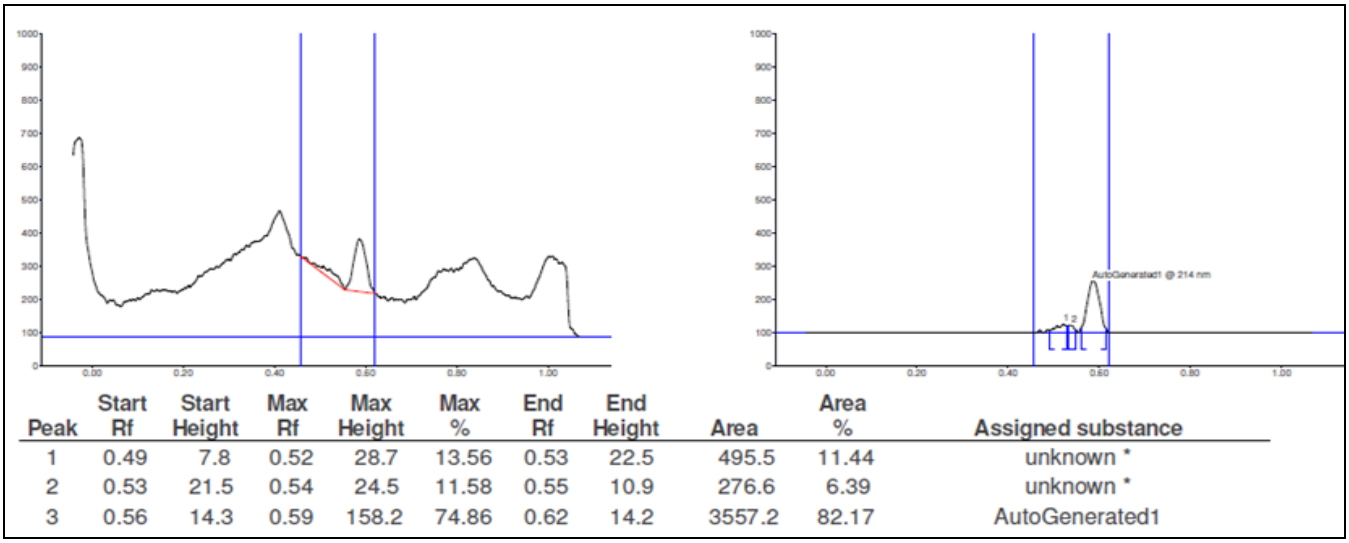
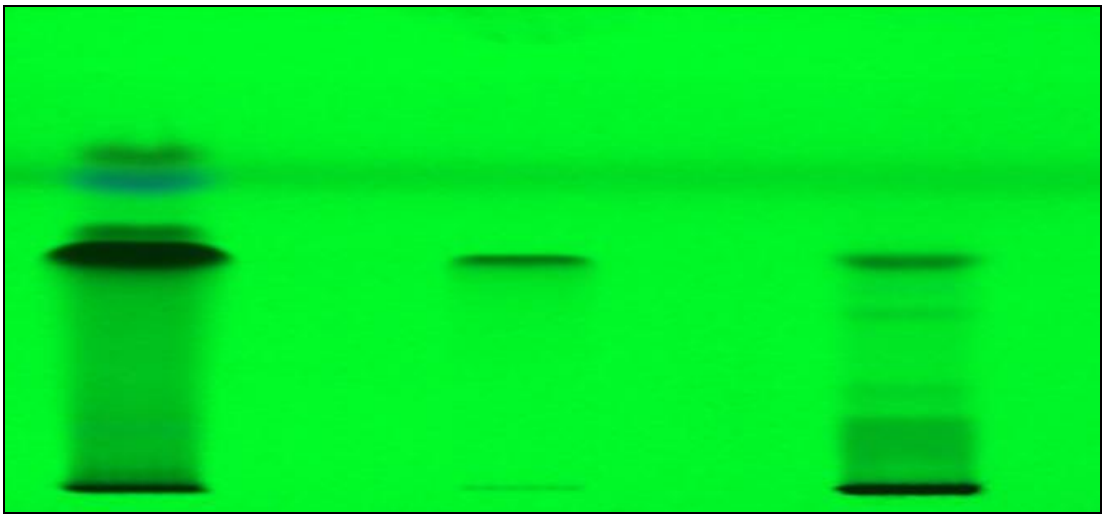


FIG. 2: CHROMATOGRAM OF STANDARD QUERCITIN COMPOUND



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.49	7.8	0.52	28.7	13.56	0.53	22.5	495.5	11.44	unknown *
2	0.53	21.5	0.54	24.5	11.58	0.55	10.9	276.6	6.39	unknown *
3	0.56	14.3	0.59	158.2	74.86	0.62	14.2	3557.2	82.17	AutoGenerated1

FIG. 3: CHROMATOGRAM OF AQUEOUS EXTRACT OF *UVARIA NARUM*



UNAL **Quercetin** **UNAQ**

FIG. 4: HPTLC FINGERPRINT PLATE OF, UNAL, QUERCITN AND UNAQ

Acute Oral Toxicity in Rats: The LD_{50} value by oral route was not determined as no mortality, or toxic symptoms were observed up to 2000 mg/kg dose level. No mortality was observed during 72 h of the observation period.

Hepatoprotective Activity Screening: Rats administered with paracetamol (2 g/kg) alone developed significant hepatocellular damage as evident from a significant increase in serum hepatic enzyme levels AST, ALT, ALP, LDH and total bilirubin with marked decrease in total protein concentration when compared to vehicle control group. Pre-treatment with silymarin (100 mg/kg), UNAQ and UNAL extracts (200 mg/kg and 400 mg/kg) followed by hepatotoxins prevented the elevation of serum marker enzymes AST, ALT, LDH, and TB and restored TP. It is confirmed that UNAQ and UNAL extracts can ameliorate hepatic function of hepatic damage induced by paracetamol **Table 3** and **4**. Furthermore, in histopathological studies, paracetamol treated liver sections showed necrosis, lymphocytes infiltration, congestion and hemorrhage of hepatocytes.

However, treatment with aqueous and alcoholic extracts at the dose of (200 and 400 mg/kg) almost prevented the infiltration of lymphocytes and congestion as compared to PCM treated rats Shown in **Fig. 5(A-G)**. Liver MDA formation (an index of lipid peroxidation) was increased significantly in paracetamol treated rats. Aqueous, alcoholic extracts and silymarin significantly prevented ($P<0.01$) increased MDA formation due to treatment **Table 4**. Depletion of GSH and the SOD and CAT were restored by pre-treatment with

UNAQ and UNAL dose-dependently. The effect of UNAQ (200 mg/kg) and UNAL (200 mg/kg) were comparable to that of silymarin 100 mg/kg **Table 5**.

TABLE 3: EFFECT OF PRE-TREATMENT WITH AQUEOUS (UNAQ) AND ALCOHOLIC (UNAL) EXTRACTS OF *UVARIA NARUM* (UN) LEAVES EXTRACTS ON SERUM MARKER ENZYMES IN PARACETAMOL-INDUCED HEPATIC DAMAGE

Drug Treatment	Parameters			
	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	LDH (IU/L)
Vehicle treated (1 % CMC, 10 ml/kg p.o)	21.83 ± 2.48	16 ± 3.4	3.1 ± 0.3	221.5 ± 19.58
Toxic control (PCM; 2g/kg p.o)	127.33 ± 6.59##	72.8 ± 5.1##	54.6± 5.5##	861.5 ± 89.5##
PCM +Silymarin (100 mg/kg)	43.33 ± 4.13**	37.1 ± 6**	15.6 ± 2.3**	338 ± 26**
PCM + 200 mg/kg p.o of aqueous extract	115.6 ± 10.19*	64 ± 5.8*	47.8 ± 3.8*	765 ± 58.39*
PCM + 400 mg/kg p.o of aqueous extract	86.5 ± 7.5**	47.8 ± 5.2**	42.2 ± 3.1**	653 ± 43.25**
PCM + 200 mg/kg p.o of alcohol extract	113.8 ± 8.8*	63.6 ± 5.5*	47 ± 3.7*	753 ± 59.49*
PCM + 400 mg/kg p.o of alcohol extract	77.3 ± 6.6**	45.6 ± 4.5**	39.6 ± 3.3**	620 ± 55.78**

*P < 0.05 and **P < 0.01 compared with PCM treated group. ## P < 0.05 compared with vehicle treated group. PCM: Paracetamol.

TABLE 4: ALTERATIONS IN THE VALUES OF TOTAL PROTEINS (TL) AND TOTAL BILIRUBIN (TB) WITH PRE-TREATMENT OF AQUEOUS (UNAQ) AND ALCOHOLIC (UNAL) EXTRACTS OF *UVARIA NARUM* (UN) LEAVES EXTRACTS TO RATS INTOXICATED WITH PARACETAMOL (PCM)

Drug Treatment	Parameters	
	Total Proteins (mg/dL)	Total Bilirubin (mg/dL)
Vehicle treated (1 % CMC, 10 ml/kg p.o)	13.3 ± 1	0.88 ± 0.13
Toxic control (PCM; 2gm/kg p.o)	6.4 ± 0.9##	2.21 ± 0.24##
PCM + Silymarin (100 mg/kg)	11.98 ± 1.1**	1.16 ± 0.24**
PCM + 200 mg/kg p.o of aqueous extract	8.16 ± 0.88*	1.8 ± 0.2*
PCM + 400 mg/kg p.o of aqueous extract	9.58 ± 0.75**	1.23 ± 0.23**
PCM + 200 mg/kg p.o of alcohol extract	7.96 ± 0.67*	1.78 ± 0.23*
PCM + 400 mg/kg p.o of alcohol extract	11.73 ± 1**	1.35 ± 0.2**

*P < 0.05 and **P < 0.01 compared with PCM treated group. ## P < 0.05 compared with vehicle treated group. PCM: Paracetamol.

TABLE 5: ALTERATIONS IN THE VALUES OF SOD, GSH, CAT AND MDA WITH PRE-TREATMENT OF AQUEOUS (UNAQ) AND ALCOHOLIC (UNAL) EXTRACTS OF *UVARIA NARUM* (UN) LEAVES EXTRACTS TO RATS INTOXICATED WITH PARACETAMOL (PCM)

Drug Treatment	Parameters			
	SOD U/mg of Protein	GSH µg/g of Protein	CAT µmole of H_2O_2/mg of Protein	MDA nmole/gm of Protein
Vehicle treated (1 % CMC, 10 ml/kg p.o)	33.66 ± 3	2.76 ± 0.27	29 ± 2.3	4.2 ± 0.34
Toxic control (PCM; 2gm/kg p.o)	13.5 ± 1.64##	1.33 ± 0.9##	10.15 ± 1.48##	29 ± 3.6##
PCM + Silymarin (100 mg/kg)	26.33 ± 2.58**	2.5 ± 0.2**	24.5 ± 2**	9.26 ± 1.1**
PCM + 200 mg/kg p.o of aqueous extract	17.6 ± 1.6*	1.73 ± 0.1*	13.83 ± 1.7*	25.5 ± 1.8*
PCM + 400 mg/kg p.o of aqueous extract	25.8 ± 2.6**	2.2 ± 0.26**	18.5 ± 2**	16.5 ± 1.6**
PCM + 200 mg/kg p.o of alcohol extract	17.5 ± 1.8*	1.7 ± 0.12*	13.8 ± 2*	25.5 ± 1.6*
PCM + 400 mg/kg p.o of alcohol extract	25.6 ± 2.3**	2.2 ± 0.2**	19.3 ± 2.5**	16.5 ± 1.8**

*P < 0.05 and **P < 0.01 compared with PCM treated group. ## P < 0.05 compared with vehicle treated group. PCM: Paracetamol.

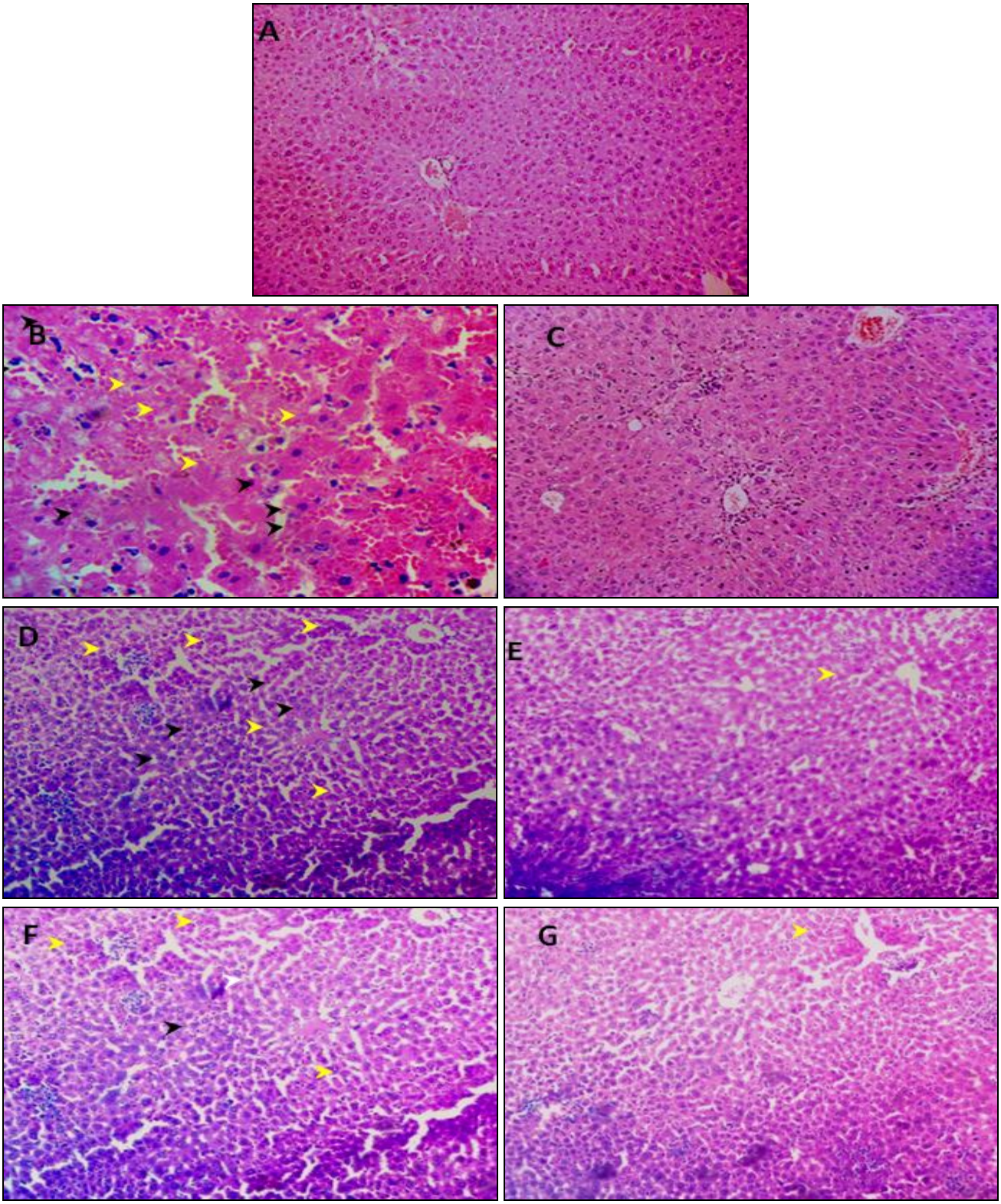


FIG. 5: EFFECT OF *UVARIA NARUM* (UN) ON HISTOPATHOLOGIC EXAMINATION OF RAT LIVER IN PARACETAMOL INDUCED LIVER DAMAGE. (A) Normal control: section shows normal hepatocytes (B) Paracetamol treated rat: section showed areas of necrosis, (yellow arrows), lymphocytes infiltration (black arrows), congestion and hemorrhages paracetamol control: section showed areas of necrosis, (yellow arrows), lymphocytes infiltration (black arrows), congestion and hemorrhages (C) Silymarin (100 mg/kg) + paracetamol (2 g/kg) mg/kg) showed mild congestion only, (D) Section of rat treated with UNAQ 200 mg/kg showed vascular dilatation, mild infiltration of lymphocytes, (E) Section of rat treated with UNAQ 400 mg/kg, showed mild congestion and lymphocytic infiltration, (F) Section of rat treated with UNAL 200 mg/kg showed necrosis, lymphocytes infiltration and congestions, (G) Section of rat treated with UNAL 400 mg/kg showed mild congestion and infiltration of lymphocytes.

DISCUSSION: In the present study efforts has been made to evaluate possible hepatoprotective and antioxidant activity of *Uvaria narum* leaves extracts, a hepatoprotective agent reported in Ayurveda, the Indian System of Medicine, in paracetamol induced hepatic damage rat model. Paracetamol (PCM) is considered a predictable hepatotoxin, where biochemical signs of hepatic damage will become apparent within 24 to 48 h after the time of overdose and produce dose-dependent centrilobular necrosis in the liver [22].

Hepatic damage induced by PCM is largely due to the biotransformation of a highly reactive and toxic metabolite N -acetyl- p- benzoquinone imine (NAPQI). NAPQI is detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. If the rate of NAPQI formation exceeds the rate of detoxification by GSH; it oxidizes tissue macromolecules such as lipid or thiol group protein and alters the homeostasis of calcium after depleting GSH [23].

The NAPQI then causes acylation or oxidation of cytosolic and membrane proteins and generation of reactive oxygen species (ROS). This leads to further oxidation of proteins thiols, lipid peroxidation, DNA fragmentation, and ultimately cell necrosis. The important sign of hepatocyte damage is largely to reflect in the leaking of cellular enzymes into the bloodstream due to disturbances caused in the transport functions of hepatocytes. Therefore determination of enzymes in the serum is a useful biological marker of the extent and nature of hepatocellular damage [24].

In the present experimental findings, the rats treated with paracetamol (2 gm/kg), showed significant hepatic damage, which can be correlated with the increased serum marker enzymes (ALT, AST, ALP, and LDH) and MDA formation in liver homogenates. Pre-treatment with aqueous and alcoholic extract at (200 and 400 mg/kg, p.o) for 7 days followed by paracetamol administration resulted in significant prevention of elevated serum marker enzymes and restored total proteins.

The prevention of elevated serum marker enzymes by aqueous and alcoholic extracts at (200 and 400 mg/kg, p.o) of *Uvaria narum* may be due to hepatocytes cell membrane stabilization, which then prevents the systolic released in circulation. These findings are in agreement with the documented fact that, serum transaminase levels return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes [25]. Furthermore, alterations of histoarchitectural changes i.e. hepatic lymphocytes infiltration and other inflammatory cells due to paracetamol are prevented with both extracts in dose-dependently.

The protective role of GSH against cellular lipid peroxidation has been well documented [26]. A substantial increase in hepatic lipid peroxidation was evident by elevated MDA formation in liver homogenate. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death and its MDA formation is increased due to oxidative stress associated with NAPQI [27]. Pre-treatment with aqueous and alcoholic extracts at (200 and 400 mg/kg, p.o) of *Uvaria narum* significantly reversed the MDA formation to normal. Hence, it may be possible that the mechanism of hepatoprotection of the extract is largely due to its antioxidant effect.

It is well reported that a deficiency of GSH within organisms can lead to tissue disorder and injury [28]. In the present study, we have demonstrated the effectiveness of the extract by using paracetamol-induced rats, which is a known model for both hepatic GSH depletion and injury. Therefore, the levels of glutathione are of critical importance in liver injury caused by paracetamol. Our results in line with this earlier report because we found that pre-treatment with UNAQ and UNAL extracts at (200 and 400 mg/kg, p.o) able to blunted to a normal level.

Biological systems have some mechanisms to protect themselves against the damaging effects of reactive oxygen species by several means. Superoxide dismutase (SOD) removes superoxide (O-2) by converting it to H_2O_2, which can be rapidly converted to water by CAT [29]. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues.

The observed increase of SOD activity suggests that the pre-treatment with UNAQ and UNAL extracts at (200 and 400 mg/kg, p.o) have an efficient protective mechanism in response to ROS. Catalase (CAT) is a hemoprotein and a key component that catalyzes the reduction of H_2O_2 and able to prevents the tissues from reactive free oxygen and hydroxyl radicals. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage.

Excessive production of free radicals may result in alterations in the biological activity of hepatic cellular macromolecules [30]. Pre-treatment with UNAQ and UNAL extracts at (200 and 400 mg/kg, p.o) restored CAT activity in paracetamol–induced hepatic damage and thus prevent accumulation of excessive free radicals. Therefore, the reduction in the activity of these enzymes may result in some deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.

The pre-treatment with UNAQ and UNAL extracts (200 and 400 mg/kg) elevated the activities of catalase in paracetamol-induced hepatic damage rats to prevent the accumulation of excessive free radicals and protects the liver from paracetamol intoxication. The ability of UNAQ and UNAL extracts to protect paracetamol-induced hepatic damage in rats might be attributed to its ability to restore the activity of antioxidant enzymes.

Thus, experimental findings together with those of earlier ones, suggest that UNAQ and UNAL extract able to prevented hepatic damage due to paracetamol by their antioxidant property. Since these models of hepatic damage in the rats simulate many of the features of human liver pathology, we suggest that natural antioxidants and scavenging agents in *Uvaria narum* leaves extracts might be effective as hepatoprotective and thus may have some obvious therapeutic implications.

Therefore, it seems logical to infer that UNAQ and UNAL extract, because of its antioxidant property, might capable of protecting the hepatic tissues from paracetamol-induced injury and inflammatory changes. The aqueous and alcoholic extracts of Uvaria narum is reported to be rich in flavonoids. Presence of Quercitin (flavonoid) in both the extracts was confirmed an agreement with our preliminary phytochemical screening and HPTLC studies. Quercitin is natural products, which have been shown to possess the antioxidant property and hepatoprotective activity. Studies have proved that indeed oxidative stress plays an important role in the initiation of liver diseases.

The results of biochemical observations are supplemented by a histopathological examination of the rat livers. Pre-treatment with UNAQ and UNAL extracts exhibited and showed regeneration of liver cells. The effect of UNAQ and UNAL extracts were comparable with those of Silymarin, a standard proven hepatoprotective. As *Uvaria narum* leaves extracts contains a large amount of flavonoids it may be suspected that the hepatoprotective activity may be due to the presence of flavonoids in the extracts.

CONCLUSION: The present experimental findings demonstrates that hepatoprotective activity of *Uvaria narum* leaves extracts through antioxidant effect (elevation of endogenous antioxidant enzymes and total proteins) and membrane stabilization of hepatocytes (reduce AST, ALT, ALP, LDH and total bilirubin) by scavenging /neutralizing free radicals. Hence, the present study justifies the traditional use of *Uvaria narum* in the treatment of liver diseases and also

points out that *Uvaria narum* warrants future detailed investigation as a promising hepato-protective agent.

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CONFLICT OF INTEREST: Nil

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