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Hepatoprotective effect of rutin and N-acetyl cysteine against isoniazid induced hepatotoxicity

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ABSTRACT

This study was carried to evaluate the protective effect of rutin and N-acetyl cysteine (NAC) against isoniazid-induced hepatotoxicity (INH). In this study, seventy-two adult male rats were divided into six equal groups as follow: control group: 1 ml saline /Kg b. wt I/P. INH group: 100 mg INH /kg b. wt. I/P. INH + Rutin group: 100 mg INH /kg b. wt. I/P with 200 mg rutin /kg orally. INH + NAC group: 100 mg INH /kg b. wt. I/P with 300 mg NAC /kg orally. Rutin group: 200 mg rutin /kg/day orally. NAC group: 300 mg NAC /kg b. wt. orally. Serum and tissue specimens were collected at 7th, 14th and 21st day form the 1st injection . IP injection of Isoniazid induced significant increase in values of ALT, AST, ALP, GGT and total bilirubin, while significant decrease in total protein and albumin was recorded. INH + Rutin showed significant decreases in the levels of ALT, AST, ALP and GGT with significant increase in albumin values and significant increase in total protein however significant decrease in the level of total bilirubin was recorded only at 21 days. INH + NAC induced significant decreases in the levels of ALT, AST, ALP and GGT with significant increase in albumin and total protein at 14 and 21 days and significant decrease in total bilirubin at 21 days. Rutin and NAC have potent protective effect against INH induced hepatotoxicity.

1. INTRODUCTION

Drug-induced liver injury is a main health problem (Yuan and Kaplowitz, 2013). Isoniazid (INH) is a commonly used and applicable primary agent for tuberculosis treatment; the main principal unfavorable reaction of INH is drug-induced hepatic injury (Jaswal et al., 2013). Classical plant medicines or herbal preparations might suggest a natural key to liver protection against xenobiotic/drug (Minani et al., 2011). Flavonoids are natural polyphenols present ubiquitously in various fruits, leaves and seeds (Kumar and Pandey, 2013). Rutin is a common dietary flavonoid that possesses a wide spectrum of biochemical and pharmacological effects attributed, at least partially, to their anti-oxidative and free-radical scavenging properties (Nkwonkam et al., 2007). N-acetyl cysteine is a well-known cytoprotective drug that has confirmed effectiveness against drug-induced liver toxicity (Bulbuloglu et al., 2006).

2. MATERIAL AND METHODS

2.1. Experimental Animals:
Seventy-two male Wister rats aging 3 weeks and weighing about (130-150) gm were obtained from Animal House at Faculty of Veterinary Medicine, Benha University. All rats were caged and maintained on a standard diet with free access to tape water and were acclimatized for 1 week before starting the experiments.

2.2. Chemicals:
Isoniazid was obtained from Medical Union Pharmaceuticals Company and was given as intraperitoneal injection of 100 mg/kg body weight once daily for 21 days as previously described *(Varkey and Vahab 2016).* Rutin was obtained from El Qahera Company and administered to rats at a dose of 200 mg/kg body weight through oral intubation, once a day for 21 days as previously described *(Abdel- Raheem 2010).* N-Acetyl cysteine was obtained from SEDICO Company and was given 300 mg/kg/day orally for 21 days as previously described *(Hemalatha et al., 2013).*

2.3. Experimental design:
In this study, 72 Wister adult male rats were divided into 6 groups each group12 rats: Control group: received 1 ml sterile saline /Kg/b. wt. I/P. INH group: received a single dose of 100 mg INH /kg b. wt./day I/P for 21 days. INH + *Rutin* group was given a single dose of 100 mg INH /kg b. wt./day I/P for 21 days and were treated with 200 mg rutin/ kg/day b. wt. orally for 21 days. INH + NAC group was given a single dose of 100 mg INH /kg b. wt./day I/P for 21 days and was treated with 300 mg NAC /kg b. wt./day orally for 21 days. *Rutin* group was given 200 mg rutin /kg b. wt./day orally for 21 days. NAC group was given 300 mg NAC /kg b. wt./day orally for 21 days.

2.3.1. Samples:
2.3.1.1. Blood samples were collected via retro-orbital bleeding from 4 rats each group at 7, 14 and 21 days (3ml) on plain tubes for serum separation.
2.3.1.2. Specimens from liver was collected from all groups after sacrificing at 21th day and preserved in neutral buffered formalin solution (10%) for histopathological examination.

2.3.2. *Measurement of biochemical parameters:*
Serum ALT and AST values were measured according to Hayashi et al, (2003), GGT according to Szasz, (1969), In addition serum values of ALP, total bilirubin, total protein and albumin according to Tietz et al, (1983), Jendrassik and Grof, (1938), Weichselbaum, (1946) and Watson et al, (1997) respectively.

2.3.3. Histopathological examination:
Small liver tissue specimens were collected from rats in all groups and immediately fixed in 10% neutral buffered formalin. After proper fixation, 5 µm tissue paraffin sections were routinely prepared and stained with H&E stain for light microscopic examination (Banchroft et al., 1996)

2.4. Statistical analysis:
SPSS 19.0 statistical package was used to perform all statistical analyses. One-way analysis of variance (ANOVA) followed by Duncan test was used for comparing control and treated groups. The results were expressed as mean ± SE. A probability (P) level of ≤ 0.05 was considered statistically significant.

3. RESULTS

3..1. Biochemical parameters:
Isoniazid group showed significant increases in serum ALT, AST, ALP, GGT and total bilirubin at 7, 14 and 21 days compared with control group. While, Isoniazid injection with rutin treatment exhibited significant decreases in ALT, AST, ALP and GGT at 7, 14 and 21 days, significant decrease in serum total bilirubin at 21 days compared with isoniazid group. While, Isoniazid with NAC treatment induced significant decreases in ALT, AST, ALP and GGT at 7, 14 and 21 days and significant decrease in total bilirubin at 21 days when compared with isoniazid group. Also, Rutin or N-acetyl cysteine administrated groups exhibited non-significant changes in ALT, AST, ALP, GGT and total bilirubin values at 7, 14 and 21 days compared with control group.
Isoniazid group induced significant decreases in serum total protein and albumin at 7, 14 and 21 days compared with control group. While, isoniazid injection with rutin treatment exhibited significant increase in albumin at 7, 14 and 21 days with significant increase in total protein at 21 days. Also, Isoniazid injection with NAC treatment induced significant increase in albumin at 7, 14 and 21 days and significant increase in total protein at 14 and 21 days. On the other hand, Rutin or N-acetyl cysteine administrated groups exhibited non-significant changes in total protein and albumin at 7, 14 and 21 days compared with control group.

3.2. Liver Histopathology:
Control group showed intact hepatocytes arranged in strands around central veins (Fig 1A). While, isoniazid group induced severe degree of hepatic hydropic and vacuolar degeneration extended from the periportal area associated with focal hepatic necrosis associated with inflammatory cells aggregation (Fig 1B). On the other hand, isoniazid injection with rutin administration induced marked decrease in hepatic degenerative changes where only centro-lobular cloudy swelling of hepatic cells was recorded (Fig 1C). Also, isoniazid with NAC administration showed moderate degree of hepatic degenerative changes (Fig 1D). Moreover, both rutin and NAC groups showed normal hepatocytes (Fig 1E and 1F).

Table 1 Serum biochemical parameters in all groups at 7, 14 and 21 days:

Time (Days)	Group	ALT U/L	AST U/L	ALP U/L	GGT U/L
7	Control	47.33 ± 4.33^{cd}	97.67 ± 8.88^{b}	574.00 ± 43^{c}	3.12 ± 0.48^{c}
	Isoniazid	85.33 ± 4.91^{a}	151.67 ± 7.80^{a}	891.00 ± 44.64^{a}	5.52 ± 0.47^{a}
	Isoniazid + Rutin	63.67 ± 3.48^{b}	121.7 ± 8.76^{b}	732.00 ± 32.35^{b}	4.32 ± 0.27^{b}
	Isoniazid + NAC	56.33 ± 4.26^{bc}	119.33 ± 8.45^{b}	713.00 ± 17.58^{b}	4.03 ± 0.47^{bc}
	Rutin	41.67 ± 4.41^{d}	99.33 ± 7.17^{b}	534.00 ± 40.26^{c}	2.95 ± 0.22^{c}
	NAC	44.33 ± 4.98^{cd}	103.70 ± 4.70^{b}	562.00 ± 27.97^{c}	3.03 ± 0.15^{c}
14	Control	50.67 ± 4.30^{cd}	102.30 ± 8.20^{d}	611.00 ± 4.00^{c}	3.56 ± 0.30^{cd}
	Isoniazid	96.67 ± 5.20^{a}	163.00 ± 8.40^{a}	1124.00 ± 68.00^{a}	6.11 ± 0.44^{a}
	Isoniazid + Rutin	68.67 ± 3.20^{b}	135.00 ± 8.10^{b}	869.00 ± 64.00^{b}	4.90 ± 0.40^{b}
	Isoniazid + NAC	63.70 ± 3.20^{bc}	131.00 ± 8.70^{bc}	830.00 ± 89.00^{b}	4.60 ± 0.32^{bc}
	Rutin	45.70 ± 4.90^{d}	105.30 ± 7.80^{d}	588.00 ± 47.00^{c}	3.14 ± 0.47^{d}
	NAC	48.00 ± 4.40^{d}	109.30 ± 4.70^{cd}	579.00 ± 26.00^{c}	3.22 ± 0.15^{d}
21	Control	54.67 ± 3.76^{c}	106.00 ± 8.08^{c}	632.00 ± 44.64^{bc}	3.82 ± 0.23^{bc}
	Isoniazid	121.33 ± 11.6^{a}	175.67 ± 7.31^{a}	1240.00 ± 58.64^{a}	6.70 ± 0.45^{a}
	Isoniazid + Rutin	75.33 ± 3.38^{b}	141.00 ± 7.81^{b}	786.00 ± 38.62^{b}	4.65 ± 0.37^{b}
	Isoniazid + NAC	69.00 ± 3.21^{bc}	139.33 ± 9.21^{b}	779.33 ± 93.85^{b}	4.31 ± 0.27^{b}
	Rutin	50.00 ± 4.04^{c}	99.33 ± 8.41^{c}	571.67 ± 33.00^{c}	3.17 ± 0.30^{c}
	NAC	52.00 ± 4.33^{c}	103.67 ± 6.44^{c}	563.00 ± 35.90^{c}	3.22 ± 0.24^{c}

Results are expressed as mean ±S.E. Different superscripts (a, b, c, d) at the same check point in the same column indicate significant differences at ($P < 0.05$).

Table 2 Serum biochemical parameters in all groups at 7, 14 and 21 days:

Time (Days)	Group	Total Bilirubin Mg/dl	Total protein g/dl	Albumin g/dl
7	Control	0.69 ± 0.03^{b}	7.71 ± 0.35^{a}	3.44 ± 0.08^{a}
	Isoniazid	0.94 ± 0.13^{a}	5.38 ± 0.09^{b}	2.67 ± 0.3^{b}
	Isoniazid + Rutin	0.84 ± 0.07^{ab}	6.18 ± 0.52^{b}	3.25 ± 0.13^{a}
	Isoniazid + NAC	0.80 ± 0.06^{ab}	6.52 ± 0.36^{ab}	3.26 ± 0.04^{a}
	Rutin	0.72 ± 0.04^{ab}	7.73 ± 0.50^{a}	3.52 ± 0.17^{a}
	NAC	0.74 ± 0.04^{ab}	7.78 ± 0.44^{a}	3.57 ± 0.21^{a}
14	Control	0.72 ± 0.03^{b}	7.59 ± 0.33^{a}	3.25 ± 0.24^{a}
	Isoniazid	1.08 ± 0.16^{a}	4.97 ± 0.12^{c}	2.33 ± 0.18^{b}
	Isoniazid + Rutin	0.87 ± 0.07^{ab}	5.85 ± 0.3^{bc}	3.04 ± 0.12^{a}
	Isoniazid + NAC	0.84 ± 0.06^{ab}	6.24 ± 0.42^{b}	3.05 ± 0.05^{a}
	Rutin	0.77 ± 0.03^{b}	7.70 ± 0.32^{a}	3.25 ± 0.12^{a}
	NAC	0.77 ± 0.03^{b}	7.67 ± 0.37^{a}	3.28 ± 0.12^{a}
21	Control	0.76 ± 0.04^{b}	7.61 ± 0.21^{a}	3.35 ± 0.21^{a}
	Isoniazid	1.17 ± 0.13^{a}	4.68 ± 0.19^{c}	2.26 ± 0.17^{b}
	Isoniazid + Rutin	0.82 ± 0.11^{b}	5.72 ± 0.27^{b}	3.47 ± 0.11^{a}
	Isoniazid + NAC	0.79 ± 0.13^{b}	5.95 ± 0.42^{b}	3.21 ± 0.2^{a}
	Rutin	0.76 ± 0.14^{b}	7.98 ± 0.19^{a}	3.32 ± 0.2^{a}
	NAC	0.77 ± 0.12^{b}	7.85 ± 0.27^{a}	3.46 ± 0.22^{a}

Results are expressed as mean ±S.E. Different superscripts (a, b, c, d) at the same check point in the same column indicate significant differences at ($P < 0.05$).

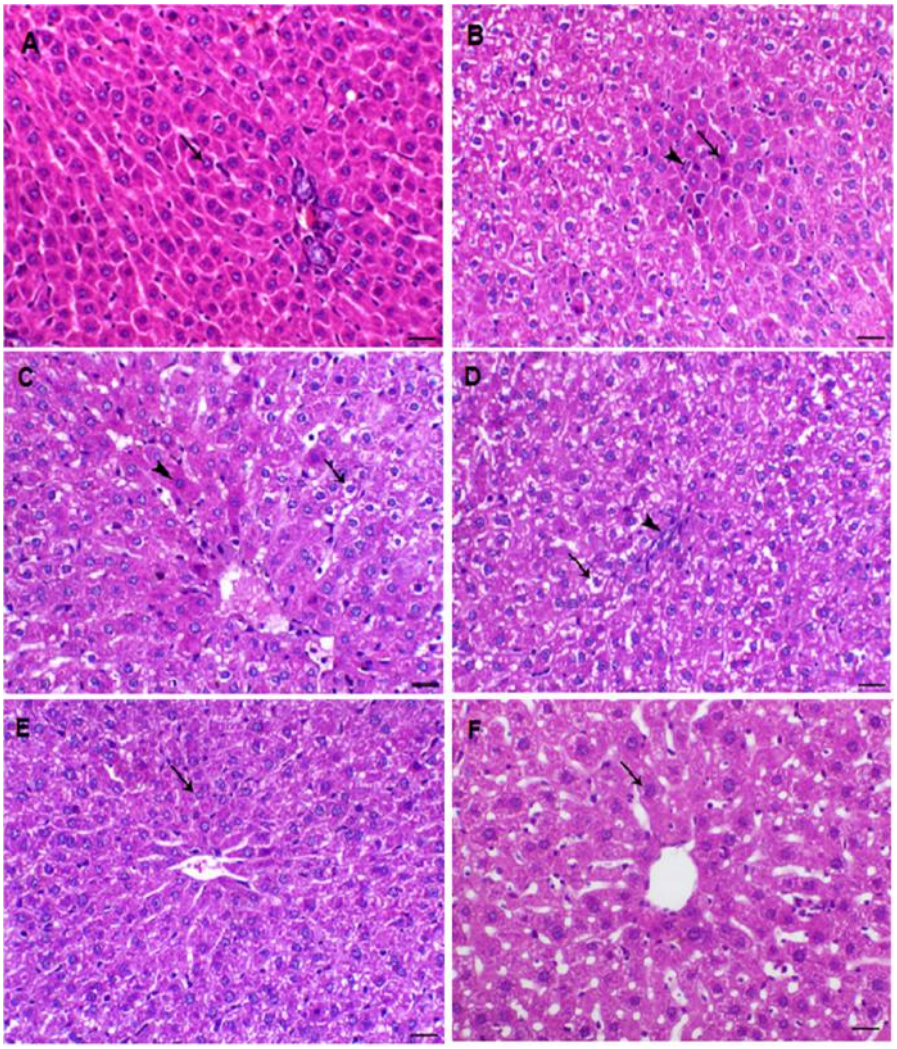

Fig 1 A: Liver of control group showing intact hepatic cells arranged in strands around central vein (arrow), H&E (X200). B: Liver of isoniazid group showing vacuolar and hydropic degeneration of hepatocytes extended from periportal area associated with focal necrotic area associated with inflammatory cells aggregation (arrowhead) (arrow indicates nuclear pyknosis and cytoplasmic eosinophilia), H&E (X200). C: Liver of isoniazid + rutin group showing marked decrease hepatic vacuolation (arrow) and mild degenerative changes represented by Centro-lobular cloudy swelling (arrowhead), H&E (X200). D: Liver of isoniazid + NAC group showing moderate degree of hepatic vacuolation (arrow), H&E (X200). E: Liver of rutin group showing normal hepatocytes (arrow), H&E (X200). F: Liver of NAC group showing normal hepatocytes (arrow), H&E (X200).

4. DISCUSSION

Drug-induced hepatotoxicity could be stimulated through variable methods such as immunological reaction; direct toxic effect or through active metabolite which is formed by a drug (Bayram et al., 2005). In the present study, compared to control group isoniazid group caused hepatocellular damage indicated by severe degree of hepatic vacuolar and hydropic degeneration with significant increases in ALT, AST, ALP, GGT and total bilirubin after 7, 14 and 21 days. These results agree with Abdel-Baset et al, (2015). The increased risk of hepatotoxicity with INH has been attributed to its metabolism which is quickly converted into its active metabolites which are related to the greater incidence of hepatic necrosis caused by INH (Hussain et al, 2003). Injury begins with alteration in the endoplasmic reticulum which leads to leakage of metabolic enzymes present in the intracellular structures (Jain et al, 2008).

Concerning to isoniazid injection with rutin administrated group, liver picture and functions improved compared with isoniazid group indicated by significant decreases in ALT, AST, ALP and GGT at 7, 14 and 21 days, significant decrease in total bilirubin at 21 days. These results were in accordance with Radwan et al, (2008). The hepatoprotective ability of rutin could be attributed to its antioxidant actions to prevent hepatic injury (Ziaee et al, 2009) so it preserves liver enzyme homeostasis by performing as a membrane-stabilizing mediator that prevent escape of enzymes because of its polyphenolic natural properties (Khan et al, 2012).

Concerning to isoniazid injection with NAC administration group improved liver functions and hepatic picture compared with isoniazid group indicated by significant decreases in ALT, AST, ALP and GGT at 7, 14 and 21 days, also there were significant decrease in total bilirubin at 21 days. These results agreed with Abdel-Reheim et al, (2017). NAC have immunomodulatory and anti-inflammatory actions that help in hepatic restoration (Lasram et al, 2014). NAC have ability to prevent hepatic damage by membrane stabilization through inhibiting the escape of liver enzymes through membranes (Kalia et al, 2007).

Concerning to serum protein, Isoniazid induced significant decrease in total protein and albumin at 7, 14 and 21 days compared with control group. Our results agree with Abd El-Raheem, et al, (2015). As the majority of plasma proteins and albumin are synthesized in the liver (Thapa

and Walia, 2007) so albumin represents a major synthetic protein and a marker for the ability of the liver to synthesize proteins. Low level indicates that the synthetic function of the liver has been markedly diminished (Green and Flamm, 2002).

The obtained data revealed that INH injection with rutin administration group exhibited significant increase in total protein after 21 days and significant increase in albumin after 7, 14 and 21 days compared with isoniazid group. These results were in agreement with Khan et al, (2010). Improvement in serum albumin concentration indicates the protecting effect of rutin on hepatic tissue maintaining albumin synthesis (Abdel-Ghaffar et al, 2017).

Isoniazid injection with NAC treated group exhibited significant increase in albumin at 7 days, significant increase in total protein and albumin after 14 and 21 days when compared with isoniazid group. These findings were in agreement with Maheswari et al, (2014). NAC has an antioxidant and hepatoprotective efficacy against the drug-induced liver injury (Saleem et al, 2018) also it has the ability to avert liver malfunction and stimulate renewal of the injured cells because it has free radical scavenging and anti-oxidant abilities (Lonare et al, 2016). Albumin is the greatest essential protein manufactured in the liver and its concentration is a beneficial guide of hepatic synthetic ability ***(Hoekstra et al, 2013).***

5. CONCULSION

In conclusion, N. acetyl cysteine and rutin have hepatoprotective effect as they preserved hepatic cells against isoniazid-induced hepatic damage with improvement of the liver functions through their anti-oxidant effects. Also, N. acetyl cysteine is superior to rutin in liver injury treatment as it improved liver functions more than rutin.

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