Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/10933
DOI URL: http://dx.doi.org/10.21474/IJAR01/10933

RESEARCH ARTICLE

HEPATOCYTE-DERIVED MICRORNAS AS BIOMARKERS OF HEPATIC INJURY IN ISONIAZID-INDUCED HEPATOTOXICITY

Khalid M.M Fararh, Mona A. El-Shemy and Ghada A.A Moussa
Clinical Pathology Department, Faculty of Veterinary Medicine, Benha University.

Manuscript Info

Manuscript History
Received: 08 March 2020
Final Accepted: 10 April 2020
Published: May 2020

Key words:-
MiRNA 122, MiRNA 125b, hepatotoxicity, MDA, GSH, GPx

Abstract

Study aimed to evaluate the diagnostic value of hepatocyte-derived microRNAs and their genes, MDA, GSH and GPX in experimental rat model of liver damage induced by isoniazid. In this study, 72 adult male rats (130-150 gm) were divided into six groups and given treatments for 21 days: control group:(1 ml saline /Kgb.wt/day I/P).INH group: 100 mgINH/kgb.wt/dayI/P. INH + Rutingroup: 100 mg INH/kg b.wt/day I/Pwith 200 mgrutin/kg b.wtorally. INH + NAC group: 100 mg INH /kg b.wt/day I/Pwith 300 mgNAC/kg/day b.wt orally. Rutingroup: 200 mgrutin/kg/day orally.NAC group: 300 mg NAC /kg b.wt orally. Tissue samples were obtained after 14 and 21 days foranti-oxidants as well as miRNAs 122 and 125b, CyclinG and STAT3 detection. Isoniazid injection into male rats induced a significant upregulation of CG1and STAT3 expression levelconcomitant with a downregulation of miRNA122 and miRNA125b.Meanwhile, ratstreated with rutin or NAC combined with INH developed a downregulation of CG1gene and STAT3 expression levelwithupregulation of miRNA122 and miRNA125b. INH induced a significant increase in MDA levelwith a significant decrease in GSH concentration and GPx activity. While INHinjection with rutin or NAC treatment induced asignificant decrease in MDA level with a significant increase in GSH concentration and GPx activity.Taken together these findings suggest that hepatocyte-derived microRNAs 122 and 125bcould beused as early sensitive biomarkers for hepatotoxicity inducedduring isoniazid treatment.

Introduction:-

MicroRNAs are small non-coding RNAs first described by (Lee, Feinbaum et al. 1993). They are approximately 20 – 27 nucleotides long(Engels and Hutvagner 2006).Hepatocyte derived miRNAs highly stable and sensitive blood based biomarkers for hepatocellular injury in animal models and in human patients with normal and high ALT activities (Laterza, Lim et al. 2009, Wang, Zhang et al. 2009). Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies(Yuan and Kaplowitz 2013). The drug-induced injury could be induced through different ways including direct toxic effect; immunological reaction or active metabolite that is formed by the drug (Bayram, Ozogul et al. 2005).Isoniazid is a widely used and effective first-line agent for treatment of tuberculosis; the most important clinical adverse reaction of INH is anti-tuberculosis drug-induced liver injury (ADLI) (Jaswal, Sinha et al.

Corresponding Author:- Ghada A.A Moussa
Address:- Clinical Pathology Department, Faculty of Veterinary Medicine, Benha University.

2013).Traditional plant medicines or herbal formulations might offer a natural key to hepatoprotective effect against xenobiotic/drug(Mukazayire, Minani et al. 2011). Flavonoids are natural polyphenols found 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 actions due to their anti-oxidative and free-radical scavenging properties (Kampkötter, Nkwonkam et al. 2007).It is a well-established that N-acetyl cysteine is a cytoprotective drug with high efficacy against drug induced hepatotoxicity (Cetinkaya, Bulbuloglu et al. 2006).

Therefore, we aimed in the present study to evaluate the diagnostic value of hepatocyte-derived microRNAs in detection of experimentally induced liver affection by INH in rats via evaluation of microRNAs, their genes, oxidants and anti-oxidants.

Materials and Methods:-

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

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 andadministered 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 300mg/kg/day orally for 21 days as previously described(Hemalatha, Reddy et al. 2013).

Experimental design:
72 adult male Wistar rats (130-150 gm) were divided into six groups each group12 rat:Control group:received 1ml sterile saline /Kg/b.wtI/P.INHgroup: received a single doseof 100 mg INH /kgb.wt/day I/Pfor 21 days.INH + Rutingroup: wasgiven a single doseof 100 mg INH /kgb.wt/day I/P for 21 days and were treated with 200 mgrutin/kg/day b.wtorally 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 /kgb.wt/day orally for 21 days.Rutingroup:was given 200 mgrutin/kgb.wt/day orally for 21 days.NAC group: was given 300 mg NAC /kg b.wt/day orally for 21 days.

Assay methods:
Tissue Samples:
Specimens from liver was collected from all groups after sacrificing at 14 and 21 days and preserved frozen for miRNAs, genes measurements, oxidants and anti-oxidantsdetermination.

Molecular investigation using real time PCR:
Cyclin G1 and STAT 3 genes:
RNA extraction from liver tissues
Pure RNA was extracted using total RNA Purification Kitaccording to the manufacturer protocol (Laudadio, Manfroid et al. 2012K0731).

cDNA synthesis:
Reverse transcription kits (Thermo Scientific, Fermentas, #EP0451)
This technique was done using Revert Aid H minus Reverse Transcriptase whichis a genetically modified M-MuLV RT, to convert RNA into complementary DNA (cDNA).

Real time PCR:
Real-time PCR with SYBR Green was used to measure expression of mRNAs of target genes in the liver tissues, with β- actin as an internal reference Table (1).The isolated cDNA wasamplified using 2X Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221)

MiRNA 122 and 125b:
MiRNA extraction:
mirVana PARIS kits (Ambion, Life Technologies, USA, #AM1556)
To isolate miRNA from liver tissues, samples are homogenized in Lysis Buffer. The lysate is then mixed with 25% ethanol. Mixture is passed through a glass-fiber filter, large RNAs are immobilized. The ethanol concentration of the filtrate is then increased to 55%, and it is passed through a second filter where the small RNAs become immobilized, washed a few times, and eluted.

cDNA synthesis
Quanti-Mir RT kit (SBI, System Biosciences, Cat. # RA420A-1)
This kit depends on the poly(A) method. In this method, a poly(A) tail is added to the 3' end of each mature miRNA done by poly (A) polymerase. Tailed miRNAs are then subjected to RT using a universal RT primer containing 2 to 3 degenerate nucleotides at 3' end followed by an oligo and universal reverse primer sequence. The synthesized cDNA is amplified with specific forward and universal reverse primers.

Real time PCR:
Real-time PCR with SYBR Green was used to measure expression of miRNA122 and miRNA125b in the liver tissues, with miRNA16 as an internal reference Table (2).The isolated cDNA were amplified using 2X Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221).

Oxidants and anti-oxidants parameters:
MDA, GSH and GPxwere measured according to manufacturer's instructions.

Statistical analysis:
Statistical analysis was performed using the statistical software package SPSS for windows Version 19. ANOVA-test was used to determine significant differences between experimental groups followed by Duncan. Results was expressed as the mean ± standard error of mean.

Results:-
The present study showed that miRNA122 as well as miRNA125b expression levels in liver tissues were significantly reduced inisoniazid group at 14 and 21 days as compared to the control group (Tables 3 and 4). This downregulation of miRNA122 as well as miRNA125b expression levels was significantly upregulated following co-administration of isoniazid with rutin or N-acetyl cysteine. However, rutin or N-acetyl cysteine administrated groups did not change miRNA122expression level in rat liver.

The obtained results revealed a significant upregulation of CG1and STAT 3genes expression level in liver tissues of isoniazid groupas compared to control group (Table 5 and 6). This elevated expression was significantly downregulated following co-administration of isoniazid with rutinorN-acetyl cysteine. Results showed significant downregulation in isoniazid group treated with NAC compared with isoniazid group treated with rutin. Moreover, non-significant difference was noticed among rutin and NAC administrated groups compared with control group.

Isoniazid group showed significant increase in MDA level while, there was significant decrease in GSH concentration and GPxactivity when compared with control group (Table 7).

In comparison to isoniazid group, Isoniazid injection withrutin or N-acetyl cysteinetreatment, there wasa significant decrease in MDA level concomitant with a significant increase in the GSH concentrationand GPx activitybut did not reach that of control.

It was noticed that rutin group showed significant decrease in MDA level while, there were significant increase in GSH concentration and GPxactivity compared with control, Meanwhile, N-acetyl cysteine group showed non-significant changes in MDAlevel, GSHconcentration and GPxactivity compared with control.

Discussion:-
MicroRNAs (miRNAs) are a class of small noncoding RNAs that regulate post-transcriptional gene expression (Bartel 2009). Regarding to molecular analysis, Isoniazidinjection exhibited significant downregulation in

miRNA122 and miR-125bwhile there wasupregulation in cyclin G1 and STAT 3 genes at 14 and 21 days when compared with control group. Our results agree with Li, (2018).Hypermethylationof CpG islands in the promoter region of miRNAgenes is one of most important mechanisms that inducemiRNA expression downregulation (Lynch, 2016). Since miR-122 is the most abundant miRNA in the liver and its expression in the liver wasdecreased in advanced liver diseases, such as primary biliary cirrhosis (PBC) and hepatocellular carcinoma (HCC). Mice treated with CCl4 for 6 weeks causes liver fibrosis that led to significant downregulation of miR-122 in mouse liver. This result was similar to that observed in human patients with primary biliary cirrhosis (Esau, 2006).Also, Expression level of miR-122 decreased significantly in activated HSCs, which contribute to the progression of liver fibrosis throughupregulating the expression of prolyl 4-hydroxylase and the subsequent production of overlycross-linked collagen. It should be noted that miR-122 is downregulated in the livers of patients with primary biliary cirrhosis (Padgett, 2009) as well as in the livers of mice treated with CCl4 (Marquez, 2010). Moreover, cell cycle protein G1(Cyclin G1) and cationic amino acid transporter-1 (CAT-1) which are miR-122 targets gene also increased at the mRNA and protein levels,which suggests that downregulation of miR-122 due to the upregulation of Cyclin G1 and CAT-1 and might play a role in INH induced hepatic injury(Li, 2018). On the other hand, MiR-125bis one of miRNAs identified to be associated with inflammation (Li, 2018).Also, STAT3 the common target gene of miR-125b(Carraro, 2009). STAT3 is an important marker in the differentiation of T-helper cells and regulates the transcription of inflammatory genes (Harris, 2007).T-helper cells mainlysecrete pro-inflammatory cytokine IL-17 (McGeachy, 2008) which stimulate the activation of various pro-inflammatory cytokines and chemokines and participate in drug-induced liver injury (Wang, 2014). TNF-α and IL-6 are key pro-inflammatorycytokines that initiate the inflammatory response and induce massive hepatocyte apoptosis. Also, liver can activate Kupffer cells to release inflammatory cytokines (IL-6 and TNF- α) when stimulated by toxic substances (Yano, 2012)so STAT3 can be activatedin hepatocytes following TNF-α and IL-6 stimulation then STAT3 activation plays an important role in cell survival, differentiation, transformation, apoptosis and inflammation (Bromberg, 2000).

Isoniazid injection with rutin treatment showed significant upregulation in MiRNA 122 and MiRNA 125b but showed significant downregulation in cyclin G1 and STAT 3 genes at 14 and 21 days compared with isoniazid group. Cyclin G1 downregulation may be due to anti-inflammatory effect of rutin by downregulating IL-6 and TGF-β1, which promotes extracellular matrix deposition and fibrosis (Edranov, 2012). Also, rutin produced anti-inflammatory effects by inhibiting pro-inflammatory cytokines in adjuvant-induced arthritis in rats (Kauss, 2008).MiRNA 122 upregulation may be due to downregulation of TGF-β1 and Cyclin G1.Moreover, Rutin inhibits inflammatory responses in ultraviolet-irradiated mouse by preventing the phosphorylated STAT3 levels (Choi, 2014).This indicates that rutin play an important role in upregulation of MiRNA 125b and inprotection against isoniazid induced hepatotoxicity.

Isoniazid injection withN-acetyl cysteine treatmentshowed significant upregulation in MiRNA 122 and MiRNA 125b with significant downregulation in cyclin G1 and STAT 3 genes at 14 and 21 days when compared with isoniazid group. Studies have shown that activation of thearyl hydrocarbon receptors increases expression of CD36, a scavenger receptor involved in fatty acid uptake, andtumor necrosis factor alpha (TNFα), a mediator of inflammation (Lee, 2010, Vondracek, 2011). Both genes have been implicated in liver steatosis (Greco, 2008).Since,NAC possible targets genes areCD36 and TNFα, so it has beenreduced both liver CD36 and TNFα expression in mouse non-alcoholic steatohepatitis (NASH)(Baumgardner, 2008) andinhibition of TNF is regarded as a therapy to block fatty liver and relieve liver injury (Feagins, 2015).Moreover,NAC is an effective hepatic antioxidant that inhibited non-alcoholic steatohepatitis induced lipid peroxidation, increased hepatic GSH, blocked non-alcoholic steatohepatitis autoimmune responses, inhibited production of TNF**α**, and attenuated inflammation leading to reduction in cellular damage, hepatocyte injury, and fibrosis(Baumgardner, 2008).

Concerning to oxidative stress, INH induced significant increase in MDA level withsignificant decrease in GSHconcentrationand GPxactivity when compared with control, our results agree withErgul, Erkan et al. 2010.INH is metabolized by acetylation induced by the hepatic enzyme N-acetyl transferase to acetyl isoniazid, which is hydrolyzed into acetyl hydrazine and isonicotinic acidwhich are toxic metabolites that generate free radicals (Tostmann, Boeree et al. 2008).

INH injection withrutin treatment induced a significant decrease in MDA and significant increase in GSH and GPx when compared with INH group. Our results agree with Vadapalli, Muvvala et al. 2017.Also rutin administrated group showed significant decrease in MDA level with significant increase in GSH concentration and GPxactivity

when compared with control group, Results agree with Khan, (2017). Rutin has antioxidant effects protect cell membrane from lipid oxidation (López-Revuelta, 2006) due to scavenging of free radicals whichinduces the lipid peroxidation chain process (Nafees, Rashid et al. 2015).

Isoniazid combined with NAC caused significant decrease in MDA level and significant increase in GSH concentration and GPxactivity compared with INH. Our results agree with Eşrefoğlu, (2006). NAC has been tried in the treatment of various liver injuries because of its known antioxidant properties (Baniasadi, Eftekhari et al. 2010). NAC exerts its antioxidant action by facilitating glutathione biosynthesis and scavenging the ROS formed during oxidative stress (Ocal, Avlan et al. 2004).

Conclusion:-

Isoniazid induced downregulation expression of microRNAs (122 and 125b) and upregulation of their genes (Cyclin G and STAT3). Therefore, hepatic microRNAs (122 and 125b) aresensitive biomarkers in INH hepatic injury. Moreover, N.acetylcysteine and rutin has hepatoprotective effect due to their anti-oxidant effects which alleviate isoniazid hepatic injury.Also, N.acetylcysteineis more potent than rutin in liver injury treatment.

Table 1:- Forward and reverse primers sequence for primers used in qPCR.

Gene	Forward primer (′5 ------ ′3)	Reverse primer (′5 ------ ′3)
Cyclin G1	CTGCACGACAACTGAAGCAC	CTGCGGTACACAGTGAATGC
STAT3	TCTGTGTGACACCAACGACC	AGGCGGACAGAACATAGGTG
B actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

Table 2:- Forward and reverse primers sequence for real time PCR.

Gene	Primer sequence (′5 ------ ′3)
miRNA122	TGGAGTGTGACAATGGTGTTTG
miRNA125b	TCCCTGAGACCCTAACTTGTGA
miRNA16	CGGTAGCAGCACGTAAATATTGGCGA

Table 3:- Changes in relative expression of miRNA122 in liver tissues fromall groups at 14 and 21 days:

Fold change / **Group**	**14 days**	**21 days**
Control	1.00 ± 0.06^{a}	1.00 ± 0.06^{a}
Isoniazid	0.23 ± 0.02^{d}	0.35 ± 0.02^{d}
Isoniazid + Rutin	0.46 ± 0.03^{c}	0.62 ± 0.02^{c}
Isoniazid + NAC	0.60 ± 0.04^{b}	0.77 ± 0.03^{b}
Rutin	0.99 ± 0.05^{a}	1.09 ± 0.06^{a}
NAC	1.04 ± 0.06^{a}	1.05 ± 0.07^{a}

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

Table 4:- Changes in relative expression of miRNA125b in liver tissues fromall groups at 14 and 21 days.

Fold change / **Group**	**14 days**	**21 days**
Control	1.00 ± 0.05^{a}	1.00 ± 0.07^{a}
Isoniazid	0.26 ± 0.02^{c}	0.15 ± 0.02^{c}
Isoniazid + Rutin	0.49 ± 0.03^{b}	0.44 ± 0.04^{b}
Isoniazid + NAC	0.53 ± 0.03^{b}	0.48 ± 0.03^{b}

Rutin	1.02 ± 0.04^{a}	1.03 ± 0.07^{a}
NAC	1.01 ± 0.04^{a}	1.04 ± 0.06^{a}

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

Table 5:- Changes in relative expression of CG1gene in liver tissues from all groupsat 14 and 21 days.

Fold change Groups	**14 days**	**21 days**
Control	1.00 ± 0.06^{c}	1.00 ± 0.08^{d}
Isoniazid	2.66 ± 0.1^{a}	4.59 ± 0.24^{a}
Isoniazid + Rutin	1.89 ± 0.09^{b}	3.07 ± 0.13^{b}
Isoniazid + NAC	1.93 ± 0.11^{b}	2.07 ± 0.11^{c}
Rutin	1.13 ± 0.07^{c}	1.13 ± 0.11^{d}
NAC	0.98 ± 0.08^{c}	1.15 ± 0.11^{d}

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

Table 6:- Changes in relative expression of STAT 3gene in liver tissues from all groupsat 14 and 21 days.

Fold change Groups	**14 days**	**21 days**
Control	1.00 ± 0.09^{c}	1.00 ± 0.07^{c}
Isoniazid	4.89 ± 0.32^{a}	3.05 ± 0.13^{a}
Isoniazid + Rutin	2.73 ± 0.14^{b}	2.16 ± 0.1^{b}
Isoniazid + NAC	2.14 ± 0.15^{b}	2.07 ± 0.09^{b}
Rutin	0.99 ± 0.08^{c}	1.01 ± 0.07^{c}
NAC	1.09 ± 0.11^{c}	1.06 ± 0.08^{c}

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

Table 7:- MDA level, GSH concentration and GPx activity at 14 days:

Groups	**MDA (nmol/mg)**	**GSH (µg/ml)**	**GPx (u/ml)**
Control	0.17 ± 0.01^{c}	0.30 ± 0.01^{b}	0.30 ± 0.01^{b}
Isoniazid	0.37 ± 0.03^{a}	0.10 ± 0.004^{d}	0.11 ± 0.01^{e}
Isoniazid + Rutin	0.26 ± 0.01^{b}	0.24 ± 0.01^{c}	0.24 ± 0.01^{d}
Isoniazid + NAC	0.18 ± 0.02^{c}	0.27 ± 0.01^{c}	0.28 ± 0.01^{c}
Rutin	0.11 ± 0.01^{d}	0.39 ± 0.02^{a}	0.40 ± 0.01^{a}
NAC	0.14 ± 0.01^{cd}	0.32 ± 0.01^{b}	0.32 ± 0.01^{b}

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

References:-

1. Abdel-Raheem, I. T. (2010). "Gastroprotective Effect Of Rutin Against Indomethacin-Induced Ulcers In Rats." Basic & Clinical Pharmacology & Toxicology 107(3): 742-750.

2. Baniasadi, S., P. Eftekhari, P. Tabarsi, F. Fahimi, M. R. Raoufy, M. R. Masjedi And A. A. Velayati (2010). "Protective Effect Of N-Acetylcysteine On Antituberculosis Drug-Induced Hepatotoxicity." European Journal Of Gastroenterology & Hepatology22(10): 1235-1238.
3. Bartel, D. P. (2009). Micrornas: Target Recognition And Regulatory Functions. Cell, 136(2), 215-233.
4. Baumgardner, J. N., K. Shankar, L. Hennings, E. Albano, T. M. Badger And M. J. Ronis (2008). "N-Acetylcysteine Attenuates Progression Of Liver Pathology In A Rat Model Of Nonalcoholic Steatohepatitis." The Journal Of Nutrition138(10): 1872-1879.
5. Bayram, M., C. Ozogul, A. Dursun, Z. S. Ercan, I. Isik And E. Dilekoz (2005). "Light And Electron Microscope Examination Of The Effects Of Methotrexate On The Endosalpinx." European Journal Of Obstetrics And Gynecology And Reproductive Biology120(1): 96-103.
6. Bromberg, J. And J. E. Darnell (2000). "The Role Of Stats In Transcriptional Control And Their Impact On Cellular Function." Oncogene19(21): 2468.
7. Carraro, G., Et Al. (2009). "Mir-17 Family Of Micrornas Controls Fgf10-Mediated Embryonic Lung Epithelial Branching Morphogenesis Through Mapk14 And Stat3 Regulation Of E-Cadherin Distribution." Developmental Biology333(2): 238-250.
8. Cetinkaya, A., E. Bulbuloglu, E. B. Kurutas And B. Kantarceken (2006). "N-Acetylcysteine Ameliorates Methotrexate-Induced Oxidative Liver Damage In Rats." Medical Science Monitor12(8): Br274-Br278.
9. Choi, K.-S., J. K. Kundu, K.-S. Chun, H.-K. Na And Y.-J. Surh (2014). "Rutin Inhibits Uvb Radiation-Induced Expression Of Cox-2 And Inos In Hairless Mouse Skin: P38 Map Kinase And Jnk As Potential Targets." Archives Of Biochemistry And Biophysics559: 38-45.
10. Edranov, S. And P. Motavkin (2012). "Apoptosis As A Mechanism Of Maxillary Sinus Mucosa Injury In Rats After Experimental Transection Of The Maxillary Nerve." Bulletin Of Experimental Biology & Medicine153(4.(
11. Engels, B. M. And G. Hutvagner (2006). "Principles And Effects Of Microrna-Mediated Post-Transcriptional Gene Regulation." Oncogene25(46): 6163.
12. Ergul, Y., T. Erkan, H. Uzun, H. Genc, T. Altug And E. Erginoz (2010). "Effect Of Vitamin C On Oxidative Liver Injury Due To Isoniazid In Rats." Pediatr Int52(1): 69-74.
13. Esau, C., S. Davis, S. F. Murray, X. X. Yu, S. K. Pandey, M. Pear, L. Watts, S. L. Booten, M. Graham And R. Mckay (2006). "Mir-122 Regulation Of Lipid Metabolism Revealed By In Vivo Antisense Targeting." Cell Metabolism3(2): 87-98.
14. Eşrefoğlu, M., Et Al. (2006). "Ultrastructural Clues For The Protective Effect Of Ascorbic Acid And N-Acetylcysteine Against Oxidative Damage On Caerulein-Induced Pancreatitis." Pancreatology 6(5): 477-485.
15. Feagins, L. A., Et Al. (2015). "Nonalcoholic Fatty Liver Disease: A Potential Consequence Of Tumor Necrosis Factor-Inhibitor Therapy." European Journal Of Gastroenterology & Hepatology27(10): 1154.
16. Greco, D., A. Kotronen, J. Westerbacka, O. Puig, P. Arkkila, T. Kiviluoto, S. Laitinen, M. Kolak, R. M. Fisher And A. Hamsten (2008). "Gene Expression In Human Nafld." American Journal Of Physiology-Gastrointestinal And Liver Physiology294(5): G1281-G1287.
17. Harris, T. J., J. F. Grosso, H.-R. Yen, H. Xin, M. Kortylewski, E. Albesiano, E. L. Hipkiss, D. Getnet, M. V. Goldberg And C. H. Maris (2007). "Cutting Edge: An In Vivo Requirement For Stat3 Signaling In Th17 Development And Th17-Dependent Autoimmunity." The Journal Of Immunology179(7): 4313-4317.
18. Hemalatha, P., A. G. Reddy, Y. R. Reddy And P. Shivakumar (2013). "Evaluation Of Protective Effect Of N-Acetyl Cysteine On Arsenic-Induced Hepatotoxicity." Journal Of Natural Science, Biology, And Medicine4(2): 393.
19. Jaswal, A., N. Sinha, M. Bhadauria, S. Shrivastava And S. Shukla (2013). "Therapeutic Potential Of Thymoquinone Against Anti-Tuberculosis Drugs Induced Liver Damage." Environmental Toxicology And Pharmacology36(3): 779-786.
20. Kampkötter, A., C. G. Nkwonkam, R. F. Zurawski, C. Timpel, Y. Chovolou, W. Wätjen And R. Kahl (2007). "Investigations Of Protective Effects Of The Flavonoids Quercetin And Rutin On Stress Resistance In The Model Organism Caenorhabditis Elegans." Toxicology234(1-2): 113-123.
21. Kauss, T., Et Al. (2008). "Rutoside Decreases Human Macrophage-Derived Inflammatory Mediators And Improves Clinical Signs In Adjuvant-Induced Arthritis." Arthritis Research & Therapy 10(1): R19.
22. Khan, R. A., Et Al. (2017). "Effects Of Rutin On Testicular Antioxidant Enzymes And Lipid Peroxidation In Rats." Indian Journal Of Pharmaceutical Education And Research 51: 412-417.
23. Kumar, S. And A. K. Pandey (2013). "Chemistry And Biological Activities Of Flavonoids: An Overview." The Scientific World Journal2013.

24. Laterza, O. F., L. Lim, P. W. Garrett-Engele, K. Vlasakova, N. Muniappa, W. K. Tanaka, J. M. Johnson, J. F. Sina, T. L. Fare And F. D. Sistare (2009). "Plasma Micrornas As Sensitive And Specific Biomarkers Of Tissue Injury." Clinical Chemistry55(11): 1977-1983.
25. Laudadio, I., I. Manfroid, Y. Achouri, D. Schmidt, M. D. Wilson, S. Cordi, L. Thorrez, L. Knoops, P. Jacquemin And F. Schuit (2012). "A Feedback Loop Between The Liver-Enriched Transcription Factor Network And Mir-122 Controls Hepatocyte Differentiation." Gastroenterology142(1): 119-129.
26. Lee, J. H., T. Wada, M. Febbraio, J. He, T. Matsubara, M. J. Lee, F. J. Gonzalez And W. Xie (2010). "A Novel Role For The Dioxin Receptor In Fatty Acid Metabolism And Hepatic Steatosis." Gastroenterology139(2): 653-663.
27. Lee, R. C., R. L. Feinbaum And V. Ambros (1993). "The C. Elegans Heterochronic Gene Lin-4 Encodes Small Rnas With Antisense Complementarity To Lin-14." Cell75(5): 843-854.
28. Li, Y., Et Al. (2018). "Involvement Of Methylation Of Microrna-122, 125b And-106b In Regulation Of Cyclin G1, Cat-1 And Stat3 Target Genes In Isoniazid-Induced Liver Injury." Bmc Pharmacology And Toxicology 19(1): 11.
29. López-Revuelta, A., Et Al. (2006). "Membrane Cholesterol Contents Influence The Protective Effects Of Quercetin And Rutin In Erythrocytes Damaged By Oxidative Stress." Chemico-Biological Interactions 161(1): 79-91.
30. Lynch, S. M., K. M. O'neill, M. M. Mckenna, C. P. Walsh And D. J. Mckenna (2016). "Regulation Of Mir-200c And Mir-141 By Methylation In Prostate Cancer." The Prostate76(13): 1146-1159.
31. Marquez, R. T., Et Al. (2010). "Correlation Between Microrna Expression Levels And Clinical Parameters Associated With Chronic Hepatitis C Viral Infection In Humans." Laboratory Investigation 90(12): 1727.
32. Mcgeachy, M. J. And D. J. Cua (2008). "Th17 Cell Differentiation: The Long And Winding Road." Immunity28(4): 445-453.
33. Mukazayire, M.-J., V. Minani, C. K. Ruffo, E. Bizuru, C. Stévigny And P. Duez (2011). "Traditional Phytotherapy Remedies Used In Southern Rwanda For The Treatment Of Liver Diseases." Journal Of Ethnopharmacology138(2): 415-431.
34. Nafees, S., S. Rashid, N. Ali, S. K. Hasan And S. Sultana (2015). "Rutin Ameliorates Cyclophosphamide Induced Oxidative Stressand Inflammation In Wistar Rats: Role Of Nfκb/Mapk Pathway." Chemico-Biological Interactions231: 98-107.
35. Ocal, K., D. Avlan, I. Cinel, A. Unlu, C. Ozturk, F. Yaylak, M. Dirlik, H. Camdeviren And S. Aydin (2004). "The Effect Of N-Acetylcysteine On Oxidative Stress In Intestine And Bacterial Translocation After Thermal Injury." Burns30(8): 778-784.
36. Padgett, K. A., R. Y. Lan, P. C. Leung, A. Lleo, K. Dawson, J. Pfeiff, T. K. Mao, R. L. Coppel, A. A. Ansari And M. E. Gershwin (2009). "Primary Biliary Cirrhosis Is Associated With Altered Hepatic Microrna Expression." Journal Of Autoimmunity32(3-4): 246-253.
37. Tostmann, A., Et Al. (2008). "Antituberculosis Drug-Induced Hepatotoxicity: Concise Up-To-Date Review." Journal Of Gastroenterology And Hepatology 23(2): 192-202.
38. Vadapalli, U., S. Muvvala, R. Alluri And B. Lakshmi (2017). "Antiproliferative Activity Of Rutin On Hela Cell Line Induced Cervical Cancer In Rats." International Journal Of Pharmaceutical Sciences And Research8(11): 4803-4811.
39. Varkey, J. A. And A. A. Vahab (2016). "Evaluation Of Hepatoprotective Activity Of The Commerson's Anchovy (Stolephorus Commersonnii)." Indian Journal Of Pharmacology48(1): 69.
40. Vondracek, J., L. Umannova And M. Machala (2011). "Interactions Of The Aryl Hydrocarbonreceptor With Inflammatory Mediators: Beyond Cyp1a Regulation." Current Drug Metabolism12(2): 89-103.
41. Wang, K., S. Zhang, B. Marzolf, P. Troisch, A. Brightman, Z. Hu, L. E. Hood And D. J. Galas (2009). "Circulating Micrornas, Potential Biomarkers For Drug-Induced Liver Injury." Proceedings Of The National Academy Of Sciences106(11): 4402-4407..
42. Wang, X., Z. Jiang, M. Xing, J. Fu, Y. Su, L. Sun And L. Zhang (2014). "Interleukin-17 Mediates Triptolide-Induced Liver Injury In Mice." Food And Chemical Toxicology71: 33-41.
43. Yano, A., S. Higuchi, K. Tsuneyama, T. Fukami, M. Nakajima And T. Yokoi (2012). "Involvement Of Immune-Related Factors In Diclofenac-Induced Acute Liver Injury In Mice." Toxicology293(1-3): 107-114.
44. Yuan, L. And N. Kaplowitz (2013). "Mechanisms Of Drug-Induced Liver Injury." Clinics In Liver Disease17(4): 507-518.