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RESEARCH ARTICLE

HONEY ENHANCES THE HEPATIC MULTI-FUNCTIONAL DETOXIFICATION PROCESS.

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Abstract

Reactive oxygen species-induced cell damage is involved in numerous diseases, including cancer. Reducing oxidative stress is the strategy of chemoprevention via antioxidant-dependent induction of detoxifying enzymes. Honey contains bioactive constituents such as organic acids, trace elements, vitamins, amino acids, proteins and a wide range of polyphenolic phytochemicals. In this study the effect of oral honey solution administration is examined on the activation of the hepatic drug metabolizing enzymes. Oral honey was administered in doses of 2.5 and 5 g/Kg body weight to adult male albino rats for 30 days. Results indicated that the lower dose of honey is more effective, that it increases significantly the activity of all of the three phases. Phase I represented in cytochromeP450 2E1, phase II metabolizing enzymes as epoxide hydrolase-1, glutathione-S-transferase and UDP-glucuronosyltransferase-1 and phase III transporters as nuclear factor erythroid derived 2 like protein.

Introduction:-

Free radicals are atoms or molecules that have unpaired electrons, usually unstable and highly reactive. In biology system, oxygen based radicals and nitrogen based radicals are two types of free radicals (Finkel and Holbrook, 2000).

Oxygen free radicalsare generated during the metabolism process of oxygen such as superoxide, hydroxyl radicals, and peroxyl radicals, moreover with the addition of non-radicals, other species are formed as hydrogen peroxide, hypochlorous acid and ozone, are known as reactive oxygen species (ROS) (Erejuwa et al., 2012a).

Reactivenitrogenspecies(RNS),includingnitrogenbasedradicalsandnon-radicals,suchasnitrogen dioxide, nitric oxide radicals and peroxynitrite, are derived from nitric oxide and superoxide via inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, respectively (Apel and Hirt, 2004).

ROS/RNS can initiate lipid peroxidation, cause DNA strand breaks, and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury due to their special chemical characteristics. Under physiological conditions,ROS/RNS are not considered asa threat to the body because it is able to remove these reactive species to a certain degree. Oxidative/nitrosative stress represents the bodies' imbalance in the production and the elimination of ROS and RNS as well as decreased production of antioxidants (Li et al., 2015).

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Liver is a major organ attacked by ROS. Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The mitochondrion, microsomes and peroxisomes in parenchymal cells can produce ROS, regulating on PPARα, which is mainly related to the liver fatty acid oxidation gene expression. Moreover, Kupffer cells, hepatic stellate cells and endothelial cells are potentially more exposed or sensitive to oxidative stress-related molecules (Sanchez-Valle et al., 2012).

A variety of cytokines like TNF-α can be produced in Kupffer cells induced by oxidative stress, which might increase inflammation and apoptosis. With regard to hepatic stellate cells, the proliferation and collagen synthesis of hepatic stellate cells is triggered by lipid peroxidation caused by oxidative stress (Cichoz-Lach and Michalak, 2014). When the ROS is excessive, the homeostasis will be disturbed, resulting in oxidative stress, which is not only triggers hepatic damage by inducing irretrievable alteration of lipids, proteins and DNA contents but also bymodulating pathwaysthatcontrolnormalbiologicalfunctions. Sincethesepathwaysregulategenestranscription, protein expression, cell apoptosis, and hepatic stellate cell activation. Moreover the oxidative stress is regarded as oneofthepathologicalmechanismsthatresultsininitiationandprogressionofvariousliverdiseases,suchaschronicviralhepatitis,alcoholicliverdiseasesandnon-alcoholicsteatohepatitis(Singal et al., 2011).

Honey is a natural product formed from nectar by honeybees. Its composition is rather depending on the floral source and other external factors, such as seasonal and environmental conditions and processing (Alvarez-Suarez et al., 2013). It consists of primarily sugars such as monosaccharides, disaccharides, oligosaccharides and polysaccharides. It contains enzymes such as glucose oxidase, diastase, invertase, catalase and peroxidase (Bogdanov et al., 2008). Honey also contains other bioactive constituents such as organic acids, trace elements, vitamins, amino acids, proteins (Erejuwaet al., 2012a) as well as a wide range of polyphenolic phytochemicals (Alvarez-Suarez et al., 2013).

Honey can exert several health-beneficial effects such as gastroprotective(Gharzouli et al., 2002),hepatoprotective(Al-Waili et al., 2006), reproductive (Zaid et al., 2011 and Mohamed et al., 2012), hypoglycemic and antioxidant (Erejuwa et al., 2010), antihypertensive (Al-Waili, 2003), antibacterial (Tan et al., 2009), anti-fungal (Koc et al., 2011) and anti-inflammatory (Kassim et al., 2010) effects.

The main aim of this study was to investigate the potential of honey as an improvement factor on the hepaticdetoxification via its three phases.

Materials and methods:-

Materials:-

Honey was purchased from Al-Safa for bees breeding and honey production, El-Menia, Egypt. Filled up to Egyptian Standard Specifications number 355/2005,4,5(2/1,3/1)

Animals and treatment:-

Thirty six adult male albino rats "Sprague Dawely" 186-210 g were kept in stainless steel cages in the well-ventilated animal house of the Medical Research Center, Faculty of Medicine, Ain Shams University. The rats had been kept in the room for 1 week prior to the beginning of the experiment for acclimatization. They had access to 12h cycle of light/dark and provided with standard diet prepared by AIN (1993) and tap water *ad libitum.*

The animals were divided into the following three groups: Group 1, control was supplemented with plain water; group 2,was supplemented with honey 2.5g/Kg body weight and group 3, was supplemented with honey 5g/Kg body weight. Both honey and water were given orally using oral gavage for 30 days (Yao et al., 2011).

Sample collection:-

At the end of the experimental period, animals were sacrificed under ether anesthesia. Livers were excised, washed, dried and stored at -20^{o}C till analysis is done.

Hepatic biochemical assays:-

Phase I enzyme activity assay:-

The hepatic activity of cytochrome P450 2E1 (CYP2E1) was extracted by homogenizing the tissues in PBS on ice. The resulting suspensions were subjected to two freeze-thaw cycles to further break the cell membrane. After that; the homogenates were centrifuged for 5 min at 5000 x g for extracting the supernatants which were used in the

determination using the enzyme-linked immunosorbent assay kit (Cloud-Clone Corp., USA)according to themethod of Tonkiri et al. (2014).

Phase II enzyme activities assay:-
The hepatic activity of uridine-5\-diphosphate glucuronosyltransferase-1 (UGT) was extracted from liver tissue by homogenizing the tissues in PBS on ice. The resulting suspensions were subjected to two freeze-thaw cycles to further break the cell membrane. Then; the homogenates were centrifuged for 5 min at 5000 x g for extracting the supernatants which were used in the determination of the enzyme activity using the enzyme-linked immunosorbent assay kit (Cloud-Clone Corp., USA)according to themethod of Miyagi and Collier (2011).

The hepatic epoxide hydrolase-1 (EPH) was extracted from liver tissue by homogenization in PBS and stored overnight at -20^{o}C. After two freeze-thaw cycles were performed to break the cell membrane. The homogenates were centrifuged for 5 min at 5000 x g, 2-8^{o}C. The supernatants were used in the determination of the enzyme activity usingthe sandwich enzyme immunoassay technique byCusabio Kit, USA according to the method of Gill et al. (1982).

The hepatic glutathione-S-transferase (GST) was extracted from tissues by homogenizationin PBS pH 6.5.Then the suspensions were centrifuged at 10000g for 30 min at 4°C. The activity of the enzyme was assayed in the supernatant calorimetrically using Sigma Kits, USA according to the method of Pour et al. (2014).

Phase III enzyme activity assay:-
The hepatic activity of nuclear factor erythroid derived 2 like protein (Nrf2) was extracted from liver tissue by homogenization in PBS on ice. The resulting suspensions were subjected to two freeze-thaw cycles to further break the cell membrane. Then; the homogenates were centrifuged for 5 min at 5000 x g for extracting the supernatants which were used in the determination of the enzyme activity using the enzyme-linked immunosorbent assay kit (Cloud-Clone Corp., USA)according to themethod of Ebihara et al. (2016).

Statistical analysis:-
Results were expressed as mean ± Standard deviation (S.D.) of the mean. Differences among means were tested for statistical significance by one-way analysis of variance using SPSS package version 16. Statistical significance was considered when $P < 0.05$

Results:-

The activity of hepatic CYP2E1activity represented in figure (1) showed that oral administration of 2.5g honey/Kg body weight honey solution resulted in a significant increase ($P<0.05$) (49.07± 1.88) in enzyme activity compared with the control group that record (43.92±2.00) of enzyme activity. While the group that administered 5g honey/Kg body weight honey solution showed a non-significant decrease (42.58±3.10) at $P<0.05$ compared to control.

Oral administration of honey solution at dose 2.5g honey/Kg body weight also showed a significant increase in the activity of phase II hepatic EPH by 106.02% compared with control group as listed in table (1), while dose of 5g honey/Kg body weight showed a non-significant decrease ($P<0.05$) in activity of enzyme as showed in figure (2) compared with control.

The activity of the two transferases enzymes of phase II detoxification(UGT and GST) are increased significantly ($P<0.05$) by the administration of each of the honey doses comparing with the control group as showed in table (1) and figures (3 and 4).Moreover; administration of the 5 g honey/Kg body weight exerted more potent increase in the activity as showed by its higher % of change from control than the other dose (table 1).

Figure (5) showed the activity of hepatic Nrf2, that the oral dose of 2.5g honey/Kg body weight is more effective than the dose of 5g honey/Kg body weight, since it increased the activity significantly ($P<0.05$) to (6.59±.0.53) when compared with control (5.15±0.43), while the dose of 5g honey/Kg body weight showed non-significant increase (5.20±0.44) compared to control group table (1).

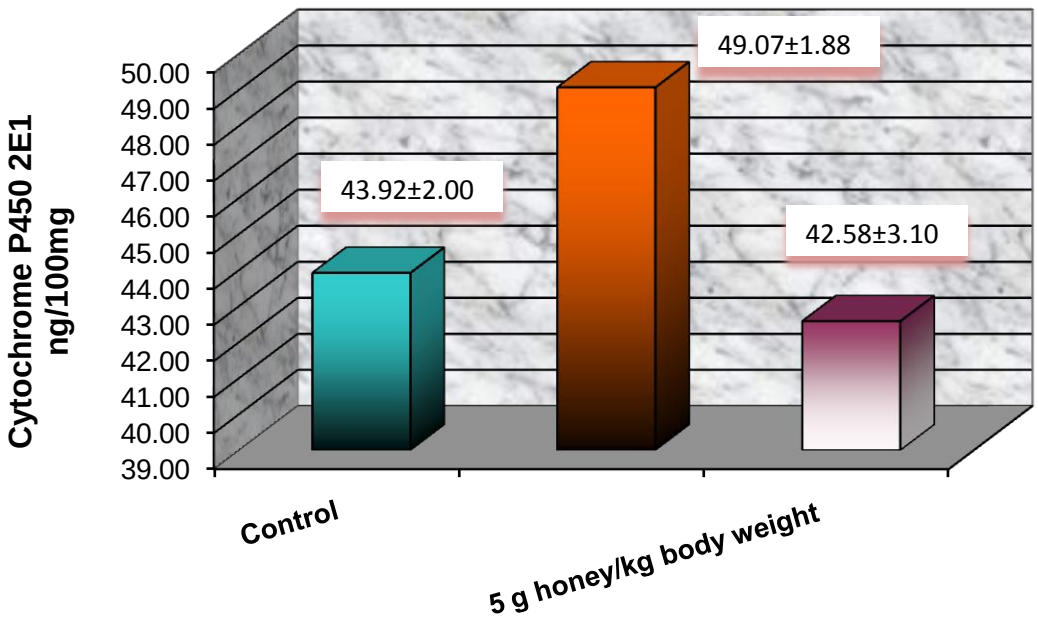

Figure 1:- Effect of oral honey solutions administration on hepatic CYP2E1 activity.

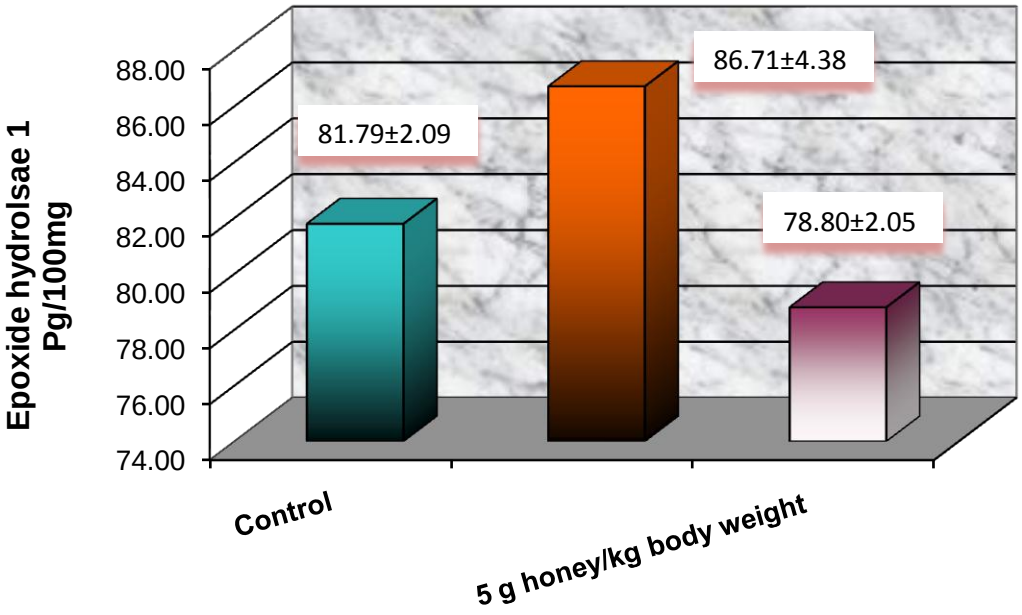

Figure 2:- Effect of oral honey solutions administration on hepatic EPH activity.

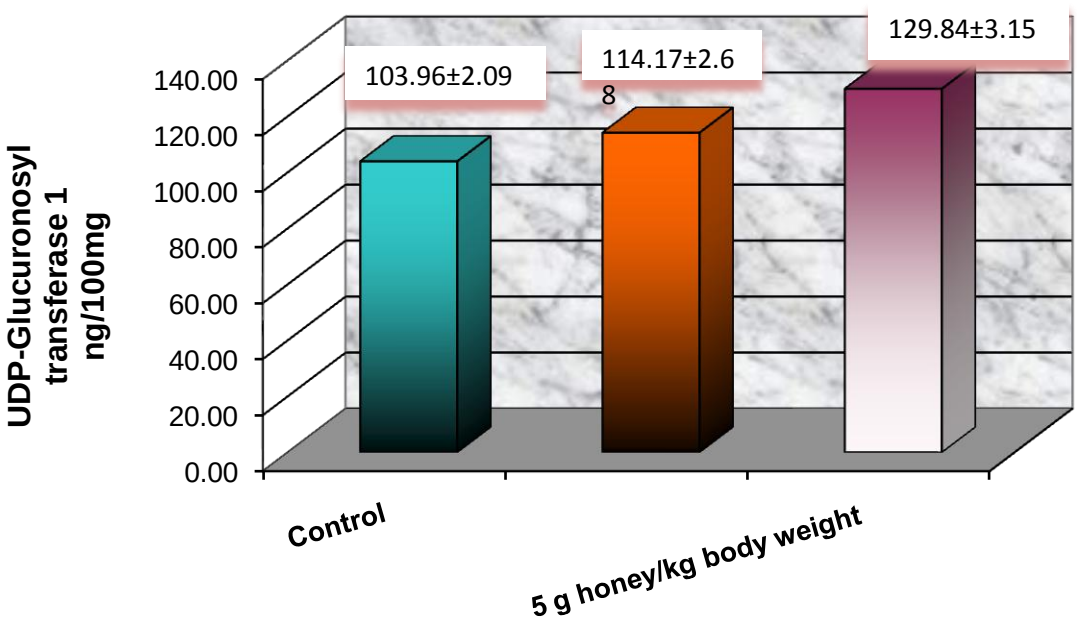

Figure 3:-Effect of oral honey solutions administration on hepatic UGT activity.

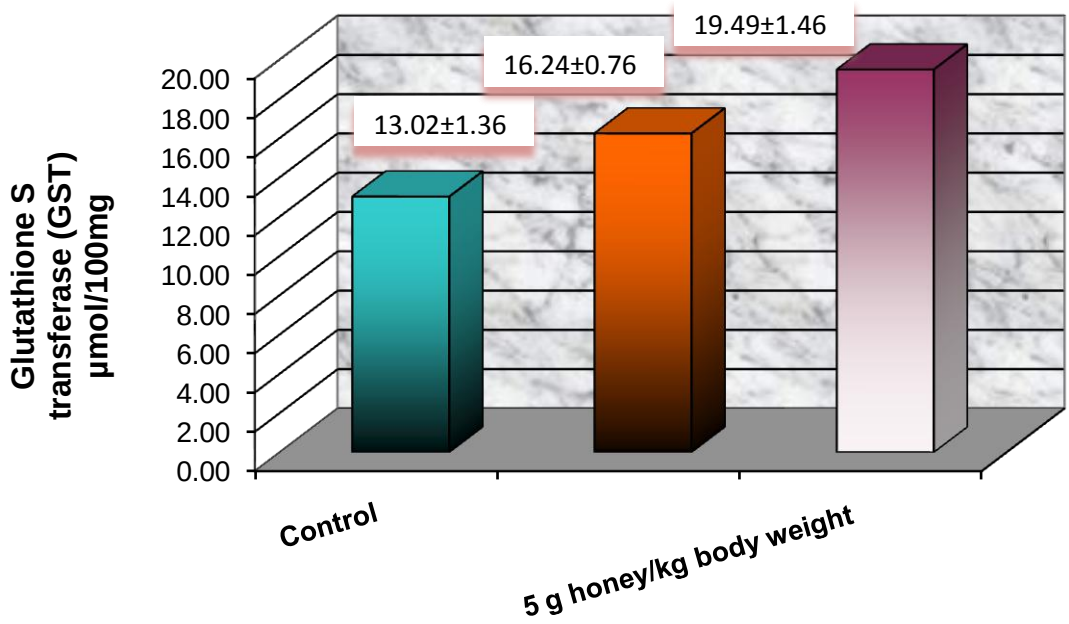

Figure 4:- Effect of oral honey solutions administration on hepatic GST activity.

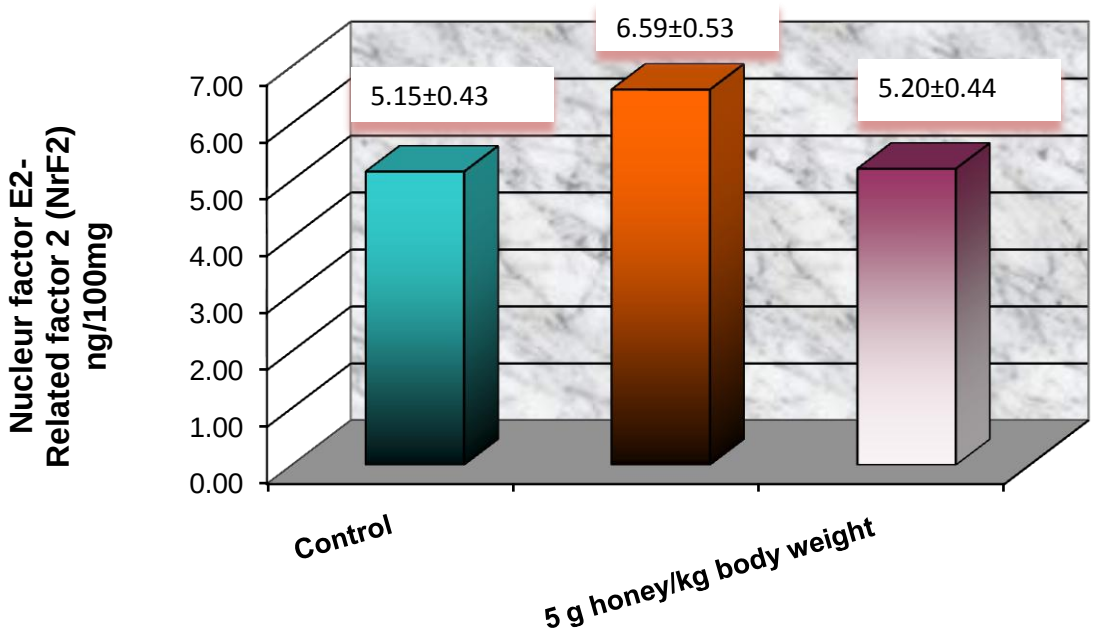

Figure 5:-Effect of oral honey solutions administration on hepatic Nrf2 activity.

Table 1:-Effect of oral honey solutions administration on some hepatic detoxification enzyme activities of phases I, II and III compared with control.

Groups Parameters	G1 Control	G2 2.5g honey/Kg body weight	G3 2.5g honey/Kg body weight
CYP2E1(ng/100mg)	43.92±2.00[a]	49.07±1.88[b]	42.58±3.10[a]
% of change from control	-	111.73%	-96.95%
EPH (Pg/100mg)	81.79±2.09[a]	86.71±4.38[b]	78.80±2.05[a]
% of change from control	-	106.02%	-96.34%
UGT (ng/100mg)	103.96±2.09[a]	114.17±2.68[b]	129.84±3.15[c]
% of change from control	-	109.82%	124.89%
GST (µmol/100mg)	13.02±1.36[a]	16.24±0.76[b]	19.49±1.46[c]
% of change from control	-	124.73%	149.69%
Nrf2 (ng/100mg)	5.15±0.43[a]	6.59±0.53[b]	5.20±0.44[a]
% of change from control	-	127.96%	100.98%
Values are represented as means ± S.D., The mean differences are significant at P<0.05			
There is no significant difference between means have the same letter in the same row.			

Discussion:-

Honey is claimed to be of therapeutic benefits in the management of chronic diseases commonly associated with oxidative stress even if it is administered alone or in combination with conventional therapy (Erejuwa et al., 2012a). The action of honey has been related to the agents' abilities to enhance the activities of the drug metabolizing enzymes (DMEs) (Alvarez-Suarez et al., 2013).

The DMEs play central roles in the metabolism, elimination and/or detoxification of xenobiotic or exogenous compounds introduced into the body. In general; DMEs protect the body against the potential harmful exposure to xenobiotic from the environment as well as certain endobiotics. In order to minimize the potential injury caused by these compounds,most of the tissues and organs are well equipped with DMEs including phase I, phase II metabolizing enzymes as well as phase III transporters which are present in abundance either at the basal uninduced level, and/or inducible at elevated level after xenobiotic exposure (Rushmore and Kong, 2002 and Wang and LeCluyse, 2003).

In the present study honey enhanced hepatic CYP2E1 activity which is one of the microsomal cytochrome P450 (CYP450) enzymes superfamily, indicating that honey has an effect for initiating the first phase in detoxification. CYP2E1 which represents 6.6% of total CYP450 in human liver (Vermeulen, 1996), plays crucial roles in hepatic metabolism and elimination of xenobiotic and drugs (Rannug et al., 1995, Lewis, 2003 and Pascussi et al., 2003). CYP2E1is induced by ethanol, carbon tetrachloride, dimethylnitrosamine, acetone and isoniazid and some drugs as chlorzoxazone,enflurane and halothane (Liska, 1998). In a typical phase I reaction, a CYP450 enzyme uses oxygen and, as a cofactor, NADH, to add a reactive group, such as hydroxyl radical. As a consequence of this step in detoxification, reactive molecules which may be more toxic than the parent molecules are produced, which must be metabolized by phase II conjugation to avoid damage to proteins, RNA and DNA within the cell (Garg et al., 2008).

Interestingly, data in the present study demonstrated that honey solution administration-mediated enhancement in the activities of phase II enzymes;EPH, UGT and GST indicating the beneficial and powerful detoxifying role of honey in liver via eliminating the toxic effect of many substrates either from its original state or which produced from CYP450 reactions. As previously stated by Erejuwa et al. (2010) that, honey administration to diabetic rats increased significantly GST compared with diabetic-non treated rats. Moreover; Erejuwa et al. (2012b) concluded that honey supplementation restored the elevated renal GST of the hypertensive rats.

The increased hepatic phase II enzymes activities are explained according to the bioactive components of honey. Once honey is absorbed by the intestinal epithelium and before crossing into the bloodstream, flavonoids undergo some degree of phase II metabolism with the generation of different conjugated products, predominantly sulphates, glucuronides and methylated derivatives through the action of sulfotransferases (SULTs), UGT, and catecholO'Methyltransferase (COMTs), respectively (Del Rio et al., 2013). Besides the metabolic biotransformation of flavonoids, which occurs by the intestinal microflora and the gut-liver pathways, their bioavailability and cell/tissue accumulation have been closely associated with the multidrug-resistance-associated proteins like MRP-1 and MRP2 (i.e. ATP-dependent efflux transporters), also named phase III metabolism (Leonarduzzi et al., 2010) and with their tissue distribution and substrate affinity in the various organs. It has been proposed that MRP-2, localized on the apical membrane of cells of the small-bowel epithelium, transports the already intracellular flavonol back to the intestinal lumen, thus modulating the actual intestinal importation of these compounds. On the contrary, MRP-1, situated on the vascular pole of enterocytes, favors transport of the flavonoid from inside the cells into the blood (van Zanden et al., 2007 and Singh et al., 2008).

In general; conjugation with phase II DMEs generally increasehydrophilicity and thereby enhance excretion in the bile and/or the urine and consequently a detoxification effect (Rushmore and Kong, 2002). EPH catalyzes epoxides by the reaction with water forming intermediates can be excreted from body (Liska, 1998). However; GST catalyzed the conjugation of reactive electrophiles with glutathione (GSH), forming reactive intermediates in particular when GSH levels in the cells are attenuated, consequently resulting in toxicological effects (Bolton and Chang, 2001). On the other hand; UGT catalyzes glucuronidation, may play roles in the conjugation and ultimately excretion and elimination of many drugs and xenobiotic containing hydroxyl functional group either present in the parent structure as phenols, thiols, amines and carboxylic acids and/or after biotransformation by the phase I enzymes(Xu et al., 2005).

The present study indicated that, honey administration increased the hepatic activity of Nrf2 as one of the receptors of phase III detoxification. This finding revealed that, honey administration exerted a closed detoxifying chain via Nrf2 activation beside the activation of each of the CYP2E1, EPH, UGT and GST, even if its degree of activation is changed by the dose administered. It was previously reported that reduced or impaired Nrf2 activity or expression contributes to increased susceptibility of kidney to oxidative stress in rats with chronic renal failure (Kim and Vaziri, 2010) or hypertension (Erejuwa et al., 2011 and Erejuwa et al., 2012b).Honey exerted a beneficial ameliorationof the oxidative stress via up-regulation of Nrf2 activity or expression (Erejuwa et al., 2009, Erejuwa et al., 2011 and Erejuwa et al., 2012b). This elevation in the Nrf2 activity as a result of honey administration may be due to quercetin, one of its flavonoids, as reported by Panchal et al. (2012). Theyreported that rats supplemented with quercetin presented a higher protein expression of Nrf2 compared with the control group with decrease in oxidative stress and inflammation.

Nrf2 is a basic leucine zipper redox sensitive transcriptional factor which is responsible for one of the physiological important stress response mechanisms (Motohashi and Yamamoto, 2004). Under basal conditions, Nrf2 binds to kelch-like ECH associated protein1 (keap1) in the cytoplasm. However under oxidative stress conditions;

electrophiles and oxidative stress disrupt the Nrf2-keap1 complex, translocate it to the nucleus (Nakata et al., 2006). Then Nrf2 binds to antioxidant responsive element with heterodimeric combinations with other basic leucine zipper proteins, such as small Mafs, and regulates gene expression of detoxifying enzymes, such as GST, NAD(P)H:quinone oxidoreductase-1, UGT, γ-glutamylcysteinesynthetase and heme oxygenase-1 (Jaiswal, 2004, So et al., 2006 and Shen and Kong, 2009).The transcription of these genes subsequently induces free radical scavenging enzymes and other detoxifying enzymes which swiftly neutralize, detoxify and eliminate the oxidants or xenobiotics(Kobayashi et al., 2009).

Conclusion:-

The present study revealed that honey administration is beneficial natural detoxifying via eliminating of some specific toxic agents and oxidative radicals through activation of a chain initiating from phase I (CYP2E1) passing through phase II (EPH, UGT and GST) then ending with phase III (Nrf2). Moreover; the lower dose of honey is more effective, hence it plays significant roles in each of the three phases than the higher dose.

Conflict of interest:-

The authors have declared that there is no conflict of interest.

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